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Par
Suzanne DUFRESNE

Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models

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Rapporteurs avant soutenance :

Alice Carrier, Directrice de Recherche – Centre de Recherche en Cancérologie de Marseille,, équipe stress cellulaire, Marseille, France
Adrien Rossary, Maitre de Conférences des Universités – Unité de Nutrition Humaine, UFR Pharmacie, Université Clermont Auvergne, Clermont-Ferrand, France

Composition du Jury :

Examinateurs :
Yann S. Gallot, Maitre de Conférences des Universités – Laboratoire de Biologie de l'Exercice pour la Performance et la Santé, Université d'Evry-Val-d'Essone, Evry, France
Romain Mathieu, Professeur des Universités, Praticien Hospitalier, CHU Pontchaillou Rennes, service urologie, Rennes, France

Dir. de thèse :
Amélie Rébillard, Professeur des Universités, laboratoire Mouvement Sport Santé, Bruz, France

Co-dir. de thèse :
Christine M. Friedenreich, Directrice scientifique, Department of Cancer Epidemiology and Prevention Research, Alberta Health Services, Holly Cross Centre, Calgary, Alberta, Canada

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"A ship in harbor is safe but that is not what ships are built for"
Admiral Grace Murray Hopper

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TABLE OF CONTENTS

ACKNOWLEDGMENTS 1

TABLE OF CONTENTS 3

LIST OF ABBREVIATIONS 7

LIST OF TABLES AND FIGURES 11

INTRODUCTION 13

LITERATURE REVIEW 15

CHAPTER I: PROSTATE CANCER 16

I – Epidemiology of prostate cancer 16

A – Prostate cancer incidence and mortality across the globe 16

B – The impact of screening on prostate cancer epidemiology 17

C – Risk factors for prostate cancer 18

1 – Non-modifiable risk factors 18

2 – Modifiable risk factors 20

II – Multistep prostate carcinogenesis, cancer hallmarks and epigenetic signatures 23

A – Anatomy and physiology of the prostate gland 23

1 – Structure and function of the prostate 23

2 – Cell types composing the prostate 24

B – Prostate carcinogenesis and cancer progression 26

C – Cancer hallmarks 27

1 – Sustained proliferative signaling 28

2 – Evading growth suppressors 28

3 – Resisting cell death 28

4 – Enabling replicative immortality 29

5 – Inducing angiogenesis 29

6 – Activating evasion and metastasis 30

7 – Genome instability and mutation 30

8 – Tumor-promoting inflammation 30

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

9 – Reprogramming energy metabolism 31
10 – Evading immune destruction 32
D – Epigenetics in prostate cancer 32
1 – DNA methylation 33
2 – Histone modifications 34
3 – Micro-RNA expression 35
III – Prostate cancer treatment 36
A – Low risk localized prostate cancer 37
1 – Active surveillance 37
2 – Radical prostatectomy 37
3 – Radiotherapy 38
B – High-risk and metastatic prostate cancer 38
1 – Androgen deprivation therapy 39
2 – Chemotherapy 39
3 – Immunotherapy 40

CHAPTER II: PHYSICAL ACTIVITY 42
I – The impact of physical activity on psychosocial and physiological outcomes in prostate cancer patients 43
A – Psychosocial outcomes 43
1 – Quality of life and health-related quality of life 43
2 – Depression 44
3 – Cancer-related fatigue 44
B – Physiological outcomes 45
1 – Cardiorespiratory fitness 46
2 – Muscle mass and muscle strength 46
II – The impact of physical activity on survival and tumor growth in prostate cancer 48
A – Physical activity and survival rates in prostate cancer patients 48
1 – Physical activity pre-diagnosis 48
2 – Physical activity post-diagnosis 50
B – Physical activity and prostate tumor growth: data from preclinical models 51
1 – A wide variety of preclinical models 51
2 – The impact of physical activity on tumor growth, cell proliferation and cell death in preclinical prostate cancer models 58
3 – Physical activity and tumor growth in preclinical models: the importance of study design 63
4 – Suggested biological mechanisms mediating the anti-cancer effects of physical activity 65

III – The impact of physical activity on cancer treatment efficiency 75
A – Surgery 75
B – Hormone therapy 76
C – Chemotherapy 77
D – Immunotherapy 78
E – Radiotherapy 80

AIMS & OBJECTIVES 83

RESULTS 87
I – Exercise training improves radiotherapy efficiency in a murine model of prostate cancer 88
II – Voluntary wheel running does not enhance radiotherapy efficiency in mouse bearing PC-3 tumors 115
III – A review of physical activity and circulating miRNA expression 146
IV – Exercise training as a modulator of epigenetic events in prostate tumors 177

DISCUSSION 195
I – Epigenetic mechanisms and physical activity: a link to cancer and cancer treatments? 196
II – Interactive effects of physical activity and radiotherapy in prostate cancer: a new perspective 199
III – Physical activity in prostate cancer: one size does not fit all? 201
IV – Implications for the clinical setting and future perspectives 202

BIBLIOGRAPHY 207

APPENDIX (RESUME) 252

LIST OF ABBREVIATIONS

Standard abbreviations will be described only the first time they are mentioned in the thesis. Non-standard abbreviations will be restated at the beginning of each chapter as they appear.

5mC	Methyl group 5-carbon on Cytosine residue
ACSM	American College of Sports Medicine
ADT	Androgen-Deprivation Therapy
AKT	Protein kinase B
AMPK	AMP-activated protein Kinase
AR	Androgen Receptor
BAX	B-cell lymphoma-Associated X
BCL-2	B-Cell Lymphoma 2
BMI	Body Mass Index
BT	BrachyTherapy
cCASP3	Cleaved Caspase 3
CCL22	C-C motif Chemokine Ligand 22
CRF	Cancer-Related Fatigue
CRPC	Castration Resistant Prostate Cancer
CTLA-4	Cytotoxic T Lymphocyte Associated protein 4
DHT	DiHydroTestosterone
DNA	DeoxyriboNucleic Acid
DNMT	DNA MethylTransferase
EBRT	External Beam Radiation Therapy
ECM	ExtraCellular Matrix
ERG	ETS Related Gene
ERK	Extracellular signal-Regulated Kinase
FDA	Food and Drug Administration
FOXP3^{+}	Forkhead bOX P3^{+}
GPAQ	Global Physical Activity Questionnaire
GST	Glutathione S-Transferase
GSTP1	Glutathione S-Transferase P1
HAT	Histone AcetylTransferase
HDAC	Histone DeACetylase

HIF-1α	Hypoxia-Inducible Factor 1α
HIIT	High Intensity Interval Training
HGPIN	High-Grade Prostatic Intraepithelial Neoplasia
HRQOL	Health-Related Quality Of Life
IGF-1	Insuline-like Growth Factor 1
IL-2Rβ	InterLeukin 2 Receptor β
IL-6	InterLeukin 6
IFN-ɣ	InterFeroN ɣ
IPAQ	International Physical activity Questionnaire
KLRK1	Killer cell Lectin-like Receptor K1
MAPK	Mitogen Activated Protein Kinase
MET	Metabolic Equivalent of Task
MEK	MAPK/ERK Kinase
miRNA	Micro-RNA
MMP	Matrix MetalloProteinase
mTOR	Mammalian target of rapamycin
MDSC	myeloid-derived suppressor cells
NCCN	National Comprehensive Cancer Network
NK	Natural Killer
NMU	N-Methyl-Nitrosourea
NSAID	Non-Steroidal Anti-Inflammatory Drug
OSM	OncoStatin M
PARP	Poly ADP-Ribose Polymerase
PCa	Prostate Cancer
PD-1	Programmed Death 1
PDCD4	ProgrammeD Cell Death protein 4
PDGFR-β	Platelet-Derived Grwoth Factor Receptor β
PD-L1	Programmed Death-Ligand 1
PDX	Patient-Derived Xenograft
PET	Preoperative Exercise Therapy
PGC-1α	Peroxisome proliferator-activated receptor Gamma Coactivator 1α
PI3K	PhosphoInositide-3-Kinase
PIN	Prostatic Intraepithelial Neoplasia
PSA	Prostate Specific Antigen
PTEN	Phosphatase and TENsin homolog
QOL	Quality Of Life
RASSF1A	Ras ASSociated domain Family 1A

RB	RetinoBlastoma
RCT	Randomized Controlled Trial
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
rPB	Rat ProBasin
RT	RadioTherapy
SV40-Tag	Simian Virus 40 large T antigen
TAM	Tumor-Associated Macrophage
TGF-β	Transforming Growth Factor β
TMPRSS2	TransMembrane PRoteaSe Serine 2
TNF-α	Tumor Necrosis Factor α
TR	Treadmill Running
TRAMP	Transgenic Adenocarcinoma of the Mouse Prostate
Tregs	Regulatory T cells
TUNEL	Terminal deoxynucleotidyl transferase dUTP Nick End Labeling
SPARC	Secreted Protein Acidic and Rich in Cystein
USPSTF	United States Preventive Services Task Force
VEGF	Vascular Endothelial Growth Factor
VO_{2peak}	Peak oxygen consumption
VO_{2max}	Maximal oxygen consumption
VWR	Voluntary Wheel Running
WCRF	World Cancer Research Fund
WHO	World Health Organization

LIST OF TABLES AND FIGURES

FIGURES

Literature review

Figure L-1 : Bar Chart of Region-Specific Incidence and Mortality Age-Standardized Rates for Cancers of the Prostate in 2018 (GLOBOCAN 2018)..... **17**

Figure L-2 : Localization of the different prostate zones. Source: De Marzo et al. 2007 ..... **23**

Figure L-3: Major genetic events involved in prostate carcinogenesis. **26**

Figure L-4: Hallmarks of cancer (adapted from Hanahan and Weinberg, 2011)..... **27**

Figure L-5: Graphical representation of the major epigenetic processes (Barrès and Zierath 2016) **33**

Figure L-6: DNA methylation processes in PCa (adapted from Szyf et al. 2004)..... **34**

Figure L-7: Histone acetylation/deacetylation processes (Pant et al. 2020)..... **35**

Figure L-8: Schematic representation of common treatments used throughout the PCa continuum (adapted from Higano in Figg et al. 2010) **36**

Figure L-9: Summary hazard ratio for the highest versus lowest levels of prediagnosis physical activity on overall mortality and cancer-specific mortality (adapted from Friedenreich et al. 2020) **49**

Figure L-10: Summary hazard ratio for the highest versus lowest levels of postdiagnosis physical activity on overall mortality and cancer-specific mortality (adapted from Friedenreich et al. 2020) **51**

Figure L-11: Possible effects of physical activity on tumor vascularization (Schumacher et al. 2020) **68**

Figure L-12: Voluntary wheel running slows down tumor growth by mobilizing and redistributing NK cells to the tumor tissue in an epinephrine and IL-6 dependent manner. Source: Pedersen et al. 2016 ..... **70**

Results

Figure R-1: Study design and characteristics – STUDY I **98**

Figure R-2: Tumor volume and tumor weight – STUDY I **99**

Figure R-3: Tumor characteristics: immunohistological analysis. – STUDY I. **101**

Figure R-4: Western blots for cellular signaling in the tumor tissue – STUDY I..... **102**

Figure R-5: NK cell infiltration – STUDY I **104**

Figure R-6: Study design and characteristics - experiments #1 A – STUDY II **126**

Figure R-7: Tumor perfusion and tumor growth – STUDY II..... **127**

Figure R-8: Study design and characteristics - experiments #2 – STUDY II **129**

Figure R-9: Tumor immunohistological analyses – STUDY II **131**

Figure R-10: Western blot analysis – STUDY II **133**

Figure R-11: Study design and characteristics - experiments #3 – STUDY II. **135**

Figure R-12: Growth proliferation assay in LNCaP and PC-3 cells treated with serum from inactive or active men – STUDY II **136**

Figure R-13: Hypothesized pathways of miRNA transport from muscles to tumor cells via the circulation – STUDY III **160**

Figure R-14: Hypothesized mechanisms of action of PA on cancer risk and progression through c-miRNA modulation – STUDY III **161**

Figure R-15: Apoptosis and DNA methylation – STUDY IV. **185**

Figure R-16: Histone acetylation – STUDY IV **186**

Figure R-17: miRNA expression – STUDY IV **187**

Discussion

Figure D-1: Epigenetic modifications modified by exercise training observed in our work..... **198**

Figure D-2: Graphical representation of molecular interactions observed between treadmill running and radiotherapy in PPC-1 tumors **200**

TABLES

Litterature review

Table L-1: Preclinical studies investigating the impact of chronic exercise/physical activity on prostate tumor growth **60**

Results

Table R-1: List of western blot antibodies..... **95**

Table R-2: List of primers used for RT-qPCR analysis **96**

Table R-3: List of western blot antibodies..... **123**

Table R-4: Effect of acute physical activity on the expression of c-miRNAs in healthy and normally active subjects..... **166**

Table R-5: Effect of acute physical activity on the expression of c-miRNAs at various time points in healhy, trained subjects **167**

Table R-6: Effect of chronic/regular/long-term physical activity on c-miRNA levels in healthy subjects **168**

Table R-7: List of western blot antibodies..... **182**

Table R-8: List of primers used for RT-qPCR analysis **182**

INTRODUCTION

INTRODUCTION

The modernization of our societies and lifestyles limits our physical movements and muscular activity. For instance, the time spent in front of a screen has drastically increased over the past years, contributing to higher daily sitting times (Owen et al. 2010). As a result, both sedentary behaviors and physical inactivity have become more common, which is not without serious health consequences: physical inactivity is indeed considered a major contributor to non-communicable diseases, contributing to 9% of premature mortality (representing 5.3 million deaths in 2008), a burden comparable to smoking (Lee et al. 2012).

While physical inactivity is considered a risk factor for several cancer types, physical activity, on the other hand, is increasingly recognized as a strategy to limit treatment side effects, improve quality of life (QOL) and increase survival of cancer patients (Rock et al. 2012; Patel et al. 2019; Campbell et al. 2019; Friedenreich et al. 2020).

Engaging in physical activity challenges whole-body homeostasis and gives rise to widespread adaptations in cells, tissues, and organ systems (Hawley et al. 2014). These pleiotropic effects have been suggested to mediate possible anti-cancer effects of physical activity, but they may also hypothetically interact with specific cancer types and/or treatments (Ashcraft et al. 2019).

Prostate cancer (PCa) is the second most diagnosed cancer in men and represents a major health issue worldwide (Bray et al. 2018). Radiotherapy (RT) is a commonly used treatment for localized PCa but its efficacy can be limited by innate or acquired radioresistance. The potential of physical activity to act as a lever to reduce these resistances remains largely unknown.

The present PhD work investigates the potential for physical activity to impact tumor molecular biology and radiotherapy efficiency using different preclinical PCa models and physical activity modalities.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

LITERATURE REVIEW

CHAPTER I: PROSTATE CANCER

Prostate cancer (PCa) is a complex disease representing a major health concern around the globe, prompting the need for effective diagnostic tools and treatments. In this first chapter, we will provide an overview of the PCa epidemiology, outlining the incidence and mortality rates around the world as well as the major risk factors for this disease. Furthermore, we will describe the multistep process of prostate carcinogenesis and describe the main characteristics of malignant cells. Finally, we will summarize the currently employed diagnosis techniques and treatments used in PCa.

I – Epidemiology of prostate cancer

A – Prostate cancer incidence and mortality across the globe

In 2018, an estimated 1,276,106 new cases of PCa were diagnosed around the world, representing the second most frequent malignancy in men and accounting for 7.1 % of new cancer cases worldwide (Bray et al. 2018). The PCa incidence rates vary considerably across regions, with the highest being observed in Oceania and the lowest in Asia (Bray et al. 2018; Taitt 2018). PCa is ranked as the fifth leading cause of cancer death worldwide and was responsible for an estimated 358,989 deaths in 2018 (Bray et al. 2018).

The number of deaths attributed to PCa is higher in developing regions than in developed countries, which contrasts with PCa global incidence patterns (Figure L-1). As an example, 86.4 men in 100,000 were diagnosed with PCa in Australia and New Zealand in 2018, and only 10.2 in 100,000 died from this disease, while 35.9 men in 100,000 were diagnosed with PCa in Middle Africa in 2018 and 22.7 in 100,000 died from this disease (Bray et al. 2018). Considering the high incidence and relatively long survival rate of PCa, this disease unsurprisingly has a very strong prevalence. It is indeed the second most prevalent cancer behind breast cancer, with an estimated 10 million men living with PCa across the world in 2017 (IHME, 2018). These

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

data raise awareness of the burden caused by PCa and highlights the importance of developing strategies to improve treatment efficiency and patients' quality of life (QOL).

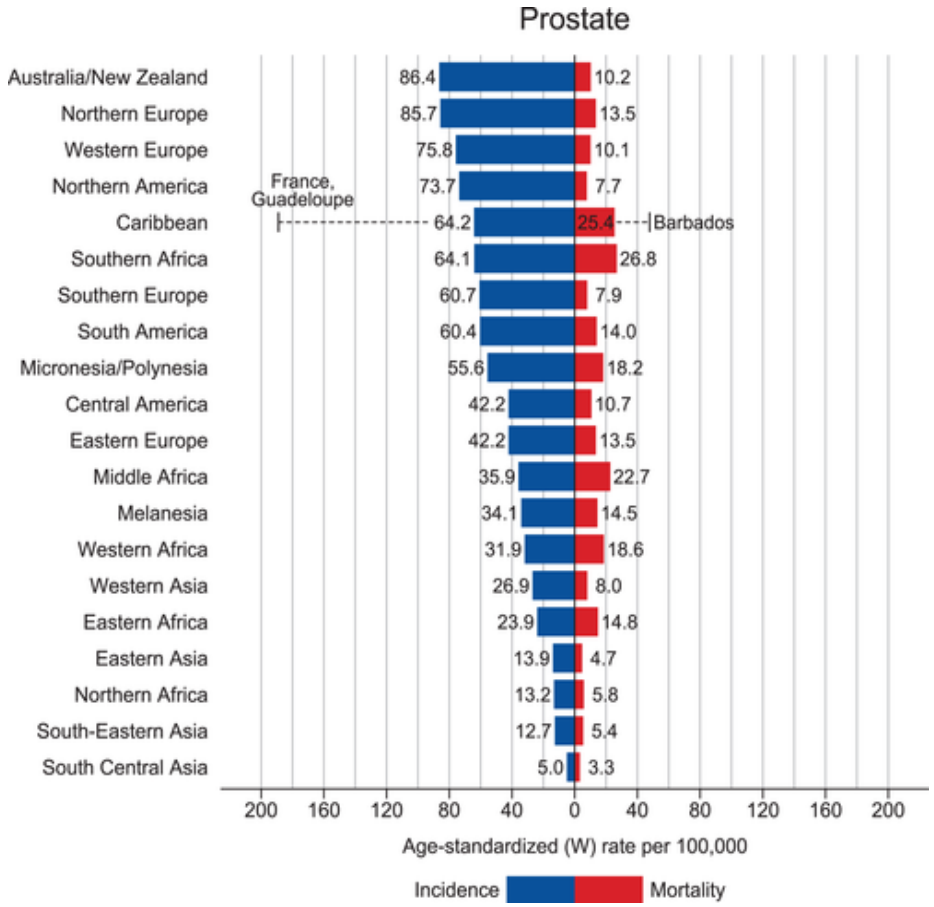

Figure L-1 : Bar Chart of Region-Specific Incidence and Mortality Age-Standardized Rates for Cancers of the Prostate in 2018. Source: GLOBOCAN 2018.

B – The impact of screening on prostate cancer epidemiology

The development of PCa screening aims to decrease the mortality rates associated with this disease. Most PCa cases are diagnosed based on elevated serum levels of prostate specific antigen (PSA) (>4ng/mL) and confirmed with a tissue biopsy. The introduction of PSA testing into the population in the 1980's has paralleled a striking increase in PCa incidence rates (Negoita et al. 2018). It also likely contributed to expanding the gap between elevated incidence rates observed in developed countries and lower rates reported in developing countries (Hassanipour-Azgomi et al. 2016), where availability and access to health care may be difficult (Quinn and Babb 2002).

PSA testing has also led to an earlier diagnosis of PCa, with an increase in the number of men diagnosed with localized disease rather than advanced stage (Thompson and Ankerst 2007; Etzioni et al. 2008). The downside of this widely used testing technique however is the increase in PCa overdiagnosis, which corresponds to cancers that may not have caused clinical consequences in the absence of screening (Loeb et al. 2014). Approximately 20 to 40% of the PCa cases in Northern America and Europe could be attributed to overdiagnosis, which questions the link between increased screening and reduced mortality (Draisma et al. 2009). Benefits from PSA testing have in fact only been recently reported: in 2018, the United States Preventive Services Task Force (USPSTF) suggested that men aged 55-69 years of age could indeed benefit from PSA testing, while it is less convincing for men over 70 years of age for whom screening may outweigh the expected harms (USPSTF 2018; Negoita et al. 2018).

C – Risk factors for prostate cancer

Epidemiologic studies have determined several risk factors for PCa, which can be grouped into two main categories: (1) non-modifiable and (2) modifiable risk factors. Identifying these risk factors helps reducing the burden of PCa by promoting behavioral changes and by facilitating the detection of men with a higher risk of developing this disease. Importantly, PCa is a clinically heterogeneous disease with some men displaying aggressive forms of PCa while most others have a slow growing form of illness. Therefore, the risk factors for total PCa and advanced PCa may differ.

1 – Non-modifiable risk factors

Non-modifiable risk factors, although irreversible, enable for a better understanding of the mechanisms involved in prostate carcinogenesis and help to identify individuals at high risk of developing this disease.

a – Older age

Aging is one of the strongest risk factors for PCa. While this disease is rare among men under 50 years of age (with an incidence of 0.1% in this population), it is estimated that 85% of PCa

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cases are diagnosed in men over 65 years of age (Patel and Klein 2009). This trend can be observed in both low and highly developed regions (Ferlay et al. 2015).

b – Ethnicity

Ethnicity represent another important risk factor for PCa. In the United Kingdom for example, Black men are three times more likely than White men to develop PCa (Ben-Shlomo et al. 2008) while men from South Asia have a 20% lower relative risk to develop PCa (Metcalfe et al. 2008). These observation may be explained, at least partly, by genetic factors (Kheirandish and Chinegwundoh 2011).

c – Height

Height attained at adulthood has been shown to be associated with higher risk of advanced cancer, but not with the risk of localized PCa (Giovannucci et al. 1997; Norrish et al. 2000; Rodriguez et al. 2001; Zuccolo et al. 2008). The World Cancer Research Fund (WCRF) considers that the evidence showing an increase risk in PCa in taller men is strong (WCRF/AIRC 2018). Height is reflective of diet and health during childhood (Perkins et al. 2016) and can influence endocrine factors. For instance, prepubertal growth is associated with increased levels of circulating insulin-like growth factor 1 (IGF-1) (Rogers et al. 2006), known to influence prostate carcinogenesis (Shi, Berkel, and Yu 2001).

d – Family history

Family history is also known to influence the risk of developing PCa (Zeegers, Jellema, and Ostrer 2003; Brandt et al. 2010). A nation-wide population based study conducted in Sweden reported that a man with a brother diagnosed with PCa had a risk twice as high of developing this disease compared to the general population, and a threefold higher risk if the father also has PCa (Bratt et al. 2016). A similar trend can be observed for the aggressiveness of the cancer: compared with a men without a positive family history, a man with a father or brother diagnosed with PCa have a two to threefold higher risk of getting an aggressive type of PCa (Bratt et al. 2016). Additionally, a family history of breast cancer could also impact PCa risk (Chen et al. 2008; Lamy et al. 2018).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

2 – Modifiable risk factors

There is a growing interest for identifying the influence of modifiable risk factors on PCa carcinogenesis in order to promote lifestyle changes and improve PCa prevention.

a – Obesity

Obesity can lead to systemic inflammation, metabolism modifications, and altered immune responses, which are known to play important roles in the initiation and progression of cancer, including PCa (Roberts, Dive, and Renehan 2010; Fujita et al. 2019). For PCa more specifically, there is consistent evidence showing that obesity increases the risk of developing an aggressive form of the disease (Bandini, Gandaglia, and Briganti 2017; Fujita et al. 2019). A meta-analysis including six different studies conducted in PCa patients reported that an increase of 5 kg/m^2 in body mass index (BMI) was associated with a 20% higher risk of PCa-specific mortality and a 21% risk of biochemical recurrence (Cao and Ma 2011). In view of these findings, the WCRF concluded that there was strong evidence that being overweight increases the risk of advanced PCa (WCRF/AIRC 2018).

b – Exposure to pesticides

The observation that farmers had increased risk of developing PCa led to the investigation of agricultural chemicals such as pesticides as potential risk factors for PCa (Van Maele-Fabry et al. 2006). Exposure to pesticides can disturb the function of different organs including the reproductive system. In fact, there is increasing evidence linking contact to pesticides with the incidence of human chronic diseases, including PCa (Mostafalou and Abdollahi 2013). For instance, exposure to the insecticide chlordecone has been associated with an increase in the risk of PCa, and with a significant relationship between exposure and response (Multigner et al. 2010). Chlordecone was widely used in from 1973 to 1993 in the French West Indies (and notably in Guadeloupe and Martinique), a region where the incidence of PCa is particularly high (Mallick, Blanchet, and Multigner 2005).

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c – Dietary factors

Data from various migrant studies have suggested for a long time that the Western lifestyle increases the risk of developing PCa (Rose, Boyar, and Wynder 1986). The Western diet consists of high intake of rich foods including animal fat, meats, and low amounts of fiber. Dairy is a major component of the Western diet containing high levels of calcium and has been identified by the WCRF as likely to increase the risk of PCa (WCRF/AIRC 2018). Even though the evidence is considered limited, a meta-analysis showed a 7% increased PCa risk per 400G of dairy products per day (Aune et al. 2015). High calcium intake may influence vitamin D and IGF-1 levels, which are involved in prostate carcinogenesis (Giovannucci 1998; Ma et al. 2001). The WRCF also reported that there was suggestive evidence for low plasma α-tocopherol and plasma selenium concentrations to increase PCa risk (WCRF/AIRC 2018). α-tocopherol represents the major vitamin E component, which acts as an antioxidant and has been suggested to reduce cancer incidence (Cardenas and Ghosh 2013). Selenium also possess antioxidant properties (Tinggi 2008) and compensate for the deficiency of vitamin E (and vice-versa). The studies investigating the effects of these two components on PCa are however highly mixed (Rock et al. 2012; Cardenas and Ghosh 2013), which may reflect the complexities of vitamin E and selenium metabolism. Of note, other dietary components may influence PCa risk, but the evidence remains inconclusive.

d – Sedentary behavior and lack of physical activity

Sedentary behavior is defined as "any waking behavior characterized by an energy expenditure $\leq$ 1.5 metabolic equivalent of task (METs), while in a sitting, reclining, or lying posture" (Tremblay et al. 2017). Importantly, sedentary behavior is associated with chronic diseases such as cardiovascular dysfunctions or type 2 diabetes (Dunstan et al. 2012), and emerging evidence suggests that it could increase cancer risk and mortality (Patel et al. 2019; Friedenreich et al. 2020). For PCa more specifically, several studies have investigated the impact of sedentary behavior on PCa risk and progression, but the results remain inconclusive. In a recent meta-analysis including 12 prospective cohort studies, Berger *et al*. concluded that sedentary behavior did not represent a strong independent risk factor associated with PCa but may however indirectly increase the risk of aggressive PCa by promoting obesity (Berger et al. 2019).

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When looking at physical activity more specifically, the conclusions remain similar. Physical activity can be defined as "any bodily movement produced by skeletal muscles that results in energy expenditure" (Caspersen, Powell, and Christenson 1985). Accumulating evidence show that physical activity decreases the risk of developing a number of different cancer types (Friedenreich 2001; Patel et al. 2019; Friedenreich et al. 2020). For PCa risk however, there seems to be no or very limited benefits of physical activity (Patel et al. 2019; McTiernan et al. 2019). A recent meta-analysis found no dose-response effect of leisure time physical activity on PCa risk (Liu et al. 2018), which is in line with the cohort study conducted by Grotta *et al*. showing no correlation between total physical activity and PCa risk/incidence (Grotta et al. 2015). However, another cohort study found that active men had a significantly reduced risk of developing advanced PCa compared to their inactive counterparts (Hrafnkelsdóttir et al. 2015).

Overall, sedentary behavior and lack of physical activity does not appear to play a major role in PCa risk but might reduce the risk of developing aggressive forms of PCa.

e – Smoking

While smoking is an important risk factors in several cancer types - such as lung or pancreatic cancers (Sasco, Secretan, and Straif 2004; Alexandrov et al. 2016) - the relationship between smoking and PCa is not as straightforward. While the evidence between smoking and PCa risk is weak, there is consistent evidence showing that PCa patients who are smokers have higher PCa-related mortality rates compared to their non-smoker counterparts (Gong et al. 2008; Huncharek et al. 2010). The largest study to date is a prospective observational study enrolling 5366 PCa patients which showed that smoking was associated with a 60% higher risk of dying from PCa (Kenfield et al. 2011). Interestingly, the time period of exposure to smoking may be an important factor, as it was suggested that quitting smoking 10 years before diagnosis resulted in similar outcomes as those of never-smokers (Kenfield et al. 2011).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

II – Multistep prostate carcinogenesis, cancer hallmarks and epigenetic signatures

Cancer is widely accepted as a disease arising from sequential genetic modifications which result in the transformation of normal cells into malignant cells. These cells possess several characteristics, called cancer hallmarks, which allow them to proliferate uncontrollably and form tumors. In addition to genetic defects, epigenetic aberrations also play pivotal roles in the establishment of these cancer hallmarks and represent a growing area of research. In the following section, the multistep carcinogenesis of PCa, the cancer hallmarks as well as the major epigenetic processes associated in PCa will be presented.

A – Anatomy and physiology of the prostate gland

1 – Structure and function of the prostate

The prostate is an accessory sexual gland found exclusively in mammals. It produces a citrate enriched fluid which contributes to making up the semen and further participates to expel the semen outwards during ejaculation (Verze, Cai, and Lorenzetti 2016). The structure of the prostate is typically divided into four zones (Figure L-2)

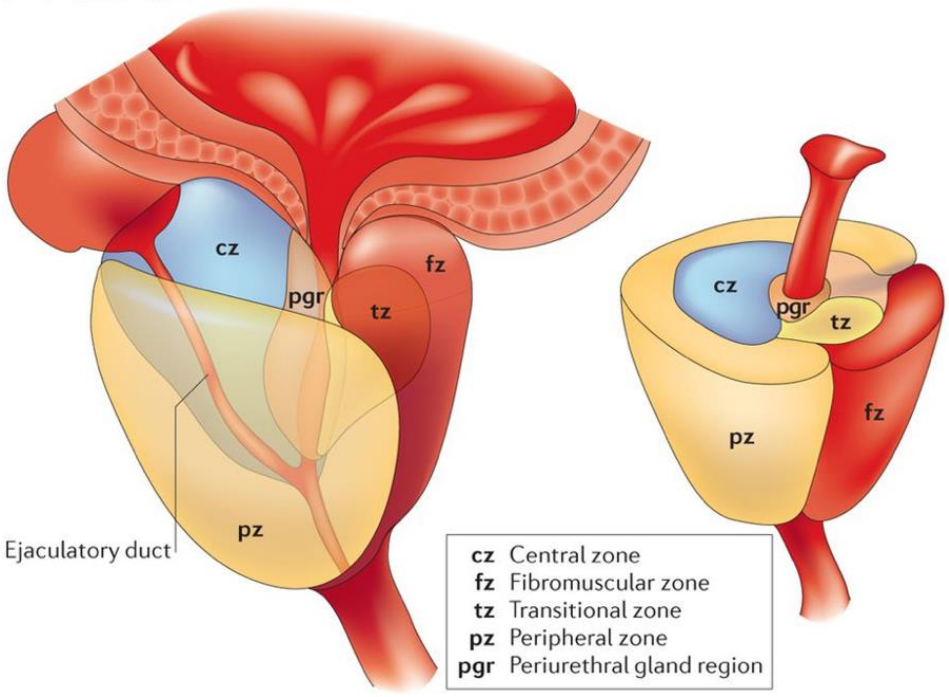

Figure L-2 : Localization of the different prostate zones. Source: De Marzo et al. 2007

These zones can be distinguished based on their embryologic roots, histologic and anatomical features, biological functions, as well as their potential to develop pathologic disorders (McNeal 1981). These four zones are comprised of an anterior fibromuscular zone and three glandular zones representing respectively 33% and 66% of the total prostate volume. The fibromuscular zone consists of fibromuscular tissue completely covering the anterior face of the prostate, hiding the glandular regions. The glandular zones include the peripheral zone, the central zone and the transition zone (McNeal 1981).

2 – Cell types composing the prostate

At the cellular level, the prostate contains (1) a glandular component and (2) a stromal component, both tightly fused within a pseudocapsule.

a – Glandular component of the prostate

The glandular component functions by producing and secreting fluids which contribute to the ejaculate. It has a tree-like organization comprised of epithelial ducts (the branches) and secretory acini (the leaves). The acini and ducts are formed by epithelial cells, which can be subdivided into three distinct cell types: luminal, basal, and neuroendocrine cells (McNeal 1988). In addition, intermediate cells expressing both the basal and the luminal lineage markers can be found in the developmental stage or in pathological conditions.

The prostate luminal cells are columnar epithelial cells that produce a variety of proteins into the lumen of the gland, including PSA. They express high levels of androgen receptor (AR) proteins and are dependent on androgen signaling for their survival and secretory function (Shen and Abate-Shen 2010). The prostate basal cells are non-secretory cells with a flat or cuboidal shape lining the basement membrane. Unlike their luminal counterparts, basal cells do not express PSA (Shen and Abate-Shen 2010). Lastly, neuroendocrine cells represent a minor epithelial cell population located in the basal layer of the prostate gland. While their precise role remains unknown, they secrete hormones and cell-signaling factors in response to neural stimulation, likely participating in the development, proliferation, and secretory activity of the prostate. Comparably to epithelial basal cells, neuroendocrine cells do not express PSA nor AR (Yuan, Veeramani, and Lin 2007; Shen and Abate-Shen 2010).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

b – Stromal component of the prostate

The stromal component of the prostate surrounds the acini and ducts. It is composed mostly of fibroblasts and smooth muscle cells. Prostatic smooth muscle cells help to expel prostatic secretions during emission. These cells are known to consistently express AR which, in the adult prostate, stimulate paracrine signaling in response to androgen and participates in maintaining prostate homeostasis (Abate-Shen and Shen 2000; Shen and Abate-Shen 2010). The prostatic fibroblasts are mainly known for providing structure to the prostate gland, but also function as immune system regulators and mediators in the wound-healing response.
Beyond fibroblasts and smooth muscle cells, the prostatic stroma also includes other cell populations such as vascular cells, nerve fibers, and circulating immune cells (Barron and Rowley 2012). This allows the stromal component of the prostate to modulate the extracellular matrix (ECM) in response to signals from the microenvironment. The ECM notably brings support and structure to the gland but also modulates cellular signaling and function. Importantly, the impact of the stroma on the ECM may play important roles in the development of growth of prostate malignancy (Barron and Rowley 2012).

c – Androgens and androgen receptors

Prostate development and homeostasis is well-known for being dependent on androgens. The major androgen in males is testosterone, which can be converted to a more potent metabolite, dihydrotestosterone (DHT) by the enzyme 5α-reductase. Testosterone and DHT mediate their actions mainly through binding to the AR. Upon ligand-binding (with testosterone or DHT for example), the AR complex undergoes a conformational change resulting in the dissociation of the heat shock proteins and a rapid nuclear-translocation of the AR. This activates gene expression leading to responses such as PSA secretion and prostate growth (Tan et al. 2015). Importantly, the regulatory function of AR involved in prostate homeostasis is thought to be aberrant in PCa. In fact, androgens and AR signaling are believed to play a major role in PCa development and growth (Heinlein and Chang 2004).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

B – Prostate carcinogenesis and cancer progression

Cancer is the result of a disruption in the homeostasis between cell proliferation and cell death. PCa more specifically are for the vast majority (more than 90%) acinar-type adenocarcinomas (cancer that develops within gland cells) (McNeal 1969; Grignon 2004). This disease is highly heterogeneous and multifocal, participating to the complexity of its understanding. PCa is also characterized by a slow growth rate: in fact, approximately one-third of men over 50 years of age exhibit pathological evidence of PCa but only a few will develop clinically significant PCa (Scardino 1989; Stangelberger, Waldert, and Djavan 2008).

Cancer is nowadays widely accepted as a disease arising from sequential genetic and epigenetic modifications which eventually result the transformation of normal cells into malignant cells (Harris 1991; Sharma, Kelly, and Jones 2010).

The multistep prostate carcinogenesis can be divided into 4 main stages (Figure L-3).

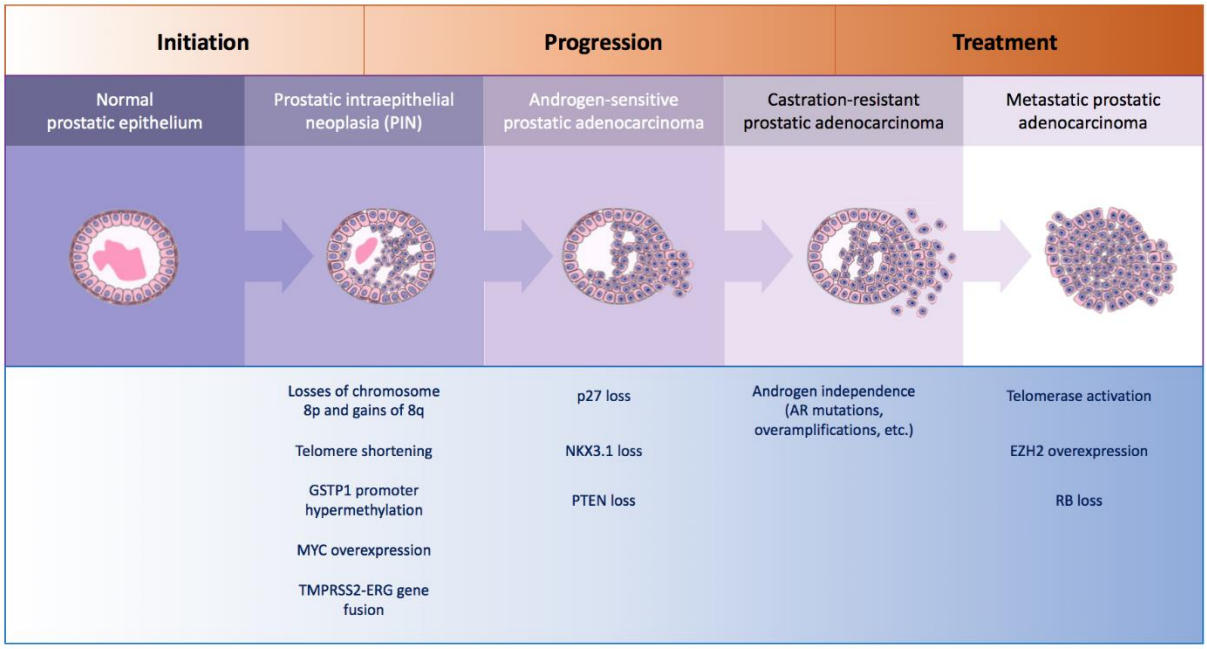

Figure L-3: Major genetic events involved in prostate carcinogenesis.

These steps include (1) prostatic intraepithelial neoplasia, characterized by dysplasia of epithelial cells and progressive loss of basal cells; (2) androgen-dependent adenocarcinoma, where the basal layer is lost which allows for cancer cells to invade and generate prostate carcinoma – with the tumor being androgen-dependent; (3) castration-resistant PCa (CRPC) characterized by a growth which does not depend on androgens, therefore remaining insensitive to androgen-deprivation therapy; and (4) metastatic prostate adenocarcinoma,

which corresponds to the spread of PCa cells into different areas of the body through the bloodstream or the lymph system.

Importantly, the progression to metastatic disease can occur either before or after the development of CRPC. It is worth clarifying that the molecular events described in the following sections can either not be observed or occur in a different order as the one outlined below. PCa is indeed a clinically heterogeneous disease and distinct molecular sub-types of PCas have been identified (Kaffenberger and Barbieri 2016; Tolkach and Kristiansen 2018).

C – Cancer hallmarks

Malignant cells acquire various hallmarks during the multiple steps of prostate carcinogenesis (described above) which distinguishes them to normal cells. In 2000, Hanahan and Weinberg proposed to regroup them into six categories (Hanahan and Weinberg 2000), which have since been revised to include four new ones (Hanahan and Weinberg 2011). These hallmarks include (1) sustaining proliferative signaling, (2) evading growth suppressor, (3) activating invasion and metastasis, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) resisting cell death, (7) avoiding immune destruction, (8) tumor-promoting inflammation, (9) genome instability and mutation, and (10) deregulating cellular energetics (Figure L-4).

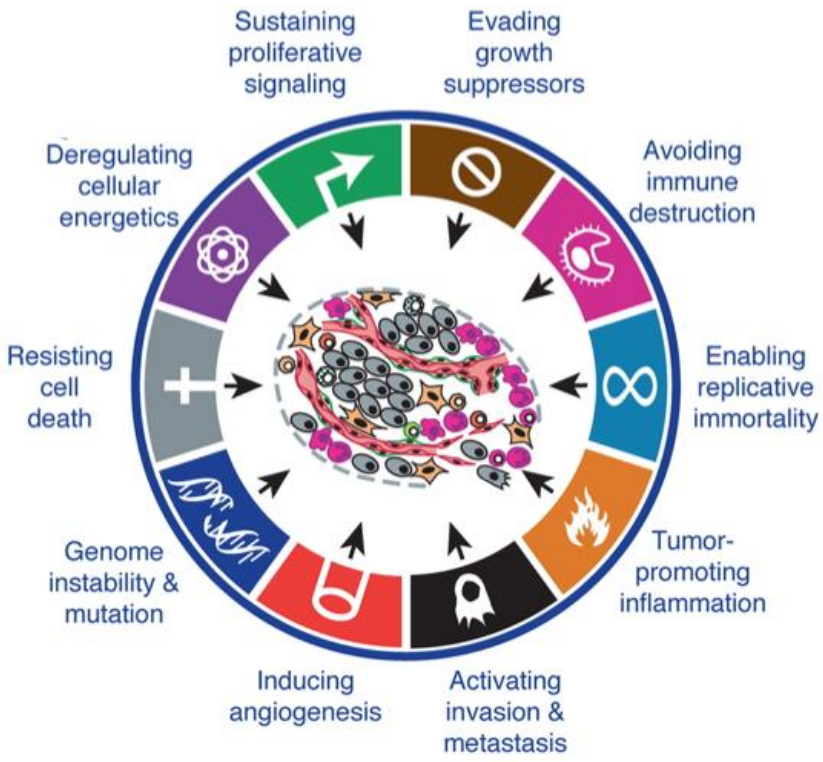

Figure L-4: Hallmarks of cancer. Source: adapted from Hanahan and Weinberg, 2011

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

1 – Sustained proliferative signaling

Sustained proliferative signaling is one of the most fundamental characteristic of cancer cells. It can be the result of several processes including (1) increased expression of growth factors and growth factor receptors; (2) hyperresponsiveness to growth factor signaling; or (3) growth factor independence, caused by constitutive activation of downstream pathways or defects in negative-feedback mechanisms (Walsh et al. 1991; Feitelson et al. 2015). In PCa, AR signaling may be altered, which contributes to cancer cell proliferation by promoting cell cycle progression and by activating other proliferative pathways such as the mitogen activated protein kinase (MAPK) cascades (Fujita and Nonomura 2019).

2 – Evading growth suppressors

Amongst the various tumor suppressors, p53, phosphatase and tensin homolog (PTEN) and retinoblastoma (RB) and have emerged as major players in growth inhibition and appear to be frequently mutated or inactivated in cancer cells. For instance, PTEN is inactivated, mainly through genomic deletion, in approximately 20% of primary PCa samples (Jamaspishvili et al. 2018). The tumor suppressor activity of PTEN primarily functions through its ability to counteract phosphoinositide-3-kinase (PI3K) activity, a family of enzymes which activates the protein kinase B (AKT) signaling cascade. AKT targets a number of key players, including mammalian target of rapamycin (mTOR) and extracellular signal-regulated kinase 1/2 (ERK1/2), involved in crucial cellular processes such as apoptosis, cell cycle regulation, cell proliferation, metabolism and motility (Song, Salmena, and Pandolfi 2012; Yamada and Araki 2001; Jamaspishvili et al. 2018). Hence, loss of PTEN represents one of the mechanisms by which PCa cells are able to evade growth suppressors and form a tumor.

3 – Resisting cell death

Malignant prostate cells can avoid apoptosis by decreasing pro-apoptotic factors (such as B-cell lymphoma-associated X; BAX) and/or increasing anti-apoptotic factors (such as B-cell lymphoma 2; BCL-2), which results in higher PCa aggressiveness and increased treatment resistance (Mackey et al. 1998; Catz and Johnson 2003). Other forms of cell death such as autophagy or necrosis also represent potential barriers to cancer but may simultaneously

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

promote tumor growth. In PCa, while autophagy can induce cell death, it also enables PCa cells to survive under stress (Farrow, Yang, and Evans 2014). Furthermore, necrotic cells release their cellular cytoplasmic contents into the extracellular space, which can activate pro-inflammatory signals and promote tumor growth (Lee et al. 2018). Indeed, the presence of necrosis in the prostate tumor is associated with high-grade PCa (Epstein et al. 2016). Apoptosis is therefore the preferred form of cell death to counteract neoplastic growth, but cancer cells develop numerous mechanisms to escape it (Fernald and Kurokawa 2013).

4 – Enabling replicative immortality

In PCa, aberrations in telomere biology are involved in the genesis and progression of the disease. PCa cells notably activate telomerase in order to maintain unlimited replicative capacity (Graham and Meeker 2017). Importantly, this replicative capacity is accompanied by extreme telomere shortening, with the average telomere length reaching 5.4kb in PCa compared with 6.6kb on average for healthy prostatic tissue (Sommerfeld et al. 1996). This telomere erosion promotes genomic alterations such as gene fusion (e.g. transmembrane protease serine 2 (TMPRSS2) and ETS related gene (ERG) gene fusion) and mutations, thereby favoring cancer growth.

5 – Inducing angiogenesis

For cancer cells, obtaining a constant supply of energy may be challenging since the diffusion of nutrients and oxygen can become difficult as the tumor grows. Angiogenesis help sustain tumor growth not only by enabling cancer cells to receive the necessary energy, but also by allowing for the disposal of metabolic and toxic waste (Hanahan and Folkman 1996). There is a correlation in PCa between tumor microvessel density and cancer progression (Strohmeyer et al. 2000). However, the blood vessels resulting from angiogenic processes induced by tumor cells exhibit aberrant characteristics including endothelial cell sprouting, vessel immaturity, alterations in the blood flow, micro-hemorrhages as well as increased permeability and leakage (Lugano, Ramachandran, and Dimberg 2019). These features allow for enough nutrient and oxygen supply to cancer cells, but also enables other aspects of cancer pathology such as formation of metastases (Carmeliet and Jain 2000). For instance, the vascular

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

endothelial growth factor (VEGF) pathway, a major pro-angiogenic signaling cascade, is highly upregulated in PCa and has been shown to promote PCa cell migration to the bone (Kitagawa et al. 2005).

6 – Activating evasion and metastasis

In PCa, cancer cells can exhibit decreased cell adhesion and increased invasiveness due to altered expression of several molecules, such as E-cadherin (Mol et al. 2007) or matrix metalloproteinases (MMPs) (Gong, Chippada-Venkata, and Oh 2014). This enables for the epithelial to mesenchymal transition (EMT) and the degradation of the ECM, which allows PCa cells to breach outside of the prostate (Jin, Dayyani, and Gallick 2011). PCa cells can either directly invade the surrounding tissues (e.g. the bladder and rectum) or disseminate via the blood circulation to reach distant organs such as bones, lung and liver.

7 – Genome instability and mutation

In cancer cells, there is an accumulation of genomic alterations (Lengauer, Kinzler, and Vogelstein 1998) which can confer selective advantage to the cells by allowing them to develop the different hallmarks presented in this section. Increased levels of oxidative stress have been implicated in the development of aggressive PCa (Kumar et al. 2008). High levels of reactive oxygen species (ROS) can lead to DNA damage which, in healthy tissues, are efficiently removed (Lindahl 1990). In malignant prostate cells however, failure of antioxidant mechanisms and alterations in the deoxyribonucleic acid (DNA) repair system results in the accumulation of genomic defects over time, thereby supporting tumor growth (Khandrika et al. 2009). For instance, DNA breaks caused by oxidative stress can promote TMPRSS2-ERG gene fusion (Mani et al. 2016), which further enhances tumor cell invasion (Tomlins et al. 2008; Tian et al. 2014).

8 – Tumor-promoting inflammation

A potential link between inflammation and cancer has been suggested as early as early 1900s based on the observation of inflammatory cells in tumor biopsies (Mantovani et al. 2008). Epidemiological data reinforced this hypothesis by revealing that chronic inflammation could

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

predispose an individual to several cancer types. Additionally, the use of non-steroidal anti-inflammatory drugs (NSAID) such as aspirin has been associated with a decreased risk of developing cancer. For PCa more specifically, a meta-analysis including several epidemiological studies concluded that NSAID may decrease PCa risk but that this effect was however relatively small (Jafari, Etminan, and Afshar 2009). These results are supported by a large case-control study which found that aspirin users had a decreased risk of PCa compared to the control group (Salinas et al. 2010), possibly through decreased inflammation levels.

At the molecular level, there is increasing evidence showing that the tumor-associated inflammatory response enhances tumorigenesis and cancer progression by modulating the microenvironment in favor of tumor growth and helping cancer cells gain the various hallmark characteristics. Indeed, inflammation leads to the supply of growth factors, such as interleukin 6 (IL-6), survival factors, including hypoxia-inducible factor 1α (HIF-1α), pro-angiogenic factors and extracellular matrix-modifying enzymes, such as VEGF and MMPs. Thus inflammation supports the sustained proliferative signaling of cancer cells, limiting their cell death and promoting angiogenesis, invasion, as well as metastasis (Colotta et al. 2009). Additionally, the tumor-associated inflammation generates ROS which causes DNA damage and eventually gene mutations, favoring the development of malignancy (Kay et al. 2019).

9 – Reprogramming energy metabolism

Cancer cells require a significant amount of energy in order to fuel their cell growth. As a consequence, they remodel their metabolism, usually through the use of glycolysis for their energy production even in the presence of oxygen (Warburg 1956). While this feature, known as the Warburg effect, is observed in most malignancies, it does not apply to PCa cells. This is mainly due to the fact that the healthy prostate gland displays a different metabolism to most tissues in the body. As early as 1944, it was reported that the prostate gland had a low respiratory rate, a high anaerobic glycolysis, and a high aerobic glycolysis (Guzman and Huggins Charles 1944; Huggins 1947). This contrasts with most cells of the body which are dependent on aerobic oxidation. One function of the prostate gland is to accumulate and secrete high levels of citrate (and zinc), which occurs at the expense of citrate oxidation and creates a high bioenergetic cost (Costello and Franklin 2000). Hence, the prostate cells secreting citrate use an alternate metabolic pathway in order to meet their energy

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

requirement and function normally, such as aerobic glycolysis (Härkönen 1981), and are less dependent on aerobic oxidation compared to most cells of the body (Costello and Franklin 2000). There is however a major metabolic shift in PCa cells, which uses the Krebs cycle and thus increases levels of oxygen consumption in order to sustain uncontrolled proliferation (Costello and Franklin 2000; Mycielska et al. 2009). Hence, PCa cells use markedly different energy metabolism pathways than healthy prostate cells, but their metabolic reprogramming differs drastically compared to the vast majority of cancer cells.

10 – Evading immune destruction

Most cancers, including PCa, develop strategies to escape from the immune system, resulting in uncontrolled cell proliferation and tumor growth. Common mechanisms include decreased recognition by the immune system, higher resistance to immune cells, and the establishment of an immunosuppressive microenvironment (Mittal et al. 2014). In PCa more specifically, immunosuppressive mechanisms are associated with more advanced tumor stages. For instance, PCa patients with metastatic disease exhibit increased levels of transforming growth factor β (TGF-β) (Adler et al. 1999), a cytokine which negatively regulates the activity of both T cell and natural killer (NK) cells (Sanjabi, Oh, and Li 2017).

Importantly, the growing interest in immune-oncology has led to some substantial advances in cancer treatment, supporting the critical role of the immune system in carcinogenesis and cancer progression.

D – Epigenetics in prostate cancer

Although cancer is typically viewed as a genetic disease, epigenetic alterations have also been shown to play crucial roles in cancer initiation and growth (Sharma, Kelly, and Jones 2010). Indeed, they can participate to the establishment of hallmarks of cancer (Flavahan, Gaskell, and Bernstein 2017). Epigenetics involves modification in gene expression but without changes in the DNA sequence. Structural changes in the DNA or chromatin may however be responsible for the epigenetic alterations, thereby enhancing or repressing the expression of associated genes. The most common mechanisms mediating these effects are (1) DNA methylation, (2) histone modifications and (3) micro-RNA (miRNA) expression (Figure L-5).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

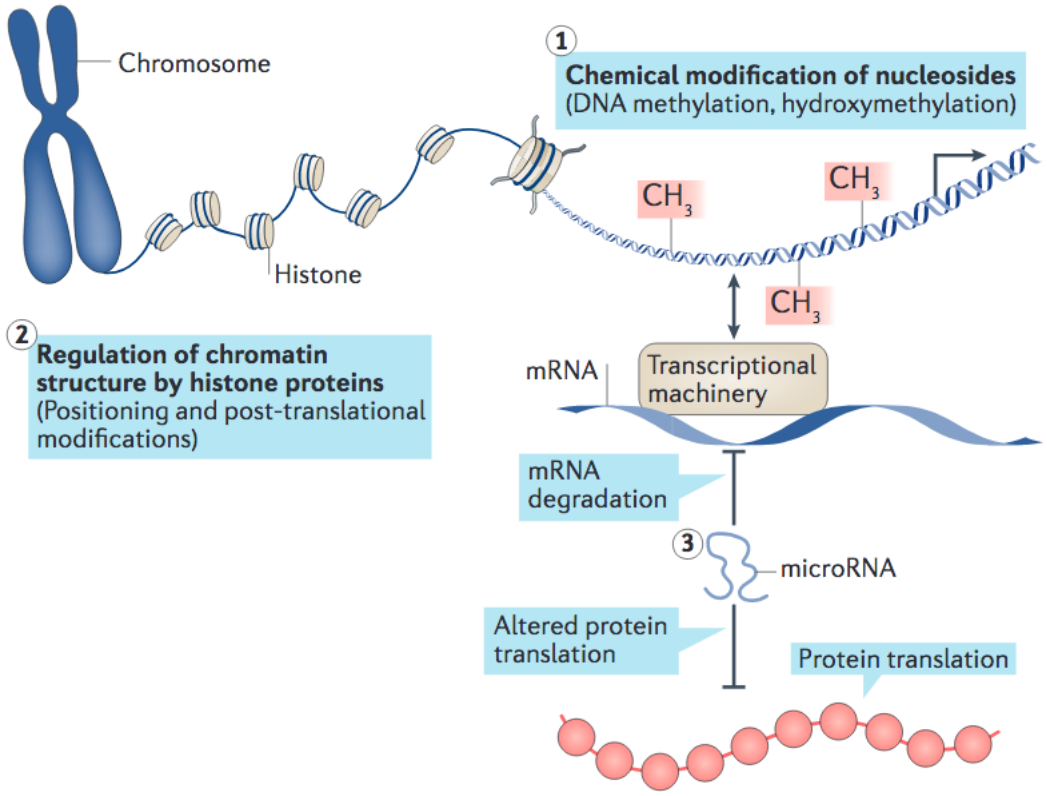

Figure L-5: Graphical representation of the major epigenetic processes: (1) Methylation (or hydroxymethylation) of DNA, (2) histone modifications, (3) Changes in small non-coding RNA expression (e.g., miRNA). DNA methylation and histone modifications regulate gene transcription through conformational modifications of the chromatin and therefore the access of DNA binding factors. miRNA usually repress gene expression by binding to their target messenger RNA or by inhibiting protein translation. Source: Barrès and Zierath 2016

1 – DNA methylation

DNA methylation corresponds to the covalent addition of a methyl group on the 5-carbon on cytosine residues (5mC) and is mediated by a class of enzymes called DNA methyltransferases (DNMTs). DNA hypomethylation and DNA hypermethylation induce gene over-expression or gene repression respectively (Figure L-6). Compared to normal cells, tumor cells exhibit genome-wide DNA hypomethylation as well as hypermethylation of specific CpG islands. Both of these processes are involved in PCa initiation and progression. Notably, hypermethylation of glutathione S-transferase P1 (GSTP1) promoter has gathered considerable attention. GSTP1 gene promoter has been found to be hypermethylated in more than 90% of prostate carcinomas and in approximately 70% of prostate intraepithelial neoplasia (PIN) lesions (Meiers, Shanks, and Bostwick 2007), concomitantly associated with loss of GSTP1 gene expression. GSTP1 is part of the glutathione S-transferases (GSTs) family, a group of enzymes

responsible for the detoxification of exogenous substances, such as carcinogens, through the conjugation of toxic compounds to reduced glutathione (Strange, Jones, and Fryer 2000). Therefore, hypermethylation of the GSTP1 promoter could predispose the prostatic epithelial cells to increased DNA damage, eventually promoting genetic instability and PCa initiation (Schnekenburger, Karius, and Diederich 2014).

PCa is also characterized by global DNA hypomethylation. Global DNA hypomethylation has been associated with increased genomic instability (Eden et al. 2003; Karpf and Matsui 2005) which, as we have seen, is a major cancer hallmark enabling tumor growth. Furthermore this feature is typically observed in advanced metastatic PCas (Bedford and van Helden 1987; Yegnasubramanian et al. 2008).

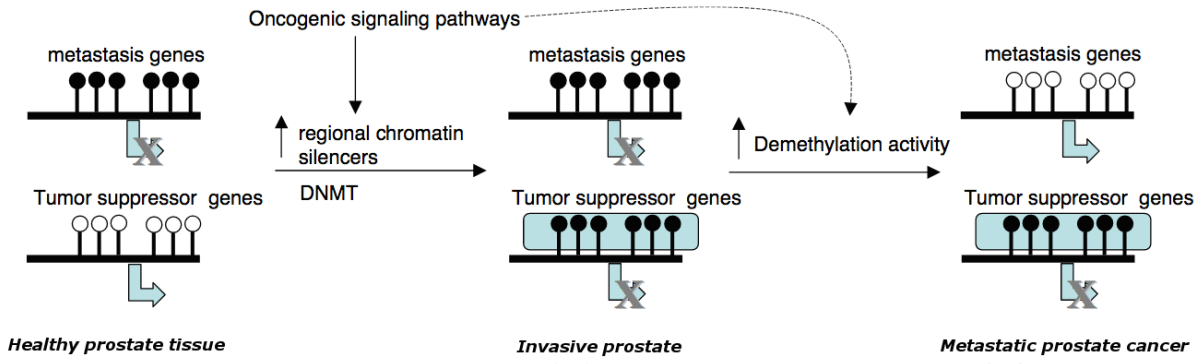

Figure L-6: DNA methylation processes in PCa. In healthy prostate tissue, promoter region of tumor suppressor genes are largely unmethylated (white circles indicate unmethylated CpGs) and active, while promoter region of oncogenes (e.g., metastasis genes) are mostly methylated and inactive (black circles). Under the action of DNMTs, tumor suppressor genes become methylated and repressed, favoring PCa initiation and progression. In more advanced (e.g., metastatic) diseases, demethylation of oncogenes occurs, enhacing their expression. Source: adapted from Szyf, Pakneshan, and Rabbani 2004.

2 – Histone modifications

The nucleosome, which represents the basic unit of chromatin, is comprised of a segment of DNA wrapped around the histones H2A, H2B, H3 and H4. Histone post-translational modifications influence the accessibility of transcription factors to DNA through chromatin remodeling, thereby regulating gene transcription (Audia and Campbell 2016). Histone modifications are multiple and include histone acetylation, histone methylation and histone phosphorylation. The most documented is histone lysine acetylation, which, when dysregulated, can promote cancer growth (Audia and Campbell 2016). Histone lysine

acetylation is dependent on the balance between two enzymes with opposite effects: histone acetyltransferase (HAT) and histone deacetylase (HDAC). HATs transfer acetyl groups of lysine residues on histones, and HDACs removes them (Figure L-7). Therefore, by loosening or tightening the chromatin structure, HATs and HDACs increase and repress gene expression respectively (Li and Seto 2016).

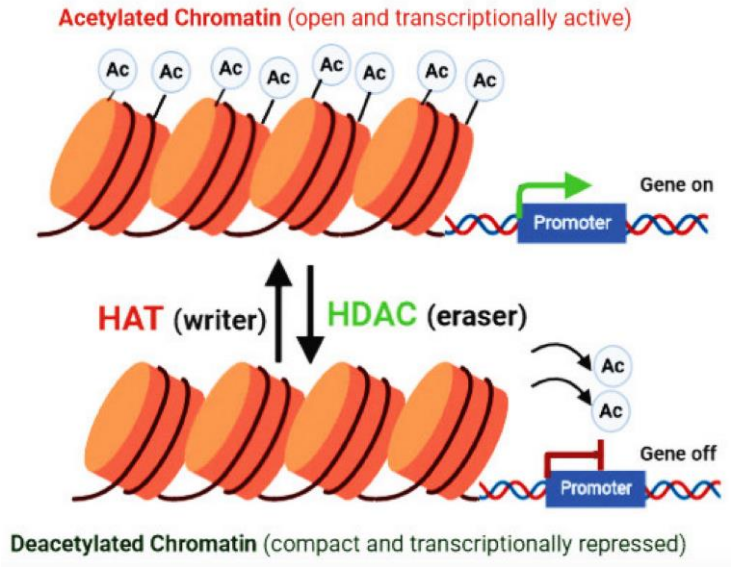

Figure L-7: Histone acetylation/deacetylation processes. HATs and HDACs regulate histone acetylation and deacetylation, respectively. Acetylated chromatin is opened and transcriptionally active while deacetylated chromatin is compact and transcriptionally repressed. Source: Pant et al. 2020

In PCa more specifically, high HDAC activity, and notably HDAC1, HDAC2 and HDAC 3, has been associated with increased serum PSA levels and poorer outcomes (Weichert et al. 2008). The use of HDAC inhibitors as a therapy for PCa is being tested in clinical trials, as it may regulate the expression of oncogenes and tumor suppressors, such as p53 (Kaushik et al. 2015).

3 – Micro-RNA expression

miRNAs are small non-coding ribonucleic acids (RNAs) that regulate gene expression through post-transcriptional modifications of its messenger RNA. The role of miRNAs in the development of human tumors is now well recognized and an increasing number of studies are investigating their potential as diagnostic, prognostic and therapeutic tools (Hayes, Peruzzi, and Lawler 2014). In PCa, the miRNA expression profile differs greatly from healthy prostate tissue and is associated with tumor aggressiveness (Porkka et al. 2007; Ambs et al.

2008). Some of these miRNAs regulate crucial gene expression. For instance, miR-21 has been shown to repress PTEN expression (Yang, Guo, and Shao 2017) which, as previously mentioned, plays a pivotal tumor suppressive role. In addition, miRNAs are being extensively studied for their potential as biomarkers and diagnostic tools for PCa (Brase et al. 2011; Velonas et al. 2013; Jackson, Grabowska, and Ratan 2014; Vanacore et al. 2017; Ghafouri-Fard, Shoorei, and Taheri 2020).

The efforts in understanding the characteristics of cancer cells and what mechanisms underlie these behaviors has resulted in the development of new treatments.

III – Prostate cancer treatment

Nowadays, several treatment options are available for men with PCa including radical prostatectomy, androgen-deprivation therapy (ADT), radiotherapy (RT), chemotherapy, and newly emerging immunotherapies (Figure L-8). These treatments can either be performed alone or in combination, depending on the clinical context. The choice of therapy depends notably on PCa aggressiveness but also on the expected longevity and the risk of death from other causes.

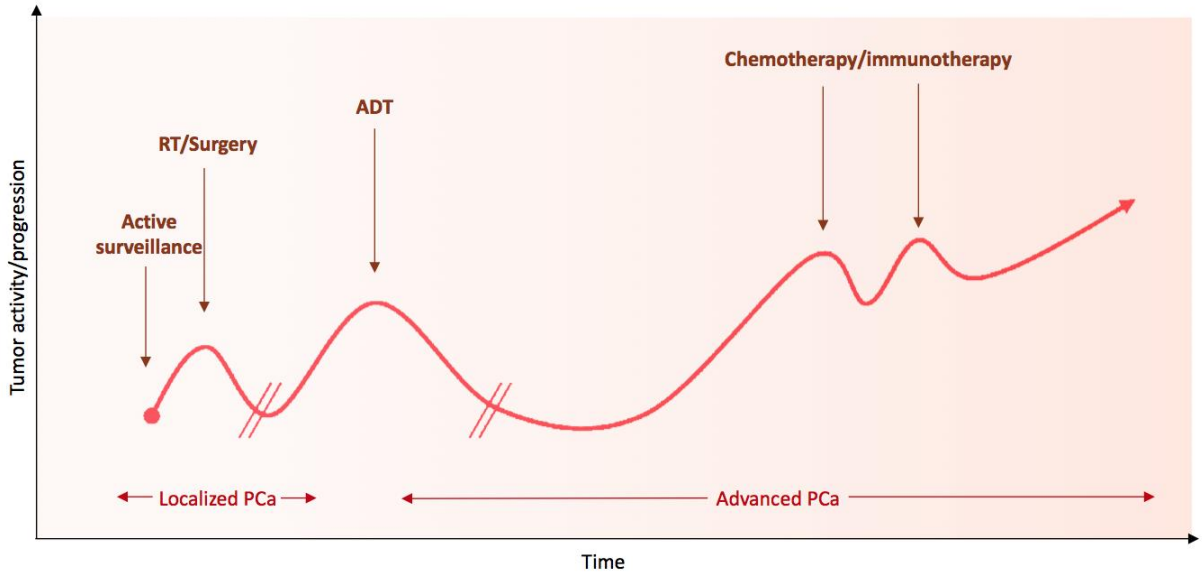

Figure L-8: Schematic representation of common treatments used throughout the PCa continuum. Patients with early stage localized PCa can undergo active surveillance. As the disease progresses, localized PCa can be treated with RT and/or surgery. In more advanced diseases, therapeutic options include ADT, chemotherapy and immunotherapy. Source: adapted from Higano in Figg, Chau, and Small 2010

A – Low risk localized prostate cancer

1 – Active surveillance

The popularization of PSA testing led to an increase in PCa diagnosis, and notably PCa at a relatively early stage. This widespread screening is however highly criticized, as the risk of dying from other causes is greater than the risk of death from PCa (Riihimäki et al. 2011; Wilt et al. 2012). Therefore, men diagnosed with PCa which would not cause morbidity or mortality can only be harmed by conventional PCa treatments known for being accompanied by numerous side effects. Therefore, it is now recommended for men with a slowly growing disease to undergo active surveillance which consists of closely watching PCa progression without giving definitive therapy. It consists of monitoring PCa progression with PSA testing, digital rectal examinations and biopsies in order to give treatment if necessary (Filson, Marks, and Litwin 2015). This approach has to date shown a relatively low risk of metastasis and PCa mortality (Tosoian et al. 2015; Klotz et al. 2015) and is therefore being used to a growing extent (Weiner et al. 2015). Importantly, a randomized clinical trial showed that men undergoing active surveillance had similar PCa mortality rates at 10 years compared to patients who went through active treatments but were however more prone to develop metastases (Hamdy et al. 2016). In this context, while expectant management appears promising, current research is aiming to refine inclusion criteria based on genetic markers.

2 – Radical prostatectomy

In patients diagnosed with PCa requiring local treatment, all clinical guidelines recommend either radical prostatectomy or RT (Mohler et al. 2010; Sanda et al. 2018). Over time, radical prostatectomy has become the preferred approach over RT (Cooperberg and Carroll 2015), even though the current literature suggests no difference on tumor progression or PCa specific mortality between the two treatment options (Hamdy et al. 2016).

Radical prostatectomy is a surgical procedure which consists of removing the entire prostate, seminal vesicles and nearby lymph nodes. This technique has been used for the treatment of PCa for over 100 years (Young 2002) and has evolved along with technological advances. Nowadays, prostatectomy is performed either by an open approach or a minimally invasive

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

technique (robotic or laparoscopic). Efforts are made to spare the nerves when possible, in order to preserve the erectile function. Approximately 30% of patients treated with radical prostatectomy will however experience biochemical recurrence (Stephenson et al. 2006).

3 – Radiotherapy

The goal of RT is to deliver a lethal dose of radiation to the tumor while sparing the surrounding tissues. RT exerts its anti-cancer effects by inducing DNA damage either directly by ionization or indirectly through radiolysis of water and generation of ROS. This results in a series of signaling events leading to either damage repair or cell death. RT also has the ability to modulate the microenvironment and enhance the immune response (Demaria, Golden, and Formenti 2015; Dar, Henson, and Shiao 2018). The danger signals (such as the release of pro-inflammatory cytokines, chemokines or tumor antigens) given by the stressed or dead cells can indeed initiate the adaptive and innate immune responses (de Andrade Carvalho and Villar 2018).

There are two major types of RT for PCa: brachytherapy (BT) and external beam radiation therapy (EBRT). EBRT can be used for any types of PCa, while BT performed alone is typically reserved for low-intermediate stage PCas. BT can however also be directed for more advanced PCas as a boost to EBRT (Podder, Fredman, and Ellis 2018). Both EBRT and BT are effective for the treatment of PCa, and advancements in technology allows for improving cancer control while sparing the surrounding tissues.

With EBRT, ionizing radiation is generated by an external source and can be delivered by various machines such as linear accelerators, cyberknife robotic systems, or tomotherapy. BT on the other hand uses radiation coming from an internal source: radioactive seeds or capsules are implanted in the prostate which allows for a high conformal dose distribution to the prostate while sparing the surrounding healthy tissues.

B – High-risk and metastatic prostate cancer

Despite the initial success of radical prostatectomy and RT, approximately one third of PCa patients will experience biochemical recurrence, characterized by a rise in PSA levels (Roehl et al. 2004). The standard choice of treatment for a patient diagnosed with an aggressive

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

and/or metastatic PCa is ADT, which can be combined with RT (Warde et al. 2011; Heidenreich et al. 2014). However, if the disease progresses to CRPC, chemotherapy or immunotherapy may also be recommended (Heidenreich et al. 2014).

1 – Androgen deprivation therapy

As previously mentioned, androgens are essential for the development and function of the prostate gland. The use of ADT for PCa was introduced by Huggins and Hodges in 1941 who observed that treating PCa patients by castration or estrogen therapy resulted in a decrease in serum acid-phosphatase levels (Huggins and Hodges 1941). Since then, the role of androgens on PCa growth has been extensively investigated, which led to the development of novel drugs aiming to achieve maximal androgen blockade while minimizing the associated side effects. These novel therapies act either by inhibiting testosterone synthesis or blocking the AR (Sharifi, Gulley, and Dahut 2005). Nowadays, ADT represents the first-line treatment for metastatic disease or locally advanced PCa (Heidenreich et al. 2014). It can also be used as a neoadjuvant or adjuvant therapy along with radical prostatectomy or RT (A. Lee et al. 2017; McClintock et al. 2019). However, while most patients initially respond to ADT, nearly all men eventually develop fatal CRPC (Feldman and Feldman 2001).

2 – Chemotherapy

While there is currently no cure for CRPC, efforts are being made to improve patients' QOL and delay PCa progression. In particular, the use of chemotherapeutic agents such as mitoxantrone, docetaxel and cabazitaxel have shown encouraging results (Body et al. 2018). Mitoxantrone was the first approved cytotoxic drug for CRPC but only reduced symptoms (Tannock et al. 1996). The use of docetaxel in the 2000s (van Soest and de Wit 2015), however, showed promising survival benefits. Docetaxel is a taxane used in various cancers and has been shown to increase the survival of CRPC patients by a couple of months. While the mechanisms behind these effects are not fully understood, docetaxel appears to interact with androgen signaling in PCa cells (Fitzpatrick and de Wit 2014). More recently, the newer taxane cabazitaxel has also been proven to increase the survival of patients with CRPC (de Bono et al.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

2010) and appears to be better tolerated than docetaxel. However, cancer cells will eventually develop resistance to chemotherapy and PCa will continue to progress.

3 – Immunotherapy

Targeting the immune system for the development of anti-cancer treatment is getting increasing attention as it shows promising results in several cancer types. Tumor cells have the ability to escape both the innate and adaptive immune system, and the aim of immunotherapy is to reacquire an effective immune response.

One of the key molecular pathways involved in the escape of tumor cells to the immune system is the programmed death-1 (PD-1) pathway (LaFleur et al. 2018). Cancer cells have the ability to bind their PD-L1 ligand to the PD-1 of T cells, which results in the abortion of the immune attack. This led to the development of PD-1 or PD-L1 blocking antibodies which aim to restore T cell function. This drug has been approved by the United States Food and Drug Administration (FDA) in approximately nine different tumor types (LaFleur et al. 2018) as it has shown objective antitumor responses.

In PCa, it was hypothesized that advanced cases could benefit from this immunotherapy, as approximately 30% of metastatic CRPC express PD-L1 (Haffner et al. 2018). However, clinical trials have shown a relatively low response rate of 3-5%, which is in contrast with results obtained in other cancer types (Venturini and Drake 2019). Similarly, the use of anti-cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), has shown promising results in cancers such as melanoma (Rotte 2019) but does appear to provide the same benefits in PCa (Venturini and Drake 2019). CTLA-4 is a critical immune checkpoint molecule which can inhibit T cell activation (Wolchok et al. 2013) but its inhibition in PCa patients has not shown any interesting clinical impact (Venturini and Drake 2019). Therefore, the use of anti-PD-1/PD-L1 or anti-CTLA-4 by themselves may not provide much benefits in PCa. However, their combination with other therapies may be promising (Venturini and Drake 2019). Indeed, the use of PD-1/PD-L1 blocking agents in combination with ADT appears to provide a much better response rate than when used in monotherapy (Graff et al. 2016; Graff et al. 2018). Similarly, preliminary results from a phase II clinical trial treating metastatic CRPC using the combination of PD-1/PD-L1 therapy with the Poly ADP-Ribose Polymerase (PARP) inhibitor olaparib found that 47% of the 25 men enrolled had an important decrease in PSA levels (Karzai et al. 2018).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

Overall, PCa appears to be relatively unresponsive to current immunotherapies compared to other cancer types, and the mechanisms mediating these resistances are largely unknown. However, combination therapies may provide additional benefits to PCa patients.

In view of the burden of PCa, efforts are being made to improve the efficiency of anti-cancer therapies. For instance, a combination of conventional therapies (e.g., immunotherapy and RT) is nowadays widely used, but interest for non-pharmacological strategies are also being investigated.

CHAPTER II: PHYSICAL ACTIVITY AND PROSTATE CANCER

Historically, cancer patients were advised to rest and avoid activity. However, there is now accumulating evidence supporting the numerous benefits of engaging in regular physical activity and exercise training after a cancer diagnosis, which will be discussed in the following sections. Defining and differentiating physical activity and exercise training therefore appears essential. Physical activity in commonly defined as “any bodily movement produced by skeletal muscles that result in energy expenditure” (Caspersen, Powell, and Christenson 1985). It was later clarified that for the activity to be considered a physical activity, the energy expenditure resulting from the bodily movements had to be superior to 1.5 MET (SBRN 2012; Tremblay et al. 2017). It includes occupational and household activities, active transportation, and recreational or leisure-time activities. Importantly, they can vary in intensity, from light (e.g., housework) to vigorous (e.g., running). Exercise is a subcategory of physical activity that is structured and planned, aiming for the improvement or maintenance of physical fitness (Caspersen, Powell, and Christenson 1985).

In this chapter, we will outline the current knowledge on how physical activity can impact PCa patients’ psychosocial and physiological outcomes, but also on the potential of physical activity as a tool to improve survival rates and reduce prostate tumor growth. The majority of the clinical and preclinical data presented will focus on PCa, but the impact of physical activity on other cancer types will also be included, notably to discuss potential biological mechanisms mediating the effects of physical activity on cancer. Indeed, the scientific literature on this specific field of research in PCa is sparse, and biological mechanisms involved in other cancer types may also apply to PCa.

I – The impact of physical activity on psychosocial and physiological outcomes in prostate cancer patients

Being diagnosed with PCa can generate psychologically stressful events, potential mood disorders, and can result in altered QOL and overall well-being. Additionally, PCa and the treatments used in the management of this disease can affect body functions (e.g., hormone imbalances), which can lead to altered muscle strength and cardiorespiratory fitness deterioration. Implementing physical activity in the health care setting has been proposed as a strategy to counteract these negative cancer- and treatment-related outcomes.

A – Psychosocial outcomes

1 – Quality of life and health-related quality of life

The definition of QOL is quite vague as it represents a broad concept covering numerous aspects of life. Hence, the notion of health-related quality of life (HRQOL) is preferred in the context of cancer since it focuses on the effects of disease and treatments. More specifically, HRQOL is defined by the United States FDA as "a multidomain concept that represents the patient's general perception of the effect of illness and treatment on physical, psychological, and social aspects of life" (U.S. FDA, 2006). The impact of physical activity on HRQOL in cancer patients has been extensively investigated both in observational and interventional studies, including in PCa (Mishra et al. 2012; Gerritsen and Vincent 2016; Burke et al. 2017). Several systematic reviews and meta-analyses reported that both aerobic and resistance training led to significant yet relatively small improvements in QOL and HRQOL in PCa patients (Keogh and MacLeod 2012; Hasenoehrl et al. 2015; Bourke et al. 2016; Baguley et al. 2017; Cormie and Zopf 2020). Interestingly, this effect may be more pronounced in supervised *versus* unsupervised training, while exercise frequency and duration does not appear to influence it (Baguley et al. 2017). However, additional studies are warranted to investigate the potential dose-response effect of exercise on QOL as it was suggested in other cancer types that moderate to vigorous exercises may provide more benefits than less intense activities (Mishra et al. 2012).

2 – Depression

Depression is a commonly observed side effect of cancer and is experienced by approximately 15-20% of PCa patients (Watts et al. 2014). Importantly, depression is associated with increased number of emergency room visits, hospitalizations, and higher mortality rates in PCa patients (Jayadevappa et al. 2012). Since physical activity have been shown to limit clinical depression in non-cancer patients, it was hypothesized that it could also benefit cancer survivors presenting depressive symptoms. Indeed, lack of physical activity is associated with increased depression in PCa patients (Erim et al. 2019) while several studies have found that exercise training interventions led to a trend towards reduced depressive symptoms in this population (Culos-Reed et al. 2010; Livingston et al. 2015; Gaskin et al. 2017), in line with findings for other cancer types (Craft et al. 2012; Fuller et al. 2018; Campbell et al. 2019). Importantly however, the exercise intervention studies in the oncology setting have been enrolling cancer patients with depressive symptoms bellow the clinical threshold, and thus the observed beneficial effects on depression associated with physical activity might only represent subclinical improvements (Christensen, Simonsen, and Hojman 2018).

3 – Cancer-related fatigue

According to the National Comprehensive Cancer Network (NCCN), cancer-related fatigue (CRF) corresponds to "a distressing, persistent, subjective sense of physical, emotional, and/or cognitive tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with functioning" (Berger et al. 2015). This symptom is commonly observed in PCa patients, with the prevalence of any fatigue being of 74% in this population (Langston et al. 2013). Importantly, CRF can discourage patients from engaging in physical activity, which will promote muscle weakness and further increase the feeling of tiredness, thereby creating a vicious circle (Schulz et al. 2017). Exercise interventions, on the other hand, may break this circle as the combination of resistance and aerobic training have been shown to improve CRF in PCa patients, notably when supervised (Baguley et al. 2017). There may be a potential dose-response effect of exercise intensity on CRF (Campbell et al. 2019) but additional studies are necessary to determine if this relationship is observed in PCa. Of importance, a systematic review highlighted the fact that

exercise training was currently the most effective strategy to reduce CRF, even when compared to pharmaceutical or psychological interventions (Mustian et al. 2017).

Overall, exercise and physical activity can provide numerous psychosocial benefits for cancer patients, including improved HRQOL and reduced symptoms of depression and CRF, but can also provide a positive impact on other factors not mentioned previously such as sleep or anxiety (Campbell et al. 2019). Importantly, supervised programs appear to induce significantly higher benefits on these different psychosocial factors compared to unsupervised activities. This may be explained by factors exceeding exercise itself, such as social inclusion favored by training in groups and/or under supervision of instructors (Buffart et al. 2017; Christensen, Simonsen, and Hojman 2018).

B – Physiological outcomes

In the healthy population, it is well recognized that physical activity and exercise training can improve muscle strength and mass, cardiorespiratory fitness, as well as physical functioning (Chou, Hwang, and Wu 2012; Hughes, Ellefsen, and Baar 2018). It is important to distinguish two main types of activities, which are often combined: (1) aerobic or endurance activities and (2) resistance or strength activities. While aerobic exercise refers to long duration and low load activities, resistance exercise causes the muscles to contract against a relatively high load for a short duration (Hughes, Ellefsen, and Baar 2018). The physiological adaptations in response to regular physical activity or exercise training will depend on the activities performed. For cancer patients however, it was hypothesized that these muscular adaptations could be different than those observed in the healthy population since cancer, host response and cancer treatments alter normal physiology (e.g., weight gain or weight loss, cardiac and pulmonary dysfunction, etc). The current literature available suggests that exercise training can improve muscle mass, strength, and cardiorespiratory function in cancer patients, but the heterogeneity of the responses observed might suggest that it is dependent on the cancer type and cancer treatment used.

Of note, the vast majority these studies investigated the impact of exercise training, but not the effect of overall physical activity levels.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

1 – Cardiorespiratory fitness

Cardiorespiratory fitness corresponds to the ability to transport oxygen from the environment to the mitochondria of the working muscles (Ross et al. 2016). It is dependent on the integrative capacity of numerous organ components, including the pulmonary, cardiovascular, and skeletal muscle systems.

Peak oxygen consumption (VO_{2peak}) is one of the main indicator of cardiorespiratory fitness (Ross Robert et al. 2016). It is measured during cardiopulmonary exercise testing and is dependent on the integrative capacity of numerous organ components, including the pulmonary, cardiovascular, and skeletal muscle systems. Cancer patients display substantial deterioration of their cardiorespiratory fitness: on average, they have a 30% lower VO_{2peak} than age- and sex- matched sedentary individuals without cancer (Jones et al. 2007; Haykowsky et al. 2009). VO_{2peak} in PCa patients has been shown to decline over the course of their treatment (Segal et al. 2009) which may be a direct result of the cancer itself or a consequence of anticancer therapies (Jones et al. 2009). These impairments in cardiorespiratory fitness can have major consequences: this parameter is considered a reflection of total body health and has been correlated to lower death rates (Sandvik et al. 1993; Blair et al. 1995; Myers et al. 2002; Arena Ross et al. 2007; Lee et al. 2010) Furthermore, it was shown that a VO_{2peak} of at least 18mL/kg/min in elderly men is necessary for living an independent life (Paterson et al. 1999). Hence, physical activity is recommended to improve or maintain cardiorespiratory fitness in the healthy population, but also in cancer patients (Lin et al. 2015; Fuller et al. 2018). In PCa, several studies have shown that exercise training could improve cardiorespiratory fitness levels (assessed through VO_{2peak} measurements or sub-maximal tests) or mitigate its decline, as highlighted by several systematic reviews and meta-analyses (Baumann, Zopf, and Bloch 2012; Keogh and MacLeod 2012; Gardner, Livingston, and Fraser 2014; Bourke et al. 2016; Bigaran et al. 2020). Interestingly, both resistance exercise training and aerobic training may provide these benefits (Segal et al. 2009).

2 – Muscle mass and muscle strength

In the healthy population, exercise training can result in major muscular adaptations such as increased muscle size, strength, and power. For cancer patients however, the cancer itself or

its treatment may counteract some of these physiological adaptations. In PCa, it is estimated that ~60% of the patients will develop cachexia (Laviano and Meguid 1996). Cachexia is characterized by a drastic weight loss – notably caused by skeletal muscle wasting and decreased body fat – and inflammation, leading to functional impairment (Fearon et al. 2011). This complex metabolic syndrome impacts numerous tissues and organs (Fearon et al. 2011; Argilés et al. 2014), which may potentially counteract gains in muscle mass and/or strength normally occurring in response to exercise training. Furthermore, cancer treatments such as chemotherapy, RT or ADT may also interfere with resistance training adaptations. ADT drastically decreases testosterone levels, a hormone implicated in muscle mass and function in men (Herbst and Bhasin 2004; Sharifi, Gulley, and Dahut 2005), eventually resulting in weight gain, muscle loss and decreased strength (Smith et al. 2002; Galvão et al. 2008; Hamilton et al. 2011; Smith et al. 2012). RT may also offset some of the exercise training adaptations as it can damage muscles and inhibit their regeneration by altering cytokine expression and impairing satellite cells (Phelan and Gonyea 1997; Jurdana et al. 2013). RT also generates muscle fibrosis (Straub et al. 2015; Stubblefield 2011), which may lead to muscle shortening, atrophy and limited mobility (Straub et al. 2015). Finally, chemotherapy can also counteract muscle adaptation in response to exercise notably through peripheral neuropathy and mitochondrial dysfunctions which in turn can lead to muscle atrophy, insulin resistance, altered muscle regenerative capacity, and overall muscle weakness (Gorini et al. 2018).

Nevertheless, several systematic reviews and meta-analyses have suggested that exercise training interventions could improve muscle strength in PCa patients compared to usual care only (Baumann, Zopf, and Bloch 2012; Keogh and MacLeod 2012; Gardner, Livingston, and Fraser 2014; Bourke et al. 2016). Furthermore, exercise interventions have been shown to prevent the treatment-induced muscle loss in PCa patients undergoing ADT (Gardner, Livingston, and Fraser 2014; Winters-Stone et al. 2015), and preserved/increased muscle strength in PCa patients treated with RT and/or ADT (Segal et al. 2009; Gardner, Livingston, and Fraser 2014; Chen et al. 2019). However, no study to date has evaluated if chemotherapy could offset exercise training adaptations in PCa patients.

Overall, exercise training appears safe for cancer patients, including those diagnosed with PCa. The American College of Sports Medicine (ACSM) indeed recognizes physical activity as generally safe for cancer survivors but still recommends them to seek advice from the oncology team when engaging in exercise while undergoing treatment (Campbell et al. 2019).

Importantly, the physiological benefits associated with physical activity in PCa patients, and notably improvements or maintenance of cardiorespiratory fitness, may in turn increase survival rates of cancer patients.

II – The impact of physical activity on survival and tumor growth in prostate cancer

A – Physical activity and survival rates in prostate cancer patients

In clinical oncology, cancer prognosis is typically determined either by estimating relative survival or cancer-specific survival. Relative survival rate (or net survival) is commonly used in clinical oncology and corresponds to the excess deaths of cancer patients compared to the expected mortality of matched cancer-free individuals. Relative survival rate includes both cancer-specific mortality, that is the deaths attributed to cancer, and non-cancer specific mortality risk, which corresponds to people dying of other causes (Mariotto et al. 2014).
Over the past decade, the impact of physical activity and exercise on mortality rates in cancer patients has raised considerable interest. There are indeed more than 130 studies investigating the impact of physical activity or exercise on mortality rates across several cancer types, with the majority focusing on breast, colorectal or PCa (Christensen, Simonsen, and Hojman 2018; Patel et al. 2019; Friedenreich et al. 2020). Importantly, distinguishing the effects of physical activity performed prior to the cancer diagnosis versus after appears essential, as they represent two distinct independent prognostic factors (Friedenreich et al. 2020). Of note, most observational studies assess overall physical activity levels through questionnaires such as the international physical activity questionnaire (IPAQ) or the global physical activity questionnaire (GPAQ), while the majority of randomized controlled trials (RCTs) implement exercise interventions, and therefore measure the impact of exercise training rather than physical activity.

1 – Physical activity pre-diagnosis

Physical inactivity is considered to be responsible for more than 5.3 million deaths worldwide (9% of premature mortality) (Lee et al. 2012). In addition, there is accumulative and strong

evidence showing physical activity provides benefits across a wide variety of diseases and decreases mortality rates in the general population (Erikssen 2001; Manini et al. 2006; Schoenborn and Stommel 2011; Pedersen and Saltin 2015). PCa patients who were physically active before their diagnosis may obtain similar benefits. Indeed, it was shown that men with PCa engaging in the highest levels physical activity pre-diagnosis had a reduction in relative mortality rates respectively compared to the ones engaging in low levels (Patel et al. 2019; Friedenreich et al. 2020). However, being physically active pre-diagnosis had no effect on PCa-specific mortality (Patel et al. 2019; Friedenreich et al. 2020) (Figure L-9).

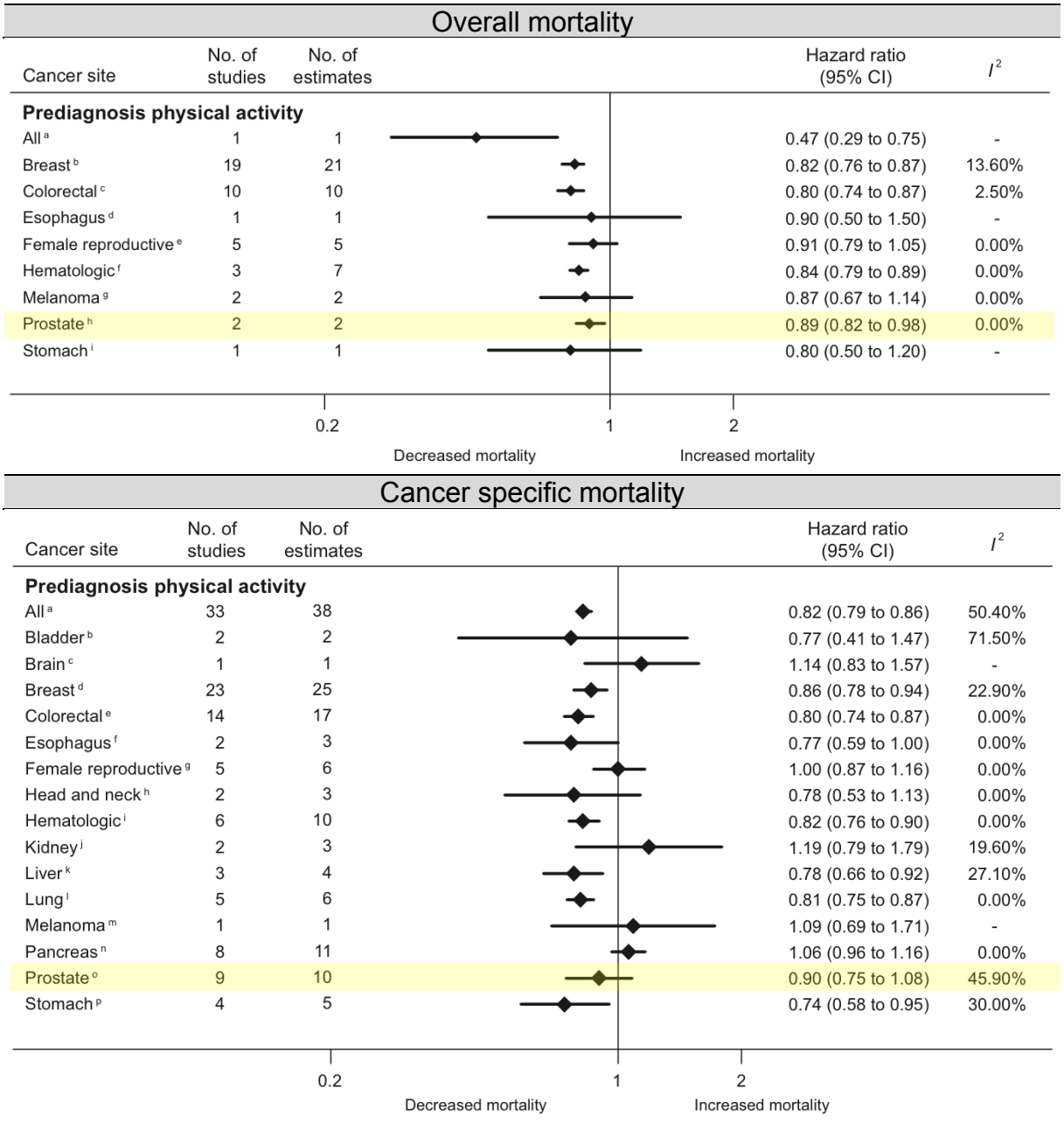

Figure L-9: Summary hazard ratio for the highest versus lowest levels of prediagnosis physical activity on overall mortality and cancer-specific mortality. Source: adapted from Friedenreich et al. 2020

Hence, amongst the three most commonly studied cancers, PCa is the only one for which physical activity levels prior to diagnosis do not appear to influence cancer-specific mortality. Nevertheless, physical activity contributes to a healthy lifestyle and decreases overall mortality rates even in patients with PCa. These results are in contrast with other cancer types such as breast and colon cancer for which both all-cause mortality and cancer-specific mortality were lower in patients undergoing high levels of physical activity pre-diagnosis (Patel et al. 2019; Friedenreich et al. 2020).

2 – Physical activity post-diagnosis

Similar to physical activity achieved pre-diagnosis, post-diagnosis physical activity is associated with improved overall survival rates in PCa patients (Patel et al. 2019; Friedenreich et al. 2020). however, physical activity following diagnosis was associated with a 30% lower PCa-specific mortality, which was not the case for pre-diagnosis physical activity (Patel et al. 2019; Friedenreich et al. 2020). Interestingly, when taking into account other cancer types, it can be observed that post-diagnosis physical activity was associated with greater decrease in both cancer-specific and all-cause mortality compared to pre-diagnosis physical activity (Friedenreich et al. 2020) (Figure L-10).

Despite the increasing evidence supporting the benefits of physical activity and exercise on cancer mortality, it remains to date largely unknown what type and dose of physical activity should be encouraged to maximize these benefits. Interestingly, a non-linear relationship between post-diagnosis physical activity levels and overall and cancer-specific mortality can be found in breast cancer patients, up to approximately 10-15 MET hours per week (Patel et al. 2019; Friedenreich et al. 2020). Additional studies are necessary to determine if a similar relationship can be found in PCa and other cancer types.

Even though the specific dose and type of activities and exercise to recommend remains largely unknown, there is some evidence supporting that regular physical activity of at least 10 METs per week should be targeted for cancer patients. This activity broadly corresponds to 150 minutes per week of moderate intensity physical activity, which is in line with the amount of physical activity recommended for healthy adults by the World Health Organization (WHO, "Global Recommendations on Physical Activity for Health" 2010). Similar physical activity

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

levels may however have a different impact on survival rates depending on the cancer type or subtype. To better understand the role of physical activity and exercise on survival in cancer patients, notably at the molecular level, preclinical models have been developed.

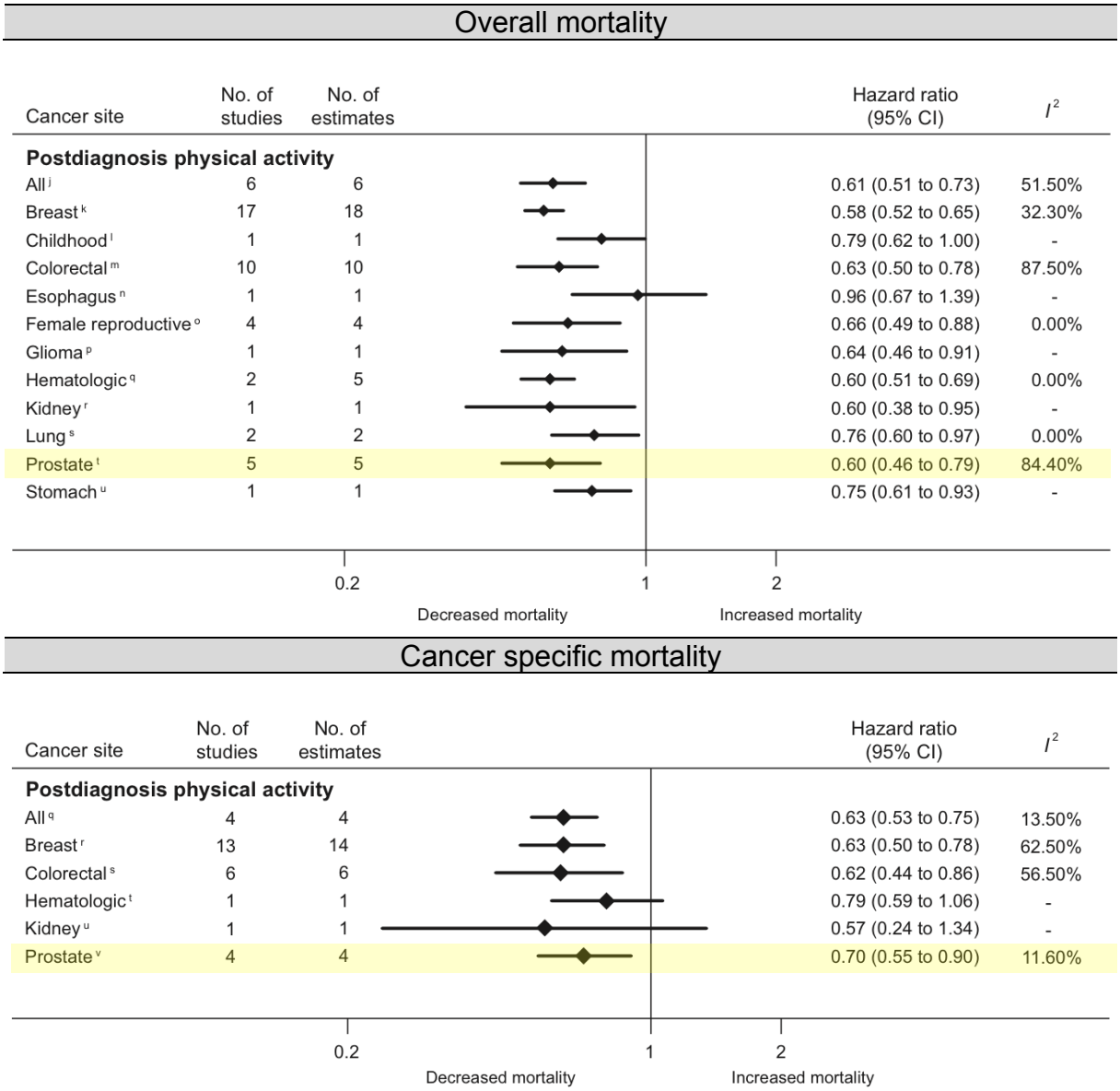

Figure L-10: Summary hazard ratio for the highest versus lowest levels of postdiagnosis physical activity on overall mortality and cancer-specific mortality. Source: adapted from Friedenreich et al. 2020

B – Physical activity and prostate tumor growth: data from preclinical models

1 – A wide variety of preclinical models

Since the early work of Rusch and Kline in the mid-1940s (Rusch and Kline 1944), a substantial number of studies have investigated the link between physical activity and tumor growth in

rodents. Their design is highly heterogeneous due to differences in tumor model but also in the modalities of exercise/physical activity.

a – Tumor models

Rodent cancer models are widely used in oncology research as they are designed to mimic the complexity of human cancers and allow for a better understanding of various biological processes involved in tumorigenesis in order to provide a rationale for therapeutic development (Van Dyke and Jacks 2002). There is a wide variety of preclinical cancer models to date, each with strengths and weaknesses.

i – Transgenic mouse models

Genetically engineered mouse models are developed through genetic modifications of the host which will eventually lead to tumor formation. For PCa, one of the commonly used transgenic models is the transgenic adenocarcinoma of the mouse prostate (TRAMP) model. Tumors in TRAMP mice result from the expression of the Simian virus 40 large T antigen (SV40-Tag) oncogene, which is driven by the androgen-dependent rat probasin (rPB) gene promoter (Greenberg et al. 1995).

The advantages of transgenic tumor models are the “natural” genesis of the tumor, which respects the interaction of the tumor with its microenvironment. However, the tumor tissue is rodent, the timing at which tumor arise can vary drastically between animals, and the tumor growth evolution is hard to follow. Furthermore, the mutations used for these models are sometimes only present in a minority of tumors (Talmadge et al. 2007). According to these strengths and weaknesses, the transgenic tumor model is very useful for studying the basic mechanisms of carcinogenesis but can have limited use in preclinical therapeutic studies due to logistical and sometimes financial barriers (Teicher 2006; Heyer et al. 2010).

ii – Transplantable syngeneic tumor models

The use of transplantable tumor models allows for a relatively quick tumor growth with relatively low variability, and has therefore been used for the evaluation of cancer treatment (Teicher 2006). In this model, cancer cell lines established from original tumors are grown *in vitro* before being injected in animal recipient. In syngeneic tumor models, the cancer cells

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

implanted initially arose from the same animal species as the recipient animal. For instance, the Dunning R3327 AT1 PCa cell line originates from the spontaneous tumor of a Copenhagen rat and its inoculation in rats constitutes a transplantable syngeneic tumor model.

Importantly, the implantation can either be ectopic (subcutaneous) or orthotopic (in the tissue of origin of the primary tumor type). Orthotopic implantations are typically preferred, as it provides an appropriate microenvironment to investigate tumor invasiveness and metastatic processes (Killion, Radinsky, and Fidler 1998; Glinskii et al. 2003; Bibby 2004). However, orthotopic implantations can involve very complex surgeries. Indeed, while orthotopic injection of breast cancer cells in the mammary fat pad is relatively easy, procedures to implant PCa tissue within the mouse prostatic gland can be far more challenging (Bibby 2004). Furthermore, while tumors implanted subcutaneously are easy to measure, orthotopic tumor volume can be difficult to estimate. This led to the development of "labeled" tumor models, where cancer cells carry a fluorescent protein (Chishima et al. 1997). This allows the assessment of tumor growth and metastasis formation with imaging technology, but these visualizing techniques require specific equipment that can be expensive. Finally, the use of syngeneic models in rodents implies that the tumor originates from a rodent, which might differ quite substantially to human tumors.

iii – Human tumor xenografts

In human tumor xenografts, the malignant cells injected in the recipient animals have a human origin, aiming to closely resemble human tumor progression. A number of these cell lines have been studied for many years and therefore a strong baseline of drug response data (Goodspeed et al. 2016). For the study of PCa, commonly used cell lines are LNCaP, PC-3 and DU145 (Wu et al. 2013). However, injecting human cells in rodents necessitate the use of immunodeficient hosts and most of the time, the tumor is implanted ectopically. Furthermore, the cell lines used might be over-subcultured, which can lead to a clonal selection that bears little resemblance to what is observed in patients (Teicher 2006; Hughes et al. 2007; Vandamme 2014). In order to establish a more clinically relevant tumor model, patient-derived xenografts (PDX) have been developed. In these models, cancer cells or tissues obtained from patient primary tumors are implanted in immunodeficient mice (Dobrolecki et al. 2016; Lai et al. 2017). PDX are increasingly used in preclinical research, notably for identifying therapeutic targets and testing of new drugs. The use of immunodeficient hosts is

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

a limit of this model, notably with the advances of immunotherapy. Humanized mouse models are currently being used to overcome this issue. This model is obtained by transplanting either human peripheral blood mononuclear cells, bone marrow, liver and thymus, or human hematopoietc stem cells (Ito, Takahashi, and Ito 2018). These efforts aim to narrow the tumor-microenvironement gap, mimicking what is observed in a clinical setting (Morton et al. 2016).

iv – Carcinogen-induced tumor models

In carcinogen-induced models, rodents are exposed to carcinogenic agents favoring the accumulation of mutations over time, transforming cells into neoplastic lesions, eventually causing tumor formation. In PCa, carcinogen-induced models are rarely used but exist. For instance, expositions of rats to N-methyl-nitrosourea (NMU) and testosterone have been shown to induce PCa (Bosland 1996).

Carcinogen-induced models have a long initiation period and thus simulates the tumor progression from the early stages. This allows for the understanding of general mechanisms of cellular alteration, and for the exploration of preventive measures. However, evaluating carcinogen-induced tumor growth can be very difficult, and the developed tumors are typically quite heterogeneous (Teicher 2006; Liu et al. 2015). For these reasons, carcinogen-induced models can be challenging if used as cancer treatment models.

Overall, recognizing the strengths and weaknesses of each model appears necessary in order to develop effective preventive or therapeutic anti-cancer strategies.

b – Physical activity and exercise models

Similar to what is observed in clinical trials, physical activity and exercise intervention models used in preclinical experiments can vary greatly between studies. Notably, there are differences observed in the timing, the type of activity, the volume and the intensity of exercise and physical activity.

i – The timing

The exercise and physical activity can be timed either before or after tumor injection/implantation. Acknowledging this parameter is important, as it differentiates the

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

studies exploring the impact of physical activity or exercise on cancer risk to the ones investigating its effect on tumor progression and survival. Engaging in regular physical activity before tumor cell inoculation could notably prepare the tissue microenvironment, and more broadly the host, against future tumor formation (Ashcraft et al. 2016). Furthermore, tumors in their early or late stage exhibit different morphological characteristics, notably in regard to vessel morphology and hypoxia, which may be dependent on tumor volume. Hence, tumors may respond differently to physical activity depending on their volume and/or stage.

Of note, exercise or physical activity was initiated either before or the same day as tumor cell injection in the vast majorities of preclinical studies, while it was started after tumor formation in a very few number of published experiments (Ashcraft et al. 2016). This may be explained, at least partly, by the fact that waiting for the tumor to form to initiate exercise or physical activity may leave a relative short amount of time for the mice to engage in this activity. Nevertheless, it is important to let the tumor form before starting physical activity when conducting experiments aiming to mimic what can be done in the health care setting.

ii – Type of activity/exercise

Several types of physical activities and exercise can be carried out by rodents in preclinical studies. The most commonly used are voluntary wheel running (VWR) and treadmill running (TR), both of which possess advantages and disadvantages. Importantly, VWR can be considered a model of physical activity, as it is not structured or programmed. TR, which is a forced activity with programmed parameters, rather is a model of exercise training (Ashcraft et al. 2016).

Voluntary wheel running

Animals engaging in VWR have a wheel installed in their cages in which they can run at their own will and pace. Voluntary running wheels are generally about 30 cm in diameter for rats and 10-15 cm in diameter for mice (Manzanares, Brito-da-Silva, and Gandra 2018; Poole et al. 2020) and the majority are attached to a revolution counter, which allows to calculate the distance run based on the wheel's circumference. Two main types of VWR model exists: upright wheels and angled wheels. The type, the size and the surface of the wheel are all parameters that will influence spontaneous wheel running activity of rodents (Banjanin and Mrosovsky 2000; Seward et al. 2018; Manzanares, Brito-da-Silva, and Gandra 2018; Poole et

al. 2020). Both mice and rats engage in the majority of their wheel running activity in the early phase of the 12 hour dark cycle (Mondon et al. 1985; De Bono et al. 2006). Mice and rats run in spurts with short intense running bouts interspersed by short breaks (De Bono et al. 2006; Goh et al. 2013; Poole et al. 2020). Importantly, their voluntary physical activity levels are dependent on sex, age and strain (Lightfoot et al. 2004; Turner, Kleeberger, and Lightfoot 2005; Coletti et al. 2013). It can vary between 1 to 10 hours per day, averaging 1-10km for mice (Lerman et al. 2002; Poole et al. 2020) and attaining 2-20 km/day for rats (Mondon et al. 1985; Rodnick et al. 1989; Poole et al. 2020). In running wheels, the average speed of mice is about 17m/min (1km/h) (Lerman et al. 2002) while it varies between 30 and 60m/min in rats (1.8-3.6km/h) (Rodnick et al. 1989). Inter-individual differences within animals of similar genetic background also exist, which led some studies to attribute the animals with high, intermediate, and low-running-activity levels, with approximately 10% of commercialized mice and rats opting not to run (Poole et al. 2020). Of note, it takes approximately 2 to 4 weeks for mice and rats to reach their maximal activity level, which they will maintain for a couple of weeks before steadily declining as they get older (Manzanares, Brito-da-Silva, and Gandra 2018; Poole et al. 2020).

Overall, VWR has the advantages of requiring minimal investigator intervention, does not require stressful stimuli (such as electric shocks), and allows the animals to engage in physical activity in accordance with their circadian rhythms and close to their natural running pattern. However, single-housing is preferred for an accurate record of the daily running distance, which can create stress for the animals (Arndt et al. 2009). Furthermore, the main disadvantage of using VWR as a physical activity intervention strategy is the lack of control over the duration and intensity of the running behavior, and subsequent heterogeneity in the total amount of physical activity achieved by the animals. Of note, motorized running wheels are now being used, allowing for the regulation of exercise duration and intensity.

Treadmill running

TR is a forced activity which allows for the control of the duration, speed and incline of each exercise performed, therefore allowing for homogeneous exercise intensity across the animals. It requires a gradual familiarization period consisting of short treadmill running bouts (5-15min) at low speed. Following the acclimation period, mice and rats often necessitate stimuli for motivation. These are more or less noxious and include short puffs of air, electric

shocks, the touch of a brush, cardboard, or a gentle tap on their back. Importantly, there is a correlation between the speed and incline of the treadmill with maximal oxygen consumption (VO_{2max}) (Fernando, Bonen, and Hoffman-Goetz 1993; Schefer and Talan 1996; Høydal et al. 2007; Chavanelle et al. 2014). For example, the intensity of TR at 18m/min with 0% incline is estimated to represent 70-75% of mice maximal oxygen uptake. Similar to VWR, TR performance differs by strain (Lerman et al. 2002).

The TR program typically includes sessions of 30-120 min, performed once a day several days per week. The parameters chosen will depend of the outcome desired, but most of the training adaptations can be observed several weeks after gradual TR. Importantly, since mice and rats are nocturnal animals, exercising during the day (their normal sleeping period) could generate additional stress to the animals. To overcome this limitation, the environmental lighting can be adjusted so that the dark phase matches with the exercising time.

Overall, the strengths of TR as an experimental exercise model is that is allows for the control of exercise timing, duration, and intensity. However, this method does not always respect the natural circadian rhythms of the animals, can lead to injuries and can be stressful, notably when an unpleasant stimulus is used for motivation. The stress generated by forced TR could interact with and confound key physiological responses produced by exercise (Moraska et al. 2000).

The major adaptations to exercise training are maximized energy delivery, increased muscle mitochondrial biogenesis and improved contractile function (Egan and Zierath 2013). Interestingly, in addition to each of their strength and weaknesses, VWR and TR, may induce distinct physiological adaptations. This is not surprising, as the total distance run and the intensity may vary between the two modalities, in addition to the stress endured by the animals. Indeed, a recent study compared TR (a classic protocol of 8 weeks) and VWR modalities in mice, and showed that animals with an access to a wheel covered weekly distances 20 to 40 times greater than the TR mice (Kim et al. 2020). The food intake of the VWR group was higher than the sedentary control mice and the TR mice, suggesting that this modality led to greater energy expenditure. However, both modalities resulted in similar changes in body composition (weight loss and reduced fat mass). Interestingly, peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α) expression was increased in the gastrocnemius of TR mice, but that was not the case for VWR mice. A possible explanation

is that TR is more intense than VWR and induces mitochondrial biogenesis through the PGC-1α pathway in glycolytic-oxidative muscle fibers, while VWR, at least in this study, is not intense enough to generate these adaptations (Kim et al. 2020). Based on this study, VWR may represent a long-duration and energy consuming activity, while TR appears to be a more brief but intense activity. However, this largely depends on the modalities used for TR (frequency, speed, duration, incline, etc) and the spontaneous activity achieved by the animals.

2 – The impact of physical activity on tumor growth, cell proliferation and cell death in preclinical prostate cancer models

Numerous preclinical studies have investigated the impact of exercise or physical activity on tumor growth and survival, and some biological mechanisms have been identified by these projects. The study designs are however highly heterogeneous between studies, making comparison between them challenging. The majority of these preclinical studies were conducted in mice or rats, and used VWR and TR as exercise/physical activity models, as reviewed by Ashcraft *et al*. (Ashcraft et al. 2016). Amongst the preclinical studies published at the time, the authors reported that physical activity was shown to reduce tumor growth in 64% of these studies, while 21% observed no effects and 9% reported an increase in tumor growth (Ashcraft et al. 2016).

a – Impact of physical activity on tumor growth in preclinical models of prostate cancer

For PCa more specifically, there are to our knowledge, 15 preclinical studies (on 13 separate sets of experiments) which have evaluated the chronic effect of physical activity on tumor growth (Table L-1). Similar to what is observed for other cancer types, preclinical studies investigating the impact of physical activity on PCa exhibit highly heterogeneous study designs. Amongst these studies, two from the same research group (and based on the same experiments) used a carcinogen-induced tumor model (Saedmocheshi et al. 2019; Vahabzadeh et al. 2020), four (including two from the same group and based on the same experiments) used a transgenic mice model (either C3(1)/SV40Tag mice, TRAMP mice or prostate-specific Pten knockout mice) (Esser et al. 2009; Patel, Abuchowski, et al. 2019; Patel, Wallace, et al. 2019; Taylor et al. 2020), four used a transplantable syngeneic tumor model (three using an orthotopic injection model, and one using a subcutaneous transplant model)

(Jones et al. 2012; McCullough et al. 2013; Gueritat et al. 2014; Baumfalk et al. 2018), and finally five used human tumor xenografts, four of which were induced subcutaneously in immunodeficient hosts (Zheng et al. 2008; 2011; 2012; Opoku-Acheampong et al. 2019; Dufresne et al. 2020) while one injected the cells orthotopically (Opoku-Acheampong et al. 2019). About half of these studies used VWR as a physical activity model (Esser et al. 2009; Zheng et al. 2008; 2011; 2012; Jones et al. 2012; Patel, Wallace, et al. 2019; Patel, Abuchowski, et al. 2019) while the other half used TR (McCullough et al. 2013; Gueritat et al. 2014; Baumfalk et al. 2018; Saedmocheshi et al. 2019; Taylor et al. 2020; Opoku-Acheampong et al. 2019; Vahabzadeh et al. 2020; Dufresne et al. 2020). The initiation of physical activity also differed between the studies. In the experiments using carcinogen-induced or transgenic models, it was not specified whether physical activity was initiated before or after the formation of tumor. However, since the mice used in these studies were younger than the expected age at which tumors are formed, it seems likely that physical activity was started before the formation of palpable tumors. Amongst the studies using transplantable syngeneic tumor models or human xenografts, the vast majority initiated physical activity several days/weeks after the tumor injection or implant, hence likely to be after tumor formation (Zheng et al. 2011; 2012; Jones et al. 2012; McCullough et al. 2013; Gueritat et al. 2014; Baumfalk et al. 2018; Opoku-Acheampong et al. 2019; Dufresne et al. 2020), with just one study starting physical activity two weeks prior to cancer cell injection (Zheng et al. 2008).

In regards to the outcomes observed, eight out of the 15 studies reported a decrease in tumor growth (Zheng et al. 2008; Esser et al. 2009; Zheng et al. 2011; 2012; Gueritat et al. 2014; Saedmocheshi et al. 2019; Vahabzadeh et al. 2020; Dufresne et al. 2020) while the remaining seven other studies showed no differences (Jones et al. 2012; McCullough et al. 2013; Baumfalk et al. 2018; Taylor et al. 2020; Patel, Wallace, et al. 2019; Patel, Abuchowski, et al. 2019; Opoku-Acheampong et al. 2019). Of note, McCullough *et al*. found a significant increase in the tumor weights of nude rats induced with a Dunning R-3327 AT-1 cancer following seven weeks of TR compared to their sedentary counterparts, while that was not the case when a similar protocol was applied in immunocompetent Copenhagen rats (McCullough et al. 2013). While the vast majority of the studies did not report or did not find metastatic lesions, Jones *et al*. observed a reduction in the number and total weight of metastases developed bearing orthotopic TRAMP-C1 prostate tumors, but that effect remained non-significant (Jones et al. 2012).

Table L-1: Preclinical studies investigating the impact of chronic exercise/physical activity on prostate tumor growth

Article	Study design				Outcomes (tumor)				
	Animals used	Cancer model	Ex/PA initiation	Ex/PA modalities	Tumor growth	Metastases	Proliferation and cell death	Plausible biological mechanisms modified with Ex/PA	Biological mechanisms reported but unchanged
Dufresne et al. 2020	Athymic nude mice Males n=8-9/gp	sc. injection of PPC-1 cells	1 wk after tumor cell injection	TR Up to 1h/d at 18m/min with 10% incline 5d/wk; 2 wks total	↓ tumor volume and weight	NR	↓ proliferation (Ki67) No changes in apoptosis (cCASP3 and BAX:BCL-2 ratio) No significant changes in necrosis (H&E)	↓ pERK1/2:ERK1/2 ratio	Tumor angiogenesis (CD31) unchanged pAKT:AKT ratio unchanged Inate immune response (NK1.1; *Klrk1*, *Il2rβ*, *Ncr1*, *Tnfα*, and *Ifnγ*) unchanged
Taylor et al. 2020	Prostate specific Pten knockout mice Males n=6-10/gp	Transgenic model (prostate specific Pten knockout)	Before tumor formation	2 modalities of TR: 1) "Endurance" Up to 70min/d at 19m/min with a 5% incline 2) "HIIT" Up to 22min (11x2min interspersed with 2in rest) at 23m/min with 5% incline 5d/wk 6 wks total	No difference on prostate (total organ) weight neither with endurance or HIIT training but ↓ lateral lobe weight with HIIT.	No metastatis identification	No difference in proliferation (Ki67) neither with endurance nor HIIT training		Metabolism unchanged (Glucose uptake and oxidation, *de novo* lipogenesis glutamine metabolism, fatty acid uptake, oxidation, and incorporation of fatty acid into cholesterol, ceramide, phospholipid, diacylglycerol, or tryaglycerol)
Vahabzadeh et al. 2020; Saedmocheshi et al. 2019	Wistar rats Males n=8/gp	Ip. Injections of cyproterone (18d, 50mg/kg) followed by injections of testosterone (3d, 100mg/kg) propionate and NMU (1d, 50mg/kg)	Before tumor formation	TR Up to 45min/d (3x15min interspersed with 2min rest) at 10m/min 5d/wk; 8 wks total	↓ in prostate (total organ) weight No effect on cancer grade nor PSA levels	NR	↓ p53 levels ↓ Nf-κB		Pro-oxidant-anti-oxidant balance unchanged No difference in Cox-2 expression
Opoku-Acheampong et al. 2019	Nude rats Males n=4/gp	Orthotopic injection of PC-3 cells	1 wk after injection	TR Up to 60min/d at 25m/min with a 6° incline 5d/wk; 10-11 wks total	No difference on tumor weight	NR			
Patel, Wallace, et al. 2019; Patel, Abuchowski, et al. 2019	TRAMP mice Males n=15/gp	Transgenic model (TRAMP mice)	Before tumor formation	VWR Access 24h/24h ; 7d/wk Average 13.56±4.1 km/d 20 wks	No difference on prostate tumor weight	NR			
Baumfalk et al. 2018	Copenhaguen rats Males n=9-10/gp	Orthotopic injection of Dunning R-2237 AT-1 cells	~1 wk after injection	TR Up to 60min/day at 15m/min with a 15° incline 5d/wk; ~5 wks total (32-35 days)	No effect on tumor weight	NR			

Gueritat et al. 2014	Copenhaguen rats Males n=10/gp	Sc. implant (surgery) of Dunning R-2237 AT-1 tumor fragment (20mg pieces)	~2 wks (15 days) after surgery	TR Up to 60min/d at 25m/min 5d/wk; 4 wks total	↓ tumor volume and ↓tumor doubling time	NR	↓ proliferation (Ki67) No effect on apoptosis (Bax:Bcl-2 ratio)	↓pERK1/2:ERK1/2 ratio Decreased DNA damage (8-oxodGuo)	Other oxidative stress markers unchanged (tumor SOD activity, MnSOD,CuZnSOD expression, carbonyl protein, nor lipid peroxidation)
McCullough et al. 2013	Copenhaguen rats and Nude rats Males n=12-15/gp	Orthotopic injection of Dunning R-2237 AT-1 cells	~1 wk (7-10 days) after surgery	TR Up to 60min/day at 15m/min with a 15° incline 5d/wk; 7 wks total	No effect on tumor weight in Copenhaguen rats ↑ tumor weight in Nude rats	NR		↓ tumor hypoxia	Number of patent vessels and vessel contractile function unchanged
Jones et al. 2012	C57BL/6 mice Males n=28-31/gp	Orthotopic injection of TRAMP-C1 cells	2 wks after tumor cell injection	VWR Access 24h/24h; 7d/wk Average ~4-6 km/d ~7-8 wks total (53 d)	No effect on tumor growth (based tumor weight of mice euthanized at different time points)	No significant effect		Alterations in pro-metastatic gene expression (↓HGFR; ↑CXCR4) ↑ hypoxia (HIF-1α) ↑in several angiogenesis markers (VEGF, ANGTP2, CD31) but ↓FIGF ↑ perfusion Borderline significant ↑ in pERK2:ERK ratio ↑ in metabolic OGDH gene	Other pro-metastatic genes unchanged (COX2, EGFR, MMP2, MMP9, IGF1R) Other MAPK pathway proteins unchanged (S6K and MEK) Other metabolism genes unchanged (ERRα, ERRɣ, PPARα, MCAD, IDH3α, GLUT1 and GLUT4) Other angiogenesis genes unchanged (ANGTP1, FGF2, EGFR, PDGFβ, PDGF-Rα, PDGF-Rβ)
Zheng et al. 2012	SCID mice Males n=5/gp	Sc. Injection of LNCaP cells	4-6 wks after injection and after castration	VWR Access 24h/24h; 7d/wk Average 2.27 km/d (1.41miles/d) 6 wks total	↓ tumor growth (percentage of initial tumor volume)	NR	↓ proliferation (mitotic cell count) ↑ apoptosis (active CASP3)	↓ IL-6 levels	
Zheng et al. 2011	SCID mice Males n=5/gp	Sc. Injection of LNCaP cells	4-6 wks after injection and after castration	VWR Access 24h/24h; 7d/wk Average 2.11±0.35km/d (1.31±0.22miles/d) 6 wks total	↓ tumor growth (percentage of initial tumor volume)	NR			
Esser et al. 2009	C3(1)Tag mice Males N=8-20mice/gp	Transgenic model (C3(1)Tag mice)	Before tumor formation	VWR Access 24h/24h; 7d/wk Average 4.03±0.39 km/day; 0.72±0.05 km/h 10 wks total	↓ aggressiveness in high (>5km/d) *vs* low (<5km/d) running mice and sed mice; no difference between sed mice and low running mice	NR			
Zheng et al. 2008	SCID mice Males n=10/gp	Sc. Injection of PC-3 cells	2 wks before injection	VWR Access 24h/24h; 7d/wk Average 2.22±0.34km/d (1.38±0.21miles/d) 9 wks total	No delay in the formation of tumors but ↓ tumor growth rates	NR	↓ proliferation (mitotic cell count) ↑ apoptosis (active cCASP3)		

Abbreviations: gp: group; sc.: subcutaneous; ip.: intraperitoneal; d.: days; wk: week; TR: treadmill running; VWR: voluntary wheel running. Symbols: ↑: increase; ↓: decrease

b – Impact of physical activity on cancer cell proliferation and cell death in preclinical models of prostate cancer

Cancer is the result of an increase in cell division combined with decreased cell death and in particular apoptosis (Green and Evan 2002). Therefore, counteracting tumor growth can be achieved by decreasing cell proliferation and/or increasing cancer cell death. Cancer cell proliferation is commonly assessed by immunohistology with a Ki67 and Dapi staining, even though only a subset of the cells expressing Ki67 will undergo mitosis (Scholzen and Gerdes 2000).

Amongst the preclinical studies investigating the impact of physical activity/exercise on prostate tumor growth, five of them evaluated tumor cell proliferation: four reported a decrease in tumor cell proliferation (Zheng et al. 2008; 2012; Gueritat et al. 2014; Dufresne et al. 2020) while one showed no effect (McCullough et al. 2013). Furthermore, some studies analyzed "core" signaling pathways, such as MAPK and PI3K cascades, which are also revealing of the tumor cells' proliferative potential (De Luca et al. 2012) and represent important targets to inhibit PCa (Kinkade et al. 2008). Interestingly, two studies showed a decrease in the activation of the MAPK cascade in response to physical activity, as observed by a reduced ratio between phosphorylated ERK1/2 and total ERK1/2 protein expression (Gueritat et al. 2014; Dufresne et al. 2020) while Jones *et al*. found a borderline significant increase in the ERK2-to-total ERK ratio as well as higher MAPK/ERK kinase (MEK) protein expression (Jones et al. 2012). These authors also reported increased PI3K and mTOR activity along with decreased AKT activity, but their results remained non-significant (Jones et al. 2012). Our group however did not find any difference in the phosphorylated AKT-to-total AKT ratio in response to exercise training in a recently published study (Dufresne et al. 2020).

In regard to cell death, apoptosis is often assessed either with a Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, where DNA fragments *in situ* are detected, or with the detection of active caspase-3 expression, a crucial mediator of apoptosis. In PCa, two animal studies found an increase in active caspase-3 expression (Zheng et al. 2008; 2012) while two others did not find any differences in the expression of apoptotic markers (Gueritat et al. 2014; Dufresne et al. 2020).

Overall, physical activity/exercise might be able to increase PCa cell death and reduce cancer cell proliferation in some settings.

The discrepancies between studies are likely a result of differences in physical activity initiation, physical activity modality and/or cancer model. No study in PCa has directly evaluated the effect of these parameters on the potential anti-cancer effects of physical activity. Their importance will be discussed using data from preclinical studies in other cancer types.

3 – Physical activity and tumor growth in preclinical models: the importance of study design

a – Physical activity initiation

A few studies have investigated whether different timing for physical activity initiation (e.g., before and after tumor inoculation) may impact tumor growth differentially.

Interestingly, three preclinical studies using transplantable breast cancer models suggest that physical activity slows down tumor growth in a similar fashion whether it is performed exclusively after, or both before and after cancer cell injection, while undergoing physical activity exclusively before tumor cell inoculation does not have an impact on tumor growth (Shalamzari et al. 2014; Betof et al. 2015; Vulczak et al. 2020). In line with this results, it was found in a preclinical leukemia model that mice engaging in exercise training both before and after tumor transplant had a slower tumor growth than sedentary control mice, while mice who stopped exercising after tumor transplant did not (Radak et al. 2002).

In contrast, other studies suggest that physical activity performed exclusively prior to cancer cell initiation is able to reduce tumor volume, while physical activity exclusively performed after cancer initiation did not impact tumor growth (Pedersen et al. 2016; Kelly et al. 2017).

Hence, while the timing of physical activity may be a key factor for its benefits on tumor growth, it is likely dependent on the cancer type.

b – Cancer model

As previously mentioned, epidemiological data suggest that physical activity may impact cancer patients differently according to their cancer type. Similar conclusions arise from preclinical studies. While several studies have shown that the use of a similar physical activity/exercise protocol in different cancer models led to similar effects of tumor growth

(Zheng et al. 2008; Betof et al. 2015; Pedersen et al. 2016; Dethlefsen et al. 2017; Pedersen et al. 2020), some have found contradictory results.

For example, a study showed that in three distinct mice models of triple negative breast cancer, the same TR program either inhibited, had no effect or accelerated tumor growth compared with sedentary controls, depending on the cancer model (Glass et al. 2017). Of note, the strain and gender of rodents may also influence the effect of physical activity on tumor growth (Mehl et al. 2005; McCullough et al. 2013; Kelly et al. 2017).

c – Physical activity modalities

There is some evidence suggesting that different physical activity models may impact tumor growth differentially.

Several preclinical studies have investigated if VWR and TR led to a similar effect on tumor growth. Amongst these studies, Colbert *et al*. found that VWR tended to slow down tumor growth (but non-significantly) in a breast cancer mouse model, while TR either had no effect or even increased tumor growth in mice following a moderate (20m/min) or a more intense (24m/min) TR program, respectively (Colbert et al. 2009). In a preclinical model of colon cancer, Mehl *et al*. found that TR but not VWR significantly reduced the total polyp number and the number of large polyps in male mice (Mehl et al. 2005). Using a similar model, Basterfield and Mathers also suggested that TR but not VWR may reduce the tumor burden (Basterfield and Mathers 2010). Indeed, this research group reported that neither VWR nor TR had a significant effect on the total number of tumors developed in the intestine. However TR mice tended to display fewer tumors in the different sections of the intestine, and led to a significant decrease in the number of tumors developed in the colon (Basterfield and Mathers 2010). Furthermore, mice undergoing TR developed fewer larger tumors than sedentary mice, while that was not the case for the VWR group (Basterfield and Mathers 2010). Another research group reported that swimming training but not resistance training (ladder climbing with a load) reduced the number of colon dysplastic lesions (Frajacomo et al. 2015), which adds to the evidence that different modalities of physical activity can result in different effects on tumor growth. However, it was also reported that motorized wheel running compared to VWR led to a similar decrease in tumor growth (Mann et al. 2010; Thompson et al. 2010), reinforcing that similarities between different physical activity models also exist.

Within each type of exercise, modulation of exercise duration, total distance covered, or intensity was also investigated. For instance, it was shown by Singh *et al*. that TR had a duration-dependent inhibitor effect on tumor growth and survival. In this experiment, mice with T-cell lymphoma were randomized to either inactive control or to an exercise training group consisting of TR for 10 days at 17m/min for 15, 30, 60, 90 or 120 min per day. The authors reported that only the mice running 60 min per day or more had a significant decrease in tumor growth, and that this effect was the most pronounced in mice running longer times (Singh, Singh, and Singh 2005). Additionally, another research group found that rats submitted to voluntary motorized wheel running set at a speed of 37m/min reduced cancer incidence, cancer multiplicity and cancer burden in a similar fashion, whether the rats ran for either 1750m/day or 3500m/day (Zhu et al. 2012; Jiang, Zhu, and Thompson 2013).

In terms of exercise intensity, two studies reported enhanced tumor growth with higher intensities in models of colon (Silva, Sivieri, and Rossi 2009) and breast cancer (Colbert et al. 2009). Others studies reported a slower tumor growth with low-to-moderate intensity but not with higher intensities in models using swimming training (Almeida et al. 2009) or TR (Siewierska et al. 2018). In contrast, other studies found slower tumor growth with intense exercise as opposed to lower intensity exercises (Paceli et al. 2012; Malicka et al. 2015; 2017). Of note, some studies found similar benefits regardless of exercise intensity (Matsuo et al. 2017) or no effect even when the intensity was modulated (Tsai et al. 2013). Hence, the impact of exercise intensity on tumor growth remains inconclusive in regard to preclinical studies. Nevertheless, the choice of physical activity modality may play a crucial role in the ability of physical activity to counteract tumor growth and is likely to be dependent on the tumor model used.

4 – Suggested biological mechanisms mediating the anti-cancer effects of physical activity

Engaging in physical activity represents a major challenge for whole-body homeostasis. In order to respond to the increased metabolic demand of contracting muscles, adaptations occur in a wide variety of cells, tissues and organs (Hawley et al. 2014). Amongst these numerous physiological processes, several ones have been suggested as potential anti-cancer mechanisms.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

a – Evidence from preclinical prostate cancer studies

In PCa, several preclinical studies have suggested potential biological pathways by which exercise or physical activity could improve cancer prognosis (Table L-1).

A study conducted by our team showed that TR slowed down tumor growth and decreased tumor DNA oxidative damage in male rats bearing AT-1 prostate tumors (Gueritat et al. 2014). In advanced PCas, several events such as metabolic alterations can result in increased ROS production which results in oxidative stress. In turn, oxidative stress creates damage to key cellular components and favors processes promoting cell survival and proliferation (Paschos et al. 2013). Hence, counteraction of oxidative damage by physical activity may delay cancer growth.

In addition to oxidative stress modulation, improved tumor vascularization has also been proposed as potential biological mechanisms underpinning the benefits of exercise in PCa. Jones *et al*. reported enhanced tumor perfusion in an orthotopic TRAMP-C1 mouse model in response to 53 days of VWR. They paradoxically also found that physical activity was associated with increased HIF-1 activation, known for being stimulated by hypoxia (Jones et al. 2012). Hypoxia promotes pro-angiogenic pathways, notably by upregulating VEGF, in order to provide sufficient oxygen and substrate delivery to the tumor cells (Krock, Skuli, and Simon 2011). However, the resulting vessels are abnormal, which causes impaired perfusion and oxygenation of the tumor, thereby creating a vicious cycle. This accelerating angiogenesis further promotes tumor invasion and metastasis (Krock, Skuli, and Simon 2011). The apparent paradox between the observed increased HIF-1 expression and the shift towards reduced metastases with physical activity led the authors to speculate that this was an adaptation to chronic activation of HIF-1 by physical activity, and that it reflected an effort to maintain cellular homeostasis (Jones et al. 2012). Interestingly, it was reported that a single bout of TR in rats bearing orthotopic prostate tumors drastically increased blood flow (~200%) and was able to halve the hypoxic tumor fraction (McCullough et al. 2014). However, this same group showed that chronic exercise was not able to impact the number of patent vessels nor modify vessel contraction, but significantly reduced hypoxia (McCullough et al. 2013). Overall, physical activity may improve tumor perfusion (Jones et al. 2012; McCullough et al. 2013), which can lead to decreased hypoxia (McCullough et al. 2013) but also quite surprisingly to

increased HIF-1 and VEGF expression (Jones et al. 2012). It is noteworthy that neither of these studies observed a decrease in tumor growth associated with physical activity.

b – Evidence from preclinical studies regardless of cancer type

Since the identification of biological pathways impacted by physical activity in PCa animal models is limited, evidence from preclinical studies conducted in various cancer types will now be presented. The mechanisms identified can be broadly classified into four main categories: (a) vascularization and blood perfusion, (b) immune function, (c) muscle-to-cancer cross-talk, and (d) tumor metabolism (Pedersen, Christensen, and Hojman 2015).

i – Vascularization and blood perfusion

As previously mentioned for PCa, improved tumor vascularization represents a well-described mechanism by which physical activity impacts tumor growth. In addition to what has been observed in preclinical models of PCa, other animal studies support the idea that physical activity can somewhat normalize tumor vascularization, which may participate in improved prognosis. Tumor growth relies partly on angiogenesis, by which new blood vessels are created to grant oxygen and nutrients to the tumor. However, aberrant angiogenic signaling driven by HIF-1 and downstream VEGF results in chaotic and immature vessel structure, which eventually leads to a discrepancy between the oxygen supply and demand, thereby resulting in hypoxia (Bertout, Patel, and Simon 2008). Hypoxia is a common feature of solid tumors, and is associated with increased metastasis potential and overall poor outcome (Vaupel and Mayer 2007; Gilkes, Semenza, and Wirtz 2014). Interestingly, several preclinical studies have suggested that physical activity and exercise were able to normalize the tumor vasculature, which in turn decreased hypoxia (Figure L-11). Indeed, physical activity/exercise was associated with higher levels of tumor VEGF and platelet-derived growth factor receptor β (PDGFR-β) within the tumor tissue of physically active rodents compared to their sedentary counterparts (Jones et al. 2012; Shalamzari et al. 2014; Betof et al. 2015; Faustino-Rocha et al. 2016). In line with this, intra-tumoral perfusion was improved with physical activity/exercise in preclinical models of breast cancer, PCa, and lymphoma (Zielinski et al. 2004; Jones et al. 2010; 2012; Betof et al. 2015; Faustino-Rocha et al. 2016), along with increased microvessel density and reduced hypoxia (Betof et al. 2015; Buss and Dachs 2017). For example, Betof *et*

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

al. reported that mice submitted to VWR reduced their hypoxic fraction by nearly 50% compared to the sedentary controls (Betof et al. 2015).

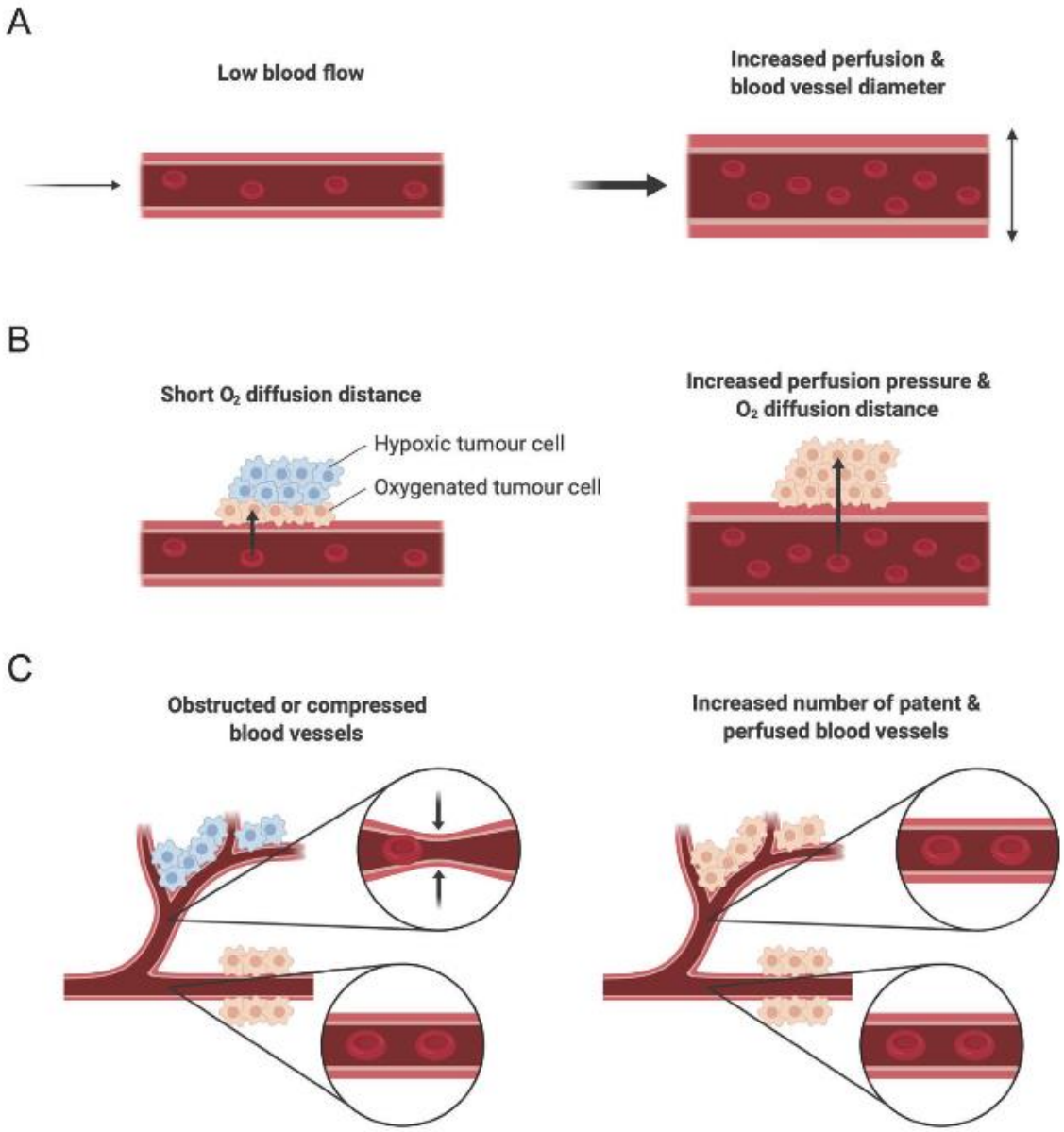

Figure L-11: Possible effects of physical activity (right) on tumor vascularization compared to rest/inactivity (left) (A) Increased blood perfusion and blood vessel diameter; (B) Increased perfusion pressure and oxygen diffusion distance; (C) increased number of patent and perfused vessels. Source: Schumacher et al., 2020

Importantly, exercise has also been shown to improve tumor vascularization in pancreatic cancer patients (Florez Bedoya et al. 2019)

Overall, normalization of the tumor vascularization with physical activity may mimic, to some extent, the pharmacological stabilization of angiogenesis induced by drugs such as bevacizumab (Kümler, Christiansen, and Nielsen 2014), and reduce tumor invasion and metastasis. Furthermore, improved tumor vascularization may also facilitate the infiltration

of immune cells within the tumor tissue, which represents another mechanism mediating the anti-cancer effects of physical activity.

ii – Immune function

As previously mentioned, tumors are able to evade immune surveillance through a variety of mechanisms allowing them to reduce immune cell infiltration and recognition (Vinay et al. 2015; Muenst et al. 2016). Interestingly, immune function may play an important role in the benefits of exercise in the oncology setting (Hojman 2017).

Infiltration of immune cells within the tumor tissue is affected by hypoxia, and therefore the previously discussed increased tumor perfusion and decreased hypoxia induced by physical activity is likely to improve accessibility of the immune cells to the tumor tissue (Zhang et al. 2019).

In addition, exercise is well recognized for directly regulating systemic concentration and functional capacity of immune cells through a characteristic bi-phasic response (Hoffman-Goetz and Pedersen 1994; Nieman and Pedersen 1999; Walsh et al. 2011). Indeed, an acute bout of exercise leads to a striking leukocytosis during or shortly after exercise completion, where white blood cells from the peripheral tissues are mobilized to the bloodstream (Simpson et al. 2015). This results in a drastic rise in circulating levels of NK cells (~10 fold increase) and T cells (~2.5 fold increase) as well a more moderate mobilization of B cells to the bloodstream (Gleeson and Bishop 2005; Campbell et al. 2009). It is noteworthy that this immune mobilization is proportional to exercise intensity and duration.

In the early recovery phase (commonly one to two hours following exercise cessation), acute leukopenia occurs characterized by a transient reduction in lymphocyte counts to sub-baseline values, before normalizing within 24 hours (Gleeson and Bishop 2005; Campbell et al. 2009). While it was largely speculated that this period was an “open window” characterized by compromised immune function, this hypothesis has recently been challenged (Campbell and Turner 2018). Notably, it was shown in mice that leukopenia following exercise was a result of T lymphocyte redeployment to the peripheral tissues (Krüger et al. 2008). It was thus proposed that the redistribution of immune cells by exercise could represent a highly specialized and systematic response, known as the acute stress/exercise immune-enhancement hypothesis (Dhabhar 2014). Therefore, the reduction in circulating immune cells post-exercise may actually reflect improved immune surveillance and regulation

(Campbell and Turner 2018). Additionally, chronic exercise in humans has been associated with increases in circulating NK cell activity and T lymphocytes, suggesting enhanced immune function, even though excessive training may be detrimental to the immune system (Sellami et al. 2018).

The potential of exercise to improve anti-tumor immunity was suggested more than 30 years ago (Mackinnon 1989; Simpson and Hoffman-Goetz 1990). It was notably shown that splenic NK cells retrieved from healthy trained mice were able to kill YAC-1 murine lymphoma cells *in vitro*, while splenic NK cells collected 30 minutes after an acute exhaustive exercise had opposite effects (Simpson and Hoffman-Goetz 1990). Subsequent preclinical studies reported that exercise or physical activity in tumor bearing rodents could increase systemic NK cell frequency and cytotoxicity *in vitro* (Hoffman-Goetz et al. 1992; MacNeil and Hoffman-Goetz 1993a; 1993b; Shewchuk, Baracos, and Field 1997). More recently, it was shown that VWR slowed-down tumor growth in a variety of cancer types through an epinephrine- and IL-6-dependent NK cell mobilization and intra-tumoral infiltration (Pedersen et al. 2016) (Figure L-12). Treatment with propanol, a β-blocker, masked the benefits of physical activity on tumor growth, while daily injections of epinephrine controlled tumor growth comparable to what was observed with VWR (Pedersen et al. 2016).

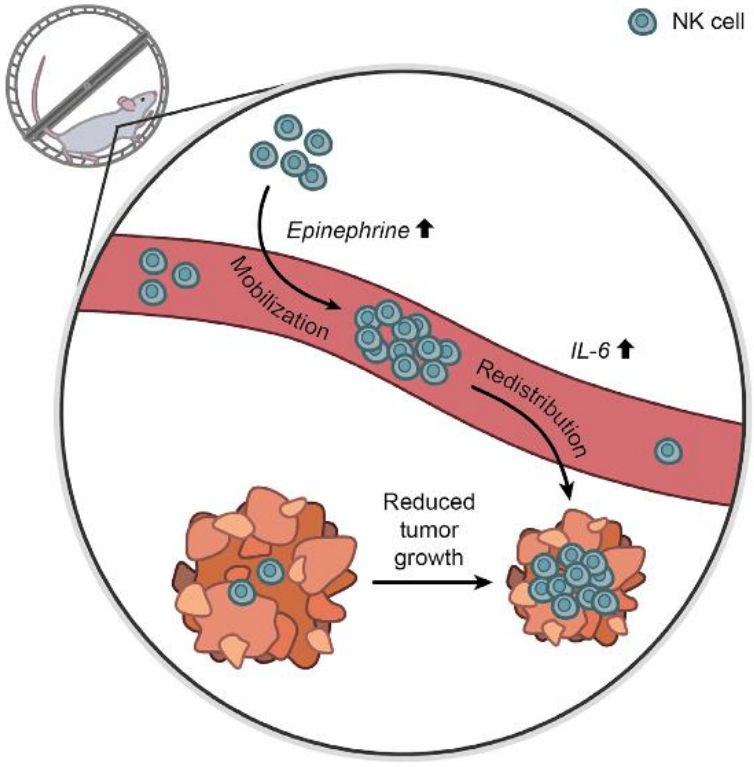

Figure L-12: VWR slows down tumor growth by mobilizing and redistributing NK cells to the tumor tissue in an epinephrine and IL-6 dependent manner. Source: Pedersen et al. 2016

Furthermore, regulation of macrophages may also play a role in the suppressive effect of exercise on neoplastic growth. Several preclinical models reported increased cytotoxicity of tumor-associated macrophages (TAMs) (Verma et al. 2010) or of macrophages isolated from either the spleen (Singh, Singh, and Singh 2005) or the peritoneal cavity (Murphy et al. 2004). Furthermore, it was found that DMBA-induced tumor incidence was reduced with exercise training, along with a shift in peritoneal macrophage phenotype towards a M1 response, as opposed to a more pronounced M2 polarization observed in sedentary mice (Abdalla, Murta, and Michelin 2013; Abdalla et al. 2014). Taken together, physical activity appears to be able to create a shift in macrophage polarization and may improve TAM anticancer activity.

In comparison to the innate immunity, the potential role of the adaptive immunity in the anti-cancer effects of exercise have received very little attention. Nevertheless, several preclinical studies suggest that physical activity may positively impact the adaptive immune system both in the circulation and in the tumor tissue (Koelwyn et al. 2015). For instance, it was reported that VWR reduced the intra-tumoral C-C motif chemokine ligand 22 (CCL22) gene expression, a cytokine which promotes the recruitment of forkhead box $P3^+$ ($FOXP3^+$) regulatory T cells (Tregs) to the tumor tissue, in a transgenic model of breast cancer (Goh et al. 2013). While the prognostic value of Tregs varies across tumor sites, their accumulation in the tumor tissue is associated with poorer outcomes in breast cancer (Shang et al. 2015), as it promotes immunosuppression by inhibiting T cell activation (Tanaka and Sakaguchi 2019). In line with these results, McClellan *et al*. found increased intestinal messenger RNA expression of both *CD8* (a marker of cytotoxic T cells) and *Foxp3* (a marker of Tregs) in trained $Apc^{Min/+}$ mice compared to the sedentary controls. Finally, a study using a preclinical lymphoma model reported increased lymphocyte density in the tumor tissue in response to two weeks of TR (Zielinski et al. 2004).

Taken together, physical activity may improve both the innate and adaptive immune responses to cancer. This effect may be observed systematically, but also within the tumor tissue directly (Koelwyn et al. 2015; Zhang et al. 2019).

iii – Tumor metabolism

As mentioned previously, cancer cells exhibit altered metabolism characterized by enhanced aerobic glycolysis, which enables them to fulfill the high energy demand necessary for rapid cell proliferation (Cairns, Harris, and Mak 2011). Physical activity on the other hand may lead

to high energy consumption, which necessitate alterations in metabolism at the cellular and whole-body levels (Egan and Zierath 2013). It is therefore plausible that physical activity alters tumor metabolism. Indeed, several preclinical studies have found metabolic alterations within the tumor tissue in response to acute or chronic exercise/physical activity, which may affect tumor growth and metastatic processes (Pedersen, Christensen, and Hojman 2015; Hojman et al. 2018).

Exercise impacts the metabolism of the whole-body through the regulation of a wide variety of hormones, but also directly by altering glucose, lipid, and amino acid metabolism in muscles as well as in other tissues such as the liver and the adipose tissues. The energy deficit caused by exercise leads to the activation of oxidative phosphorylation and energy-conserving processes (Egan and Zierath 2013). In particular, it induces AMP-activated protein kinase (AMPK) signaling, which is a major sensor and regulator of energy balance (Kahn et al. 2005). AMPK acts to restore energy levels in the muscles by activating catabolic pathways while decreasing non-essential energy-consuming pathways (Kahn et al. 2005), which may result in the inhibition of cancer cell growth. Indeed, it is suggested that AMPK activation may reprogram cellular metabolism, therefore impacting one of the necessary features of tumors to proliferate and progress, resulting in decreased tumor growth (Li et al. 2015). AMPK is increased in the skeletal muscle tissue in response to exercise, but also in other organs (Ruderman et al. 2003) and preclinical data even suggests a similar effect in the tumor tissue signaling (Zhu et al. 2008; Jiang, Zhu, and Thompson 2009; 2013; Piguet et al. 2015; Theriau et al. 2016). These studies showed increased AMPK signaling accompanied with downregulation of Akt (Zhu et al. 2008; Jiang, Zhu, and Thompson 2009; 2013; Theriau et al. 2016) as well as decreased mTOR expression (Zhu et al. 2008; Jiang, Zhu, and Thompson 2009; 2013; Piguet et al. 2015). AKT activates numerous downstream signaling involved in proliferation and survival in cancer (Fresno Vara et al. 2004) including mTOR, which promotes anabolic processes while impeding catabolic mechanisms such as autophagy (Saxton and Sabatini 2017). Therefore, the upregulation of the AMPK cascade along with the reduction in the AKT/mTOR signaling pathway in response to physical activity could participate in reprogramming the cellular metabolism of the tumor (Thompson, Jiang, and Zhu 2009).

Importantly, a recent study by Vulczak *et al*. showed that TR in mice bearing 4T1 breast cancer led to slower tumor growth which was associated with reduced mitochondrial activity and a decrease in maximum electron transport chain capacity. Furthermore, exercise training

modulated the expression of genes involved in glycolytic metabolism (Vulczak et al. 2020). Taken together, these results suggest that exercise may interfere with tumor energy input as the 4T1 triple negative breast cancer model is known for being reliant on mitochondrial metabolism and carbohydrate supply for proliferation.

Finally, a recent paper showed that mice bearing breast or lung tumors and submitted to VWR counteracted the up-regulation of muscle glutamine synthesis and transport associated with cancer, which was associated with attenuation of muscle wasting (Pedersen et al. 2020). Tumors are highly reliant on external glutamine supply (Altman, Stine, and Dang 2016) and might export glutamine from skeletal muscles to fulfill their demand, resulting in cancer cachexia. The authors also found that active mice had slower tumor growth compared to their sedentary counterparts, and suggested tumor starvation as a potential mechanism (Pedersen et al. 2020). Glutamine plays an essential role in the uptake of essential amino acids and the activation of cell proliferation *via* the mTOR pathway (Wise and Thompson 2010), and limiting its availability through physical activity may hence limit tumor growth.

iv – Muscle-to-cancer crosstalk

It is now well established that skeletal muscle is an endocrine organ which can impact other tissues in the body through either autocrine, paracrine, or endocrine signals (Pedersen and Febbraio 2012; Severinsen and Pedersen 2020). Skeletal muscle was first recognized as a secretory organ with the findings of muscle-derived IL-6 (Steensberg et al. 2000) and the following definition of myokines (Pedersen et al. 2003). Myokines have been defined as "cytokines and other peptides that are produced, expressed and released by muscle fibers and exert either autocrine, paracrine or endocrine effects" (Pedersen et al. 2003). In general, these myokines are produced, expressed and released by muscles under contraction to and induce metabolic adaptations in muscles and regulate energy exchange (Pedersen and Febbraio 2012; Severinsen and Pedersen 2020). Importantly, they are the mediators for crosstalk between the muscle and other organs such as the brain, adipose tissue, liver, pancreas, gut, etc. (Severinsen and Pedersen 2020).

To date, only few myokines have been extensively characterized in humans. Nevertheless, there is some evidence supporting their involvement in the reduction of tumor growth associated with physical activity (Pedersen, Christensen, and Hojman 2015; Hojman et al. 2018; Severinsen and Pedersen 2020). It was shown that conditioned serum obtained from

mice following an acute bout of exercise decreased MCF-7 breast cancer cell proliferation *in vitro* (Hojman et al. 2011). Furthermore, it led to increased apoptosis, which was also observed when MCF-7 cells were incubated with media from electrically stimulated myotubes. These authors further identified Oncostatin M (OSM) as a myokine playing an antioncogenic role against breast cancer cells. Indeed, it was upregulated in the serum but also in several different muscles following an acute bout of exercise, and the use of anti-OSM antibodies blunted the induction of caspase activity (Hojman et al. 2011). Furthermore, another research group identified a novel myokine, secreted protein acidic and rich in cystein (SPARC), which suppressed colon cancer cell proliferation *in vitro* and which was found to mediate, to some extent, the inhibition of colon tumorigenesis induced by exercise *in vivo* (Aoi et al. 2013). Indeed, while TR 3 times/week for 6 weeks reduced the formation of colonic aberrant crypt foci in wild-type mice, this effect was blunted in SPARC null mice (Aoi et al. 2013).

Exercise-induced myokines may also affect cancer in a more indirect manner by affecting immune cell activity. As previously mentioned, a major preclinical study in the exercise-oncology field showed that VWR led to increased NK cell mobilization to the tumor tissue and subsequent decrease in tumor growth across a variety of preclinical cancer models (Pedersen et al. 2016). Importantly, this study suggested that IL-6 may have a role in mediating the anti-cancer effect since blocking IL-6 signaling in mice subjected to VWR did not result in tumor growth inhibition. Indeed, the authors concluded that while epinephrine released during physical activity was necessary for the mobilization of NK cells into circulation, IL-6 was essential for their redistribution to the tumor tissue (Pedersen et al. 2016).

Finally, it is noteworthy that in addition to direct anti-tumor effects, some myokines may also counteract cancer-cachexia, and thereby also contribute to improve cancer. It was shown in a recent preclinical study that musclin, a myokine involved in skeletal muscle mitochondrial biogenesis in mice (Subbotina et al. 2015), counteracted cancer-induced muscle wasting (Re Cecconi et al. 2019).

Overall, by directly impacting the tumor or by acting on other organs/cells of the body, myokines released during exercise may be implicated in control of tumor growth by physical activity. Still, the list of myokines induced by exercise is continuously growing and their potential as anti-cancer agents remains to be largely investigated.

Importantly, the identification of the above-mentioned biological pathways adds to the rationale for the incorporation of physical activity in the clinical setting.

III – The impact of physical activity on cancer treatment efficiency

In view of the previously described biological mechanisms altered by physical activity in preclinical models and the observed benefits on survival rates in cancer patients, it is plausible that physical activity improves the antitumor efficacy of cancer treatments (Ashcraft et al. 2019). In particular, improved tumor perfusion and reduced hypoxia may enhance the delivery of a wide variety of treatment (Koukourakis et al. 2000; Döme et al. 2007; Ma and Waxman 2008) and physical activity has been shown to modulate these parameters, as mentioned in the previous section. Additionally, modulation of the immune system with physical activity may also impact some chemotherapy and RT treatments, as these therapies mediate some of their anti-cancer effects by activating immune responses (Ma et al. 2010). Furthermore, the effects on tumor metabolism – and notably oxidative stress – induced by physical activity may create a microenvironment favorable for cell death which could synergize or potentiate treatment efficiency (Ashcraft et al. 2019). In this section, we will review the current knowledge from preclinical and clinical studies investigating the impact of physical activity on cancer treatment efficiency.

A – Surgery

There is growing evidence that preoperative exercise therapy (PET) can improve or maintain physical capacity but also reduce morbidity in a wide variety of surgical fields, including abdominal, thoracic, cardiac and bariatric surgery (Topal et al. 2019). In the field of cancer, there is increasing evidence supporting similar benefits of PET for patients undergoing surgery. In PCa, obese patients have increased risks of poor surgical outcomes, and the combination of low-calorie diet and exercise may counteract these adverse effects (Wilson et al. 2020). The data supporting the beneficial role of PET in PCa remains insufficient, while there is accumulating evidence showing improved postoperative outcomes in lung cancer patients undergoing PET. Indeed, several systematic reviews reported that PET in lung cancer patients was effective at reducing postoperative complications and length of hospital stay (Sebio Garcia et al. 2016; Cavalheri and Granger 2017; Steffens et al. 2018; Rosero et al. 2019). In the review conducted by Steffens *et al*. for example, it was found that PET reduced postoperative complication rates by 48% and decreased the length of hospital stay by three days (Steffens

et al. 2018). Surgical resection of lungs may impair the pulmonary system and predispose to cardiopulmonary complications (including death) (Win et al. 2005; Kobayashi et al. 2017), and priming the cardiorespiratory system prior to surgery may limit the harm and improve survival rates. Evidence for other cancer types, including PCa, remains low, and additional randomized controlled trials (RCTs) are warranted before drawing conclusions (Steffens et al. 2018).

B – Hormone therapy

Numerous clinical studies have shown that exercise training was safe in PCa and breast cancer patients receiving hormone therapy, improved their physical function and decreased some of the treatment side effects (Payne et al. 2008; Furmaniak, Menig, and Markes 2016; Yunfeng et al. 2017). Notably, hormone therapy increases the risks of developing metabolic conditions such as diabetes or cardiovascular diseases, which may increase non-cancer risk mortality (Abdollah et al. 2015) and regular physical activity may reduce the risk of developing this metabolic syndrome, thereby improving overall survival rates (Kiwata et al. 2016).

There is however very little knowledge about the impact of physical activity on hormone therapy efficiency.

Two preclinical studies conducted by the same research group evaluated the impact of VWR on tumor growth in castrated PCa-bearing mice as a way to mimic the effect of ADT (Zheng et al. 2011; 2012). In both sets of experiments, SCID mice were subcutaneously injected with androgen-dependent LNCaP tumor cells before being castrated 4-6 weeks after injection, once the tumors reached a certain volume. Following castration, mice were either subjected to six weeks of VWR or remained sedentary. Under the effect of androgen-deprivation, tumor volume initially regressed over the first two weeks, before starting to grow again. Interestingly, both studies found that despite relatively low running distances (~2.0-2.5km/day on average), physical activity slowed-down tumor growth significantly (Zheng et al. 2011; 2012), and increased tumor growth delay. More precisely, the study published in 2012 reported that it took 31 days on average for active mice to gain back their initial tumor volume (at castration) compared to 24 days on average in the sedentary control group (Zheng et al. 2012). Furthermore, VWR was associated with a decrease in mitotic cell count and increased apoptosis in the tumor tissue, along with a decrease in tumor IL-6 levels (Zheng et al. 2012). Interestingly, several studies suggest that IL-6 is implicated in the progression of PCa

to the androgen-refractory stage (Chun et al. 2009; Culig 2014). Hence, physical activity in PCa patients undergoing ADT could delay the progression of PCa to androgen independence notably by decreasing IL-6 levels.

Preclinical data in breast cancer also suggests that physical activity could improve hormone therapy efficiency. In mice inoculated with MC4-L2 breast cancer cells, Isanejad *et al*. reported that TR combined with either tamoxifen or letrozol, two hormone therapies commonly used in breast cancer patients, led to greater reductions in final tumor volume than either treatment administered alone (Isanejad et al. 2016). In a similar model, the same research group showed that TR combined with tamoxifen resulted in synergistic effects in reducing miR-21 and BCL-2 (both acting as oncogenes), and increasing programmed cell death protein 4 (PDCD4) expression, a tumor suppressor (Khori et al. 2015). Even though no additional benefits on tumor growth were reported with the combination of the two interventions, physical activity may nevertheless participate in improving the cancer outcomes.

C – Chemotherapy

No studies have evaluated the impact of physical activity on chemotherapy efficiency in PCa. In breast cancer however, several researches, including a clinical study, suggest that physical activity may enhance the response to chemotherapy. The first study investigating this hypothesis however did not find any benefits of exercise on treatment response. Indeed, in 2005, Jones *et al*. reported that 8 weeks of TR did not impact tumor growth nor chemotherapy (doxorubicin) efficiency in athymic nude mice inoculated with MDA-MB-231 breast tumors (Jones et al. 2005). Strikingly however, Courneya *et al*. published a study in 2007 reporting that breast cancer patients engaging in resistance training while undergoing chemotherapy significantly improved treatment completion rates (Courneya et al. 2007). Following this pioneering study, five different preclinical experiments investigating the impact of physical activity on chemotherapy efficiency were conducted. The majority of these studies (7 out of 8) observed enhanced chemotherapy efficiency in melanoma (Sturgeon et al. 2014; Schadler et al. 2016), breast cancer (Betof et al. 2015), pancreatic cancer (Schadler et al. 2016; Florez Bedoya et al. 2019), glioblastoma (Lemke et al. 2016), Ewing sarcoma (Morrell et al. 2019) or colon cancer (Ballarò et al. 2019), while one reported no improvements in a breast cancer model (Parry and Hayward 2015). Interestingly, four studies reported no effect of physical

activity alone on tumor growth or survival, but still improved chemotherapy efficiency (Sturgeon et al. 2014; Schadler et al. 2016; Lemke et al. 2016; Florez Bedoya et al. 2019). This suggests that physical activity may induce favorable changes in the tumor tissue that are not sufficient to decrease tumor growth alone but that can improve the sensitivity of cancer cells to chemotherapy. Increased tumor perfusion with physical activity has notably been suggested as mediating these benefits. Betof *et al*. indeed reported that VWR in a preclinical model of breast cancer led to normalized tumor vascularization and increased tumor perfusion which was associated with improved tumor sensitivity to cyclophosphamide (Betof et al. 2015). Furthermore, two studies conducted on preclinical pancreatic models (including a PDX model) and one using a mouse model of Ewing sarcoma showed improved tumor vascularization in response to TR and increased gemcitabine efficiency (Schadler et al. 2016; Florez Bedoya et al. 2019; Morrell et al. 2019). In addition to improved chemotherapy delivery, increased tumor perfusion with physical activity may also facilitate immune cell mobilization to the tumor tissue (Ashcraft et al. 2019).

Physical activity has also been suggested to increase chemotherapy efficiency through modulation of oxidative stress. Lemke *et al*. indeed reported that TR improved survival and reduced tumor volumes in mice treated with temozolomide compared to mice treated with temozolomide alone, and suggested that this effect was mediated by increased levels of ROS (Lemke et al. 2016). They showed that the combination of dihydroartemisinin, known to induce oxidative stress, and temozolomide reduced the clonogenicity and proliferation of glioblastoma cells *in vitro* and hence speculated that increased oxidative stress by exercise may result in similar effects. Nevertheless, this is highly speculative as TR but not dihydroartemisinin resulted in improved chemotherapy efficiency *in vivo*, and oxidative stress markers were not measured in the tumor tissue of exercised rats. Of note, lower oxidative DNA damage and reduced lipid peroxidation have been observed in the tumor tissue of rats in response to exercise training (Gueritat et al. 2014) and therefore the link between oxidative stress and chemotherapy is yet to be fully explained.

D – Immunotherapy

Despite the relatively recent wide use of immunotherapy in cancer patients, some studies suggest that physical activity could improve this treatment's efficiency. No study to date has

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

evaluated this effect in PCa, but interesting findings have been found in other cancer types. In a recently published article, Martín-Ruíz *et al*. found that a combination of aerobic and strength training in a PDX of lung cancer slowed-down tumor growth alone, but did not impact immunotherapy (nivolumab) efficiency (Martín-Ruiz et al. 2020). Tumor cell proliferation was lower in all three intervention groups, with no added benefits from exercise training when combined with immunotherapy. Interestingly, the authors also reported that exercise associated with therapy was the only strategy which led to an increase in tumor necrosis, while it also resulted in lower apoptosis (Martín-Ruiz et al. 2020). These results suggest potential interactions between exercise training and immunotherapy, but the exact consequences are not known.

Another study also investigated the impact of exercise training on immunotherapy efficiency, but this time this treatment was associated with RT. In a mouse model of triple negative breast cancer, Wennerberg *et al*. showed that ∼3 weeks of TR initiated one week after tumor inoculation led to a slower tumor growth, and improved the response to PD-1 blockade combined with RT (Wennerberg et al. 2020). In contrast to previous findings (Pedersen et al. 2016), the authors did not report increased NK cell infiltration in the tumor tissue in response to exercise training, but rather observed a higher lymphocyte activation, as well as reduced frequency of immunosuppressive myeloid-derived suppressor cells (MDSC) in both the spleen and the tumor (Wennerberg et al. 2020). This favorable immune response observed in active mice led them to hypothesize that immunotherapy efficiency would be improved with exercise training. They subsequently evaluated the impact of TR on PD-1 blockade in a similar mouse model, along with RT to mimic what is done in the clinical setting. Interestingly, the response to this treatment was enhanced with exercise training as observed by a significantly slower tumor growth with the combination of exercise, immunotherapy and RT compared to when immunotherapy was associated with RT alone (Wennerberg et al. 2020). Exercise training combined with the treatments increased the percentage of CD8+ T cells and NK cells in the spleen compared to treated sedentary mice and reduced their PD-1 expression. In the tumor microenvironment, the group combining immunotherapy and RT with exercise exhibited lower PD-1 and higher CD69 expression on NK cells as well as reduced MDSCs compared to when treatments were performed alone (Wennerberg et al. 2020). Taken together, TR in combination with RT and PD-1 blockade led to favorable immunological changes by promoting the infiltration and activation of immune cells.

The combination of immunotherapy with RT in this study is highly interesting as it mimics what is done in the clinical setting but makes it difficult to differentiate the effect of exercise on immunotherapy *versus* RT.

E – Radiotherapy

With the exception of the above-mentioned research by Wenneberg *et al*., no other study has investigated the effect of physical activity on RT efficiency. Yet, biological mechanisms induced by physical activity may interact with this treatment (Ashcraft et al. 2019).

Interestingly, both physical activity and RT have the ability to modulate oxidative stress levels. RT induces most of its tumor toxicity through an important production of ROS (Riley 1994; Azzam, Jay-Gerin, and Pain 2012). Physical activity, on the other hand, can either participate in ROS production if performed acutely or, in contrast, can boost antioxidant defenses when achieved regularly (He et al. 2016). As previously mentioned, lower oxidative DNA damage and reduced lipid peroxidation have been observed in the tumor tissue of rats in response to TR (Gueritat et al. 2014). Of note, another study using a preclinical model of liposarcoma found no alterations in tumor oxidative stress levels in response to VWR (Assi et al. 2017). Nevertheless, physical activity in combination with RT could either enhance or reduce tumor oxidative stress levels, depending on whether the acute ROS production or the chronic increase in antioxidant defenses is considered. As a consequence, physical activity may potentially either improve, worsen, or have no effect on RT efficiency.

Impacting tumor vascularization and hypoxia represent another pathway by which physical activity may improve tumor sensitivity to RT. Indeed, hypoxic cancer cells are more resistant to radiation compared to aerobic cells (Rockwell et al. 2009). Hence, physical activity could hypothetically reduce resistance of cancer cells to RT by circumventing tumor hypoxia.

Additionally, the improved immune response associated with physical activity may increase RT efficiency. Indeed, increasing evidence suggests that the combination of RT and immunotherapy produces synergistic effects which enhance the immune response an thus result in greater cancer cell death (Demaria, Golden, and Formenti 2015). Notably, the damage inflicted by RT to cancer cells increases immune recognition and promotes the anti-tumor T cell response (Hodge et al. 2008; Wang et al. 2018). Hence, priming the immune system with physical activity may improve the RT response.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

Overall, there are several plausible biological mechanisms supporting the idea that physical activity may impact RT efficiency, and that these effects are likely to be dependent on tumor type and physical activity modality.

AIMS & OBJECTIVES

AIMS OF THE THESIS

Prostate cancer (PCa) represents a major health issue around the world, representing the second most frequent cancer in men, with 1.3 million new cases in 2018 and an estimated 359 000 deaths (Bray et al. 2018). This complex disease is characterized by a disruption in the homeostasis between cell proliferation and cell death which typically occurs following damage to the prostatic epithelium and the formation of high-grade prostatic intraepithelial neoplasia (HGPIN) (Packer and Maitland 2016). A better understanding of prostate carcinogenesis and the identification of major cancer hallmarks over the years allowed several treatments to be developed. For PCa, several therapeutic options can be considered, including active surveillance, surgery, radiotherapy (RT), androgen-deprivation therapy (ADT), chemotherapy, and more recently, immunotherapy. Amongst them, RT is the commonly administered treatment, with an estimated 48-66% of PCa patients receiving RT, irrespective of cancer stage, PSA levels or Gleason grade (Chamie, Williams, and Hu 2015). However, despite current efforts to improve RT efficiency, tumors can become resistant to this treatment, leading to subsequent disease recurrence, which represents a major challenge in the radiation oncology field (Barker et al. 2015; Kim et al. 2015).

Physical activity following cancer diagnosis is now well recognized for being associated with reductions in all-cause mortality but also in cancer-specific mortality in PCa patients (Patel et al. 2019; Friedenreich et al. 2020). In line with this, preclinical studies have identified several biological mechanisms mediating the benefits of physical activity in cancer. These mechanisms can be broadly classified into four categories: (a) vascularization and blood perfusion, (b) immune function, (c) muscle-to-cancer cross-talk, and (d) tumor metabolism (Pedersen, Christensen, and Hojman 2015). In this context, the potential for physical activity as a strategy to improve treatment efficiency in cancer appears plausible (Ashcraft et al. 2019). Notably, several preclinical studies have shown enhanced response to chemotherapy following physical activity in preclinical models of melanoma (Sturgeon et al. 2014; Schadler et al. 2016), breast cancer (Betof et al. 2015), pancreatic cancer (Schadler et al. 2016; Florez Bedoya et al. 2019), glioblastoma (Lemke et al. 2016), Ewing sarcoma (Morrell et al. 2019) or colon cancer (Ballarò

et al. 2019). More recently, it was also shown that physical activity had the ability to increase the efficiency of combined PD-1 blockade and RT in a 4T1 mammary carcinoma model (Wennerberg et al. 2020). In PCa however, the role of physical activity of the RT response remains largely unknown.

In this context, **the primary objective of this thesis was to investigate the impact of physical activity on PCa growth and RT efficiency using different physical activity paradigms and preclinical PCa models. We further aimed to identify the potential underlying mechanisms.**

Hence, the first two studies included in this thesis were conducted in order to:

1) Evaluate the impact of treadmill running (TR) on RT efficiency in a PCa xenograft model (**Study n°1**)
2) Investigate the effect of voluntary wheel running (VWR) on the RT response in a preclinical model of PCa (**Study n°2**)

As mentioned previously, there is increasing evidence linking physical activity to reduced cancer mortality risk and several pathways mediating these effects have been identified. Nevertheless, the underlying mechanisms remain incompletely understood. Epigenetics is a research field which has gained considerable interest over the past 15 years (Deichmann 2016), notably in the oncology setting. In parallel, physical activity has emerged as a lifestyle factor able to modify several epigenetic processes in a variety of body fluids and tissues (Alegría-Torres, Baccarelli, and Bollati 2011; Ling and Rönn 2014). However, few studies to date have evaluated the potential of epigenetics for mediating some of the exercise-induced benefits in cancer.

Based on these elements, **a second objective of this thesis was to evaluate the potential of epigenetic processes as mechanisms underlying the anti-cancer effects of exercise in PCa**.

Accordingly, two additional studies were included in this study aiming to:

1) Review the alterations in circulating miRNas in response to chronic physical activity and acute exercise, and discuss how these changes could potentially affect tumor growth (**Study n°3**)
2) Investigate whether or not exercise training was able to regulate epigenetic processes within the tumor tissue of PCa-bearing rats (**Study n°4**)

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

RESULTS

RESULTS

In this section, we will present the major findings obtained throughout the PhD. Some of these results have been published in or submitted to international scientific articles, while others still require additional experiments before entering the peer review process. Each study will be briefly introduced and summarized before being fully detailled.

I – Exercise training improves radiotherapy efficiency in a murine model of prostate cancer

Prostate cancer (PCa) is one of the most commonly diagnosed cancers in men (Bray et al. 2018). Amongst the various therapeutic options available, radiotherapy (RT) is commonly administered to these patients. For instance, it is estimated that 40% of PCa patients over 65 years of age will undergo RT during the course of their treatment (Skolarus et al. 2014). However, despite notable medical advances, resistance to RT can occur, resulting in treatment failure and poor outcomes. Hence, strategies are being explored to bypass treatment resistance and improve RT efficiency.

In parallel, accumulating epidemiological and preclinical data show that regular physical activity is associated with decreased overall and cancer-specific mortality and reduced tumor growth in a wide variety of cancer types (Ashcraft et al. 2016; Patel et al. 2019; Friedenreich et al. 2020). Several biological mechanisms mediating these effects have been identified and can be grouped into four main categories: (1) vascularization and blood perfusion, (2) immune function, (3) tumor metabolism and (4) muscle-to-cancer crosstalk (Pedersen, Christensen, and Hojman 2015). In view of these findings, it was further hypothesized that regular physical activity could enhance the response to commonly used anti-cancer therapies. In a randomized controlled trial including 242 breast cancer patients undergoing chemotherapy, Courneya *et al*. showed that resistance exercise, but not aerobic exercise, was able to improve chemotherapy completion rates (Courneya et al. 2007). Subsequent preclinical studies found

that physical activity was able to improve chemotherapy efficiency in rodent models of melanoma (Sturgeon et al. 2014; Schadler et al. 2016), breast cancer (Betof et al. 2015), pancreatic cancer (Schadler et al. 2016; Florez Bedoya et al. 2019), glioblastoma (Lemke et al. 2016), Ewing sarcoma (Morrell et al. 2019) or colon cancer (Ballarò et al. 2019). Physical activity may also modulate other standard anti-cancer treatments such as immunotherapy and RT (Ashcraft et al. 2019; Schumacher et al. 2020). However, this hypothesis remains to be investigated.

In this first study presented, we aimed to evaluate the impact of exercise training on RT efficiency in a murine model of prostate cancer. Mice were inoculated with PPC-1 prostate cancer cells and randomized to one of the following groups one week after injection: cancer control (CTL), cancer exercise training (ET), cancer RT (RT) or cancer RT combined with ET (RT-ET). Mice following the exercise training program ran on a treadmill running at 18m/min with a 10% incline for 1h, five days per week, while mice treated with RT received five doses of five Grays (Gy). 24 days post-cancer cells injection, all animals were euthanized.

As expected, RT reduced tumor growth. Interestingly, ET was able to slow down tumor growth either alone or in combination with RT. Hence, mice combining RT with ET had the smallest and lightest tumors, showing that ET was able to improve RT efficiency. All three intervention groups displayed reduced cancer cell proliferation (assessed with Ki67 staining). When analyzing pERK1/2:ERK1/2 protein ratio in the tumor tissue however, only ET alone was able to decrease this parameter. Our team had previously reported reduced ERK1/2 activation in response to treadmill running in rats bearing AT1 prostate tumors (Gueritat et al. 2014). However, the surprising result in the present study is that the reduction in ERK1/2 activation associated with ET was not observed in RT-ET mice.

We further analyzed apoptosis in the tumor tissue and found that apoptotic effector cleaved caspase-3 (cCASP3) remained unaltered with ET alone and were elevated with RT. Importantly, this increase was most pronounced in the RT-ET mice. Hence, ET potentiated the RT response through higher CASP3 activation. We further investigated whether the innate immune system could be implicated in the enhanced RT response with ET. Physical activity is well known for modulating the immune response in various tissues and fluids (Pedersen and Hoffman-Goetz 2000). Importantly, it was demonstrated in a preclinical study that immune cells, and notably natural killer (NK) cells, played a major role in the anti-cancer effects associated with physical activity (Pedersen et al. 2016). Furthermore, the immune system

plays a crucial role in the RT response, as brought to light with the recent advances in immunotherapy (Demaria, Golden, and Formenti 2015; Weichselbaum et al. 2017). In our study, we found that immunohistological staining of NK1.1, an activating receptor expressed on NK cells, was increased with RT, and this increase was even more important when combined with ET. In line with these results, killer cell lectin-like receptor K1 (*Klrk1*) and interleukin 2 receptor β (*Il2rβ*), two genes coding for NK cell activating receptors, and inflammatory cytokines tumor necrosis factor α (*Tfn-α*) and interferon ɣ (*Ifn-ɣ*) associated with the immune response had increased mRNA expression levels in the tumors of RT mice, and even higher expressions in the RT-ET group. Hence, ET may enhance RT efficiency by potentiating NK cell activation in the tumor tissue, resulting in increased apoptosis and slower tumor growth.

Overall, this study shows that (a) ET improves the RT response in a murine model of PCa through increased NK cell activation and apoptosis; and (b) while both ET alone and ET combined with RT had a positive impact on tumor growth, the mechanisms mediating these effects are different. Indeed, ET alone decreased cancer cell proliferation through reduced ERK1/2 activation but did not alter apoptosis in the tumor tissue, while ET combined with RT did not impact ERK1/2 activation, but potentiated tumor cell apoptosis associated with RT. Thus, exercise may interact with conventional treatments and improve their efficiency on the one hand, while on the other hand some of the anti-cancer effects of exercise may be lost when combined with treatment. Nevertheless, our results are in favor of promoting physical activity in PCa patients, even when undergoing RT.

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Exercise training improves radiotherapy efficiency in a murine model of prostate cancer

Suzanne Dufresne[1], Jordan Guéritat[1], Sophie Chiavassa[2], Caroline Noblet[2], Mohamad Assi[1], Nathalie Rioux-Leclercq[3], Françoise Rannou-Bekono[1], Luz Lefeuvre-Orfila[1], François Paris[2], Amélie Rébillard[1]

1. M2S laboratory, EA 1274, Université Rennes 2, ENS Rennes, 35170 Bruz, France
2. CRCINA, INSERM, Université de Nantes, Université d'Angers, 44000 Nantes, France; Institut de Cancérologie de l'Ouest Centre René Gauducheau, 44805 Saint Herblain, France
3. Department of Pathological Anatomy and Cytology, Université Rennes 1, 35000 Rennes, France

ABSTRACT

Engaging in exercise while undergoing radiotherapy (RT) has been reported to be safe and achievable. The impact of exercise training (ET) on RT efficiency is however largely unknown. Our study aims to investigate the interactions between ET and RT on prostate cancer growth. Athymic mice received a subcutaneous injection of PPC-1 cells and were randomly assigned to either cancer control (CaCTL), cancer ET (CaET), cancer RT (CaRT), or cancer RT combined with ET (CaRT-ET). Mice were sacrificed 24 days post-injection. All three intervention groups had reduced tumor size, the most important decrease being observed in CaRT-ET mice. Apoptotic marker cleaved caspase-3 was not modified by ET, but enhanced with RT. Importantly, this increase was the highest when the two strategies were combined. Furthermore, NK1.1 staining and gene expression of natural killer (NK) cell receptors *Klrk1* and *Il2rβ* were not affected by ET alone but were increased with RT, this effect being potentiated when combined with ET. Overall, our study shows that (1) ET enhances RT efficiency by potentiating NK cell infiltration, and (2) while ET alone and ET combined with RT both reduce tumor growth, the mechanisms mediating these effects are different.

Key words: radiation therapy; physical activity; treatment; oncology

INTRODUCTION

Prostate cancer (PCa) is one of the most predominant health issues in the aging population: it represents the second most frequently diagnosed cancer in men, responsible for 1.3 million new cases in 2018 and an estimated 359 000 deaths (1). Several therapeutic options can be considered, including surgery, radiotherapy (RT), androgen deprivation therapy, chemotherapy, and immunotherapy. RT is one of the most commonly used treatment for PCa (2) and is chosen as a definitive therapy in approximately 25% of cases (3). Despite the efforts to increase treatment tolerance, RT is associated with numerous side effects including fatigue, sore skin, declined urinary, bowel and sexual functions, resulting in a persistent adverse effect on quality of life (4–6).

Physical activity is well-known for improving the overall health and may reduce some of these undesirable side effects. Engaging in physical activity while undergoing RT treatment has been found to be safe, achievable, and may even provide several physical and mental health benefits. The American College of Sports Medicine has indeed assessed physical activity as safe in cancer patients during and after treatment (7) and appears to be a safe practice even in more advanced diseases (8). Even though novel strategies are being tested to improve the feasibility of exercise in the oncology setting (9, 10), the adherence of patients is quite high, ranging from 65 to 89% (11). In PCa more specifically, several clinical trials showed that exercise training was compatible with radiotherapy, with an adherence rate of approximately 85% and no reported adverse events (12–15).

In terms of benefits, randomized-clinical trials in PCa patients have shown a positive impact of physical activity on fitness levels as well as on cancer-related fatigue and quality of life (12, 14–16). Importantly, large cohort studies have observed that exercising regularly after a PCa diagnosis was associated with increased lifespan as well as reduced risk of both cancer-relapse and cancer-related death (17–20). For example, Bonn et al. found that walking or cycling a minimum of 20 minutes per day was associated with a 30% decrease in overall mortality rates (20). *In vivo*, six studies have reported that exercise could significantly, or border-line significantly, decrease prostate tumor growth (21–26), while three others however did not find any effect (27–29).

Several biological mechanisms mediating the effects of exercise on tumor growth have been identified and can be grouped into four main categories: (a) vascularization and blood

perfusion, (b) immune function, (c) tumor metabolism, and (d) muscle-to-cancer cross-talk (30). Interestingly, these diverse biological pathways involved in the exercise response have been suggested to improve cancer treatment efficiency (31). A few studies have shown improved chemotherapy efficiency with exercise (32–35), notably through normalized vascularization (34, 35). Yet, even though physical activity being increasingly suggested to cancer patients following a diagnosis, the combination of exercise training (ET) with anti-cancer therapies remains largely unexplored, and its effect on RT is unknown.

Against this background, we have conducted an explorative pre-clinical investigation with the primary objective to evaluate the effect of ET on RT efficiency in a murine model of PCa.

MATERIALS AND METHODS

Animals and tumor model

The use and care of animals followed the guidelines of the French Agriculture Ministry (authorization number: n° R-2012-AR-01). Mice we maintained on a 12:12 h dark-light cycle at the animal facility of M2S laboratory (n°A3523840). Experiments were conducted in accordance with the ethical standards of the European Community (directive 86/609/EEC).

Forty male athymic nude mice (Harlan, Gannat, France) aged five to seven weeks old were housed in standard laboratory conditions, with a temperature- and humidity-controlled environment. Their body weight was monitored throughout the entire study.

All mice received a dorsal subcutaneous injection of $8x10^6$ human prostate cancer PPC-1 cells (day 1). The PPC-1 cells (36, 37) were a gift from Pr. James Norris (Medical University of South Carolina, Charleston, SC, USA). The cancer cells were maintained in a humidified atmosphere at 37°C; 5% CO2 and cultured in RPMI 1640 medium supplemented with 10% FBS and 0.1% Penicillin/Streptomycin.

One week following the injection (day 7), a scanner was performed to assess tumor growth. Four mice did not develop tumors or had an uncharacteristic tumor size/shape and were withdrawn from the study. The remaining 36 mice were randomized into four experimental groups: Cancer Control (CaCTL), Cancer Exercise Training (CaET), Cancer Radiotherapy (CaRT), and Cancer Radiotherapy combined with Exercise Training (CaRT-ET). The study design is illustrated in Figure R-1.A.

Treatments

CaCTL mice (n=9) did not receive any treatment.

CaET mice (n=8) completed the following ET protocol: after two days of acclimation (20 min of treadmill running at a speed of 10 m/min and with a 10% slope) mice underwent treadmill running in the morning, five times per week for two weeks. The intensity of the exercise was the same throughout the protocol and set at 18 m/min with a 10% incline. The exercise duration however varied over time: mice ran for 25 min during their first ET session and gradually increased their running time to reach one hour from their fifth ET session onwards.

CaRT mice (n=8) underwent the following RT treatment: they received a total of 20 Grays (Gy) localized on the tumor site and fractionated in four doses of 5 Gy (XRAD 225Cx, PXI, CRCNA, Nantes, France) distributed on day 10, 12, 14 and 17, in the afternoon. The system delivered equally weighted parallel-opposed beams at 13mA with a 0.3 mm Cu filter, yielding a dose rate output of 3.06 Gy/min through 10-15mm diameter collimator. The irradiator was dosimetrically calibrated to ensure accurate radiation dosage and the tumor was targeted by 3D imaging (Cone Beam CT). 3D Cone-bean CT (CBCT) images were obtained with the on-board imager of the XRAD 225Cx. It consists of a digital imaging panel (model: XRD 0820 AN3 ES, Perkin-Elmer, Germany) mounted in front of the XRay tube on a rotating arm. CBCT were performed at 40 kVp and 2.5 mA using the small focal spot (1 mm in diameter) and the 2 mmAl filter. Mice were anesthetized upon each RT sessions with 3% halothane in 100% oxygen.

Finally, CaRT-ET mice (n=9) underwent both ET and RT procedures described above (ET protocol and RT treatment).

Throughout the treatment period, all mice had their tumor size regularly evaluated with sliding calipers. The following formula was used to calculate tumor volume: length x width x height x 0.5236 (expressed in mm^3).

Exhaustive endurance test

An exhaustive endurance test was performed at day 7 (before randomization) and at day 21 (after treatment completion). The animals had to run for the longest time possible at 18m/min with a 10% slope and the end of the test was determined by the inability of mice to go back on the treadmill (38).

Tissue preparation

On day 24, one week after RT completion and 48 hours after their last ET session, mice were anesthetized by intra-peritoneal injection of ketamine (50 mg/kg) and xylazine (4 mg/kg) before being euthanized.

Tumors, lungs, and skeletal muscles (gastrocnemius) were removed and washed in PBS. Lungs were fixed in 4% paraformaldehyde (PFA), muscles were frozen in liquid nitrogen and stored at -80°C, and tumor samples were each divided into two parts; one part being fixed in 4% PFA, and the other part being frozen in liquid nitrogen and stored at -80°C. Tissues which were frozen were then grinded in liquid nitrogen and the obtained powder was used to perform experiments.

Western Blot analysis

Tumor samples were lysed in buffer (50 mM Tris-HCl, 10 mM EDTA, 1% SDS) and protein concentration was determined using Lowry protein assay. Proteins (50 µg) were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After being blocked with 5% BSA or non-fat dry milk in TBS-Tween (0.05%), membranes were incubated overnight at 4°C with the appropriate primary antibodies (Table R-1). After being washed three times with TBS-Tween (0.05%), membranes were incubated with secondary antibodies for one hour at room temperature. Immunoreactive bands were visualized with Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA). Protein loading was normalized to HSC70 expression.

Table R-1: List of western blot antibodies

Protein	Molecular weight	Reference	Dilution	Source
Cleaved caspase-3	17, 19 kDa	Cell signaling 9661	1/1000	Rabbit
BAX	20 kDa	Cell signaling 2772	1/1000	Rabbit
BCL-2	28 kDa	Abcam ab7973	1/1000	Rabbit
p-ERK1/2	42, 44 kDa	Cell signaling 4376	1/1000	Rabbit
ERK1/2	42, 44 kDa	Santa Cruz sc-514302	1/1000	Rabbit
p-AKT	60 kDa	Cell signaling 9271	1/1000	Rabbit
AKT	60 kDa	Cell signaling 9272	1/1000	Rabbit
HSC70	70 kDa	Santa Cruz sc-7298	1/5000	Mouse

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

RNA extraction and RT-qPCR

Total RNA was extracted from tumor tissue samples using Trizol reagent according to the manufacturer's protocol (Sigma Aldrich, St Louis, MO, USA). Due to the small tumor size, only five tumor samples of each treatment group were included for the RT-qPCR experiments.

RNA concentration was determined for each sample with Nanodrop spectrophotometer and RNA quality was controlled using the Flashgel electrophoresis system (Lonza, Rockland, ME, USA).

Reverse transcription reaction was carried out on 1µg of total RNA using the iScript Reverse Transcription Supermix (Bio-Rad, Hercules, CA, USA).

For real time PCR experiments, samples were measured in duplicate, each reaction volume containing 4.8 mL SybrGreen Supermix (Bio-Rad, Hercules, CA, USA), 0.1 mL of each primer (100nM final) and 5 mL cDNA. Primer sequences are listed in Table R-2. A dilution series of 10^2, 10^3, 10^4, 10^5, and 10^6 copies of template DNA served as internal standard for quantification, and specificity of the amplification process was verified by analyzing the melting curves. The expression of target genes was normalized to the reference gene RPL4 and the relative expression was calculated using the ΔΔCt method on CFX-Manager software (Bio-Rad, Hercules, CA, USA).

Table R-2: List of primers used for RT-qPCR analysis

Gene	Forward (5' → 3')	Reverse (5' → 3')
Ifn-ɣ	ACTGGCAAAAGGATGGTGACA	TGGACCTGTGGGTTGTTGAC
Il2rβ	GTCCATGCCAAGTCGAACCT	AGGCGAAGGTTGTCAAAGGG
Klrk1	TGGCATTGATTCGTGATCGAAA	TGCACAATACTGGCTGAAACG
Ncr1	GACTAGGGCTCACAGAGGGA	CCAGAAGGCGGAGTCCTTTT
Tnf-α	GCCTCTTCTCATTCCTGCTTG	CTGATGAGAGGGAGGCCATT

Immunohistological analysis

Tumor tissues were fixed in 4% PFA for 24h and embedded in paraffin. Tissue sections of 4 µm thickness were obtained using LEICA microtome and mounted on glass slides.

Tumor sections were stained with Hematoxylin and Eosin (H&E) which colors the nucleus in dark purple and the cytoplasm in a lighter pink shade. This enabled for the evaluation of necrotic areas and immune cell infiltration. Necrotic areas were defined on the H&E slides by

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

the absence of tumor cell nuclei and were expressed as necrotic area relative to total tumor area. Immune cell infiltration was determined based on cell morphology and was characterized as either low (grade 0-1) or high (grade 2-3). Immunohistochemical staining for Ki67, CD31, and NK1.1 were performed on the Discovery XT Automated IHC stainer using the Ventana detection kit (Ventana Medical Systems, Tucson, AZ, USA). Following deparaffination with Discovery wash solution (Ventana Medical Systems, Tucson, AZ, USA) at 75 °C for 8 minutes, antigen retrieval was performed with a Tris-based buffer solution pH8 (Ventana Medical Systems, Tucson, AZ, USA) at 95°-100°C for 40 minutes. Endogen peroxidase was blocked with 3% H2O2 for 12 minutes. After rinsing, slides were incubated at 37°C for 60 minutes with the primary antibody for CD31 (DIA-310, Dianova, Hamburg, Germany; dilution at 1/50), Ki67 (NB600-1252, Novus Biologicals, Centennial, CO, USA ; dilution at 1/400) or NK1.1 (MA1-70100, Thermo Fischer, Waltham, MA, USA ; dilution at 1/100). Signal enhancement for CD31 was performed with an anti-rat HRP at 37°C for 32 minutes and using the Ventana ChromoMap Kit Slides (biotin free detection system) (Ventana Medical Systems, Tucson, AZ, USA). Slides were then counterstained for 16 minutes with hematoxylin and rinsed before being manually dehydrated and coverslipped. Signal enhancement for Ki67 and NK1.1 was performed using a goat anti-rabbit or a goat anti-mouse at 37°C for 16 minutes and Discovery Rhodamine Kit (542-568nm) for 8 minutes (Ventana Medical Systems, Tucson, AZ, USA). To visualize the nucleus, Dapi staining was added and coverslipped. CD31, Ki67 and NK1.1 were analyzed with ImageJ software. CD31 was expressed as the percentage of stained area (in brown) compared to the total tumor area whereas Ki67 and NK1.1 were reported as the percentage of positive Ki67 or NK1.1 cells (in red) relative to the total number of cells (DAPI staining, in blue).

Statistics

Statistical analyses were performed in Prism6 (GraphPad softwares, San Diego, CA, USA). The results were analyzed using a two-way ANOVA with the two main variables being (1) ET and (2) RT. When the two-way ANOVA showed a significant interaction, the main effects were analyzed and Tukey post-hoc tests were performed. Comparisons were considered significant for p values below 0.05. Data are represented as means ± SEM.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

RESULTS

ET does not counteract RT-induced weight loss but improves endurance capacities in PCa bearing mice

Throughout the protocol described in Figure R-1.A., the body weight was measured regularly. At baseline (day 3), body weight was similar between all experimental groups. On day 17, RT induced a significant weight loss (main RT effect p=0.0012) which is not counteracted by ET (Figure R-1.B.). Importantly, this effect is transient since it was erased one week after RT completion (day 24).

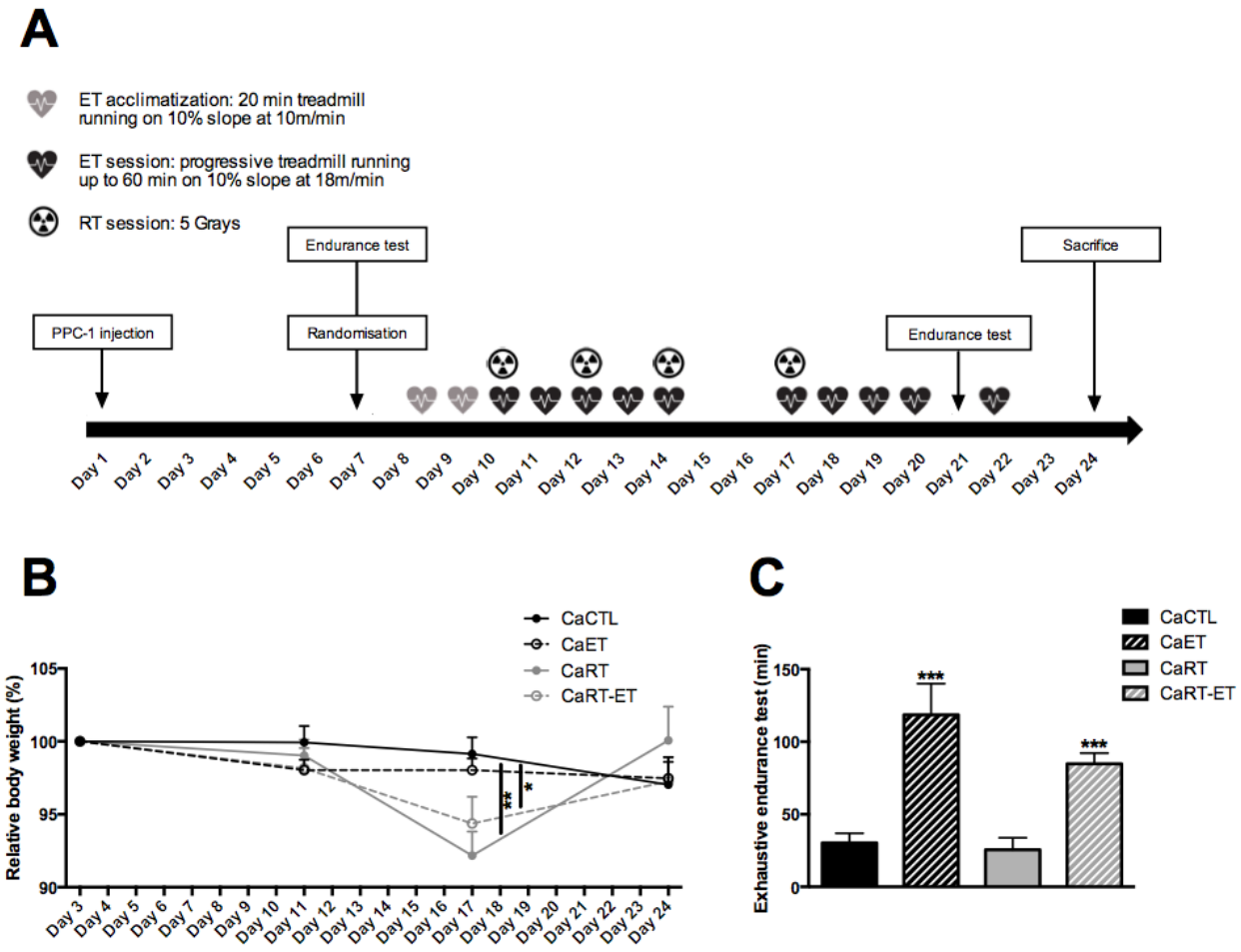

*Figure R-1: (A) Study design (B) Evolution of relative body weight (%) over the time course of the experiment (C) Measure of time to exhaustion in the exhaustive endurance test performed on day 21 at the end of the protocol. Data are represented as means ± SEM. ET main effect is represented by * p<0.05, ** p<0.01, *** p<0.001; RT main effect is represented by # p<0.05, ## p<0.01, ### p<0.001; interaction is represented by $ p<0.05, $$ p<0.01, $$$ p<0.001; Tukey post hoc tests performed when an interaction was found is represented by letters: a compared to CaCTL, b compared to CaET, c compared to CaRT.*

To test the effectiveness of our ET strategy, we measured aerobic capacities using an exhaustive treadmill running test at the beginning (day 7) and at the end (day 21) of the

protocol. No differences were observed between groups on day 7 (data not shown). On day 21, the mice from both the CaET (118.60 ± 21.45 min) and CaRT-ET groups (84.81 ± 7.40 min) had significantly higher running times than the non-exercise groups (30.32 ± 6.66 min for CaCTL and 25.53 ± 8.31 for CaRT) (ET main effect $p<0.0001$) while RT did not have any effect. ET was thus effective at developing endurance capacity despite cancer growth and RT treatment (Figure R-1.C.).

ET improves RT efficiency

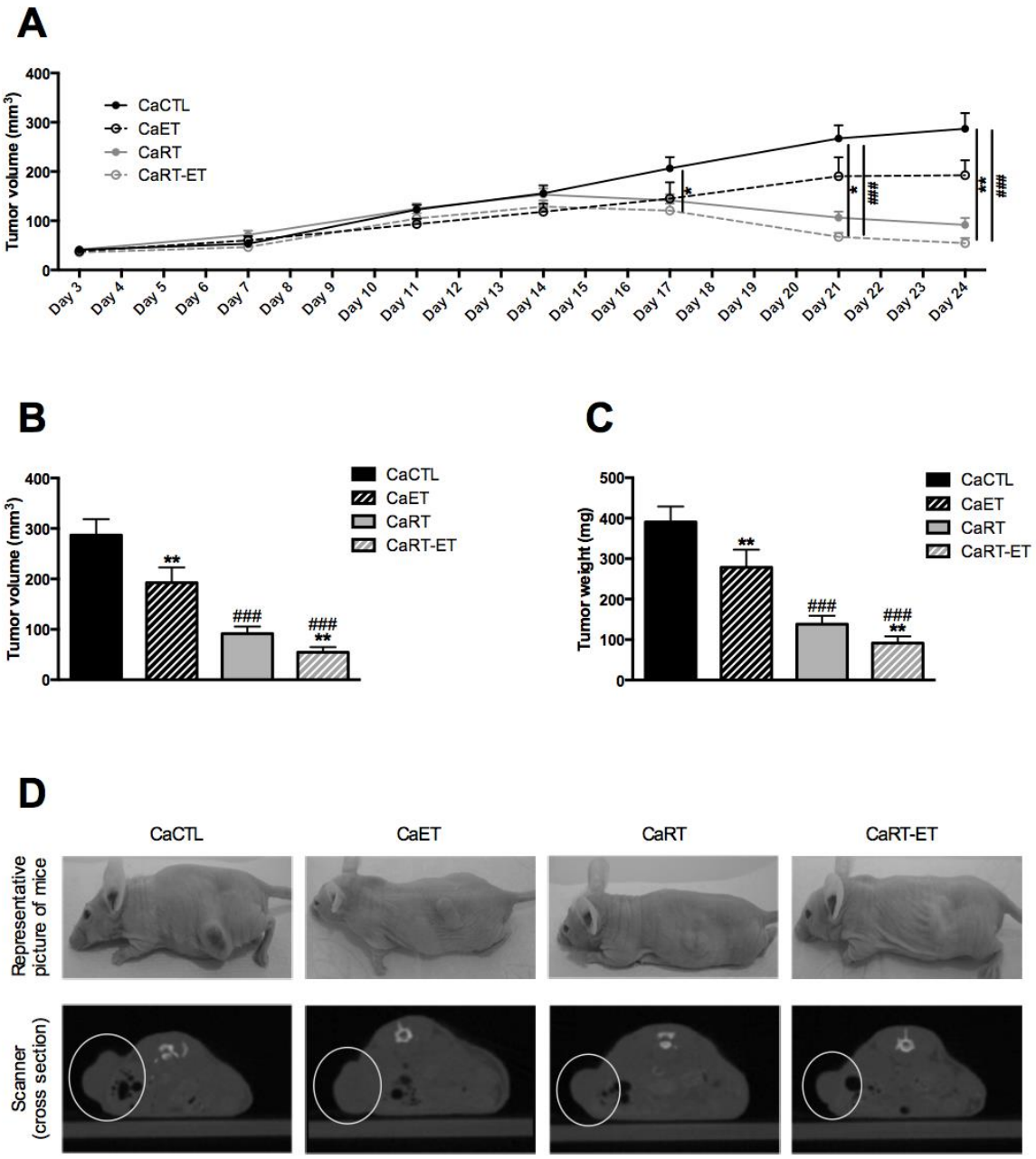

Figure R-2: Tumor volume and tumor weight (A) Evolution of tumor volume measured with sliding calipers and calculated with the formula length x width x height x 0.5236 (expressed in mm3) (B) Tumor volume and (C) tumor

*weight measured on the final day of the protocol (day 24) (D) Representative pictures of mice on day 24 and scanner taken on day 17 (last RT session) showing a cross-sectional image of the mice, with the tumor surrounded with a white circle. Data are represented as means ± SEM. ET main effect is represented by * p<0.05, ** p<0.01, *** p<0.001; RT main effect is represented by # p<0.05, ## p<0.01, ### p<0.001; interaction is represented by $ p<0.05, $$ p<0.01, $$$ p<0.001; Tukey post hoc tests performed when an interaction was found is represented by letters: a compared to CaCTL, b compared to CaET, c compared to CaRT.*

To evaluate the effect of the different strategies on tumor growth, tumor volume was regularly measured throughout the protocol. The results obtained showed that both ET and RT were able to significantly decrease the tumor volume at the end of the protocol (ET main effect p=0.0095 and RT main effect p<0.0001), but no interaction was found (p=0.2365) (Figure R-2.A. and Figure R-2.B.). There was an increase in tumor volume over time in both non-irradiated groups, but to a lesser extent in CaET mice, with the tumor reaching a mean of 286.90 ± 31.67 mm^3 for the CaCTL group and 192.60 ± 30.38 mm^3 for the CaET group. As expected, RT treatment decreased tumor volume significantly from day 17 onwards (RT main effect p=0.0442 on day 17) thereby proving its effectiveness against PCa growth, with a mean tumor volume of 91.64 ± 13.94 mm^3 in the CaRT group on day 24. The smallest tumors were observed in the CaRT-ET group a mean volume of 54.59 ± 10.35 mm^3. In the same way, the weight of resected tumors measured on the final day of the protocol showed that the lightest tumors were obtained with the combination of RT and ET (ET main effect p=0.0063; RT main effect p<0.0001) (Figure R-2.C.). Overall, our results demonstrated for the first time that ET improves RT efficiency in a model of murine PCa.

RT but not ET improves necrosis and tumor vascularization

To investigate histological alterations of tumor tissue, an H&E staining was performed. Necrotic areas observed in aggressive prostate tumors are associated with poor prognosis (39). In our study, necrotic areas represented a mean of 13.22 ± 5.01 % of the tumor sections in the CaCTL group. ET did not significantly decrease this feature (ET main effect p=0.3314), with 6.286 ± 2.68 % of the tumor area being necrotic in the CaET group. There was however a large remodeling of the tissue architecture in response to RT, with necrotic areas being almost inexistent (representing 0.68 ± 0.68% of the tumor sections for CaRT and 0.71 ± 0.71 % for CaRT-ET, RT main effect p=0.0152) (Figure R-3.A. and Figure R-3.B.).

Microvasculature was also assessed through CD31 immunohistochemistry. Vascular labeling (brown staining on the images) was only significantly increased with RT (ET main effect

p=0.4395; RT main effect p<0.001) whereas the combination with ET did not enhance nor counteract this effect (interaction p=0.8531) (Figure R-3.A. and Figure R-3.C.).

Overall, while RT was able to influence the morphological features of PCa tissue and impact tumor vasculature, its association with ET did not modify this response.

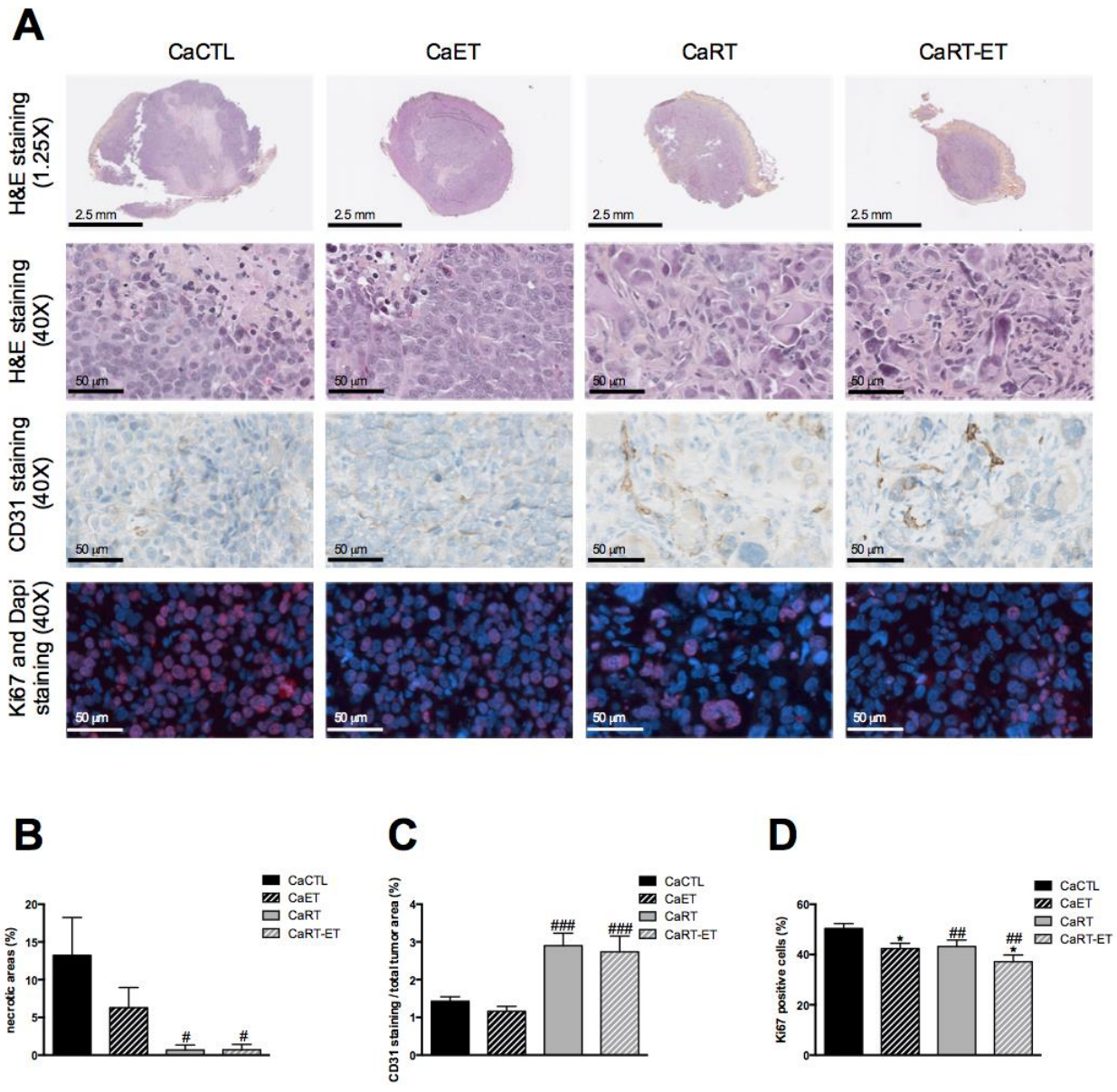

*Figure R-3: Tumor characteristics: immunohistological analysis. (A) Representative H&E staining at 1/25X and 20X, CD31 staining at 40X and Ki67 (red) with Dapi (blue) at 40X (B) necrotic areas quantification calculated as the percentage of necrotic areas compared to total tumor area (C) CD31 staining quantification and (D) Ki67 positive cells quantification. Data are represented as means ± SEM. ET main effect is represented by * p<0.05, ** p<0.01, *** p<0.001; RT main effect is represented by # p<0.05, ## p<0.01, ### p<0.001; interaction is represented by $ p<0.05, $$ p<0.01, $$$ p<0.001; Tukey post hoc tests performed when an interaction was found is represented by letters: a compared to CaCTL, b compared to CaET, c compared to CaRT.*

ET combined with RT potentiates apoptosis

Tumor cell proliferation was assessed using a co-staining for DAPI (in blue) and Ki67 (in red), a commonly used nuclear marker of proliferation. The Ki67 staining was decreased in the tumors of all three experimental groups: 50.36 ± 1.95% of CaCTL cells were Ki67 positive compared to 42.35 ± 2.10% for CaET, 43.21 ± 2.58% for CaRT and 37.19 ± 2.65% for CaRT-ET (ET main effect p=0.0062; RT main effect p=0.0144). The combination of the two strategies did not potentiate this effect (interaction p=0.6727) (Figure R-3.A. and Figure R-3.D.)

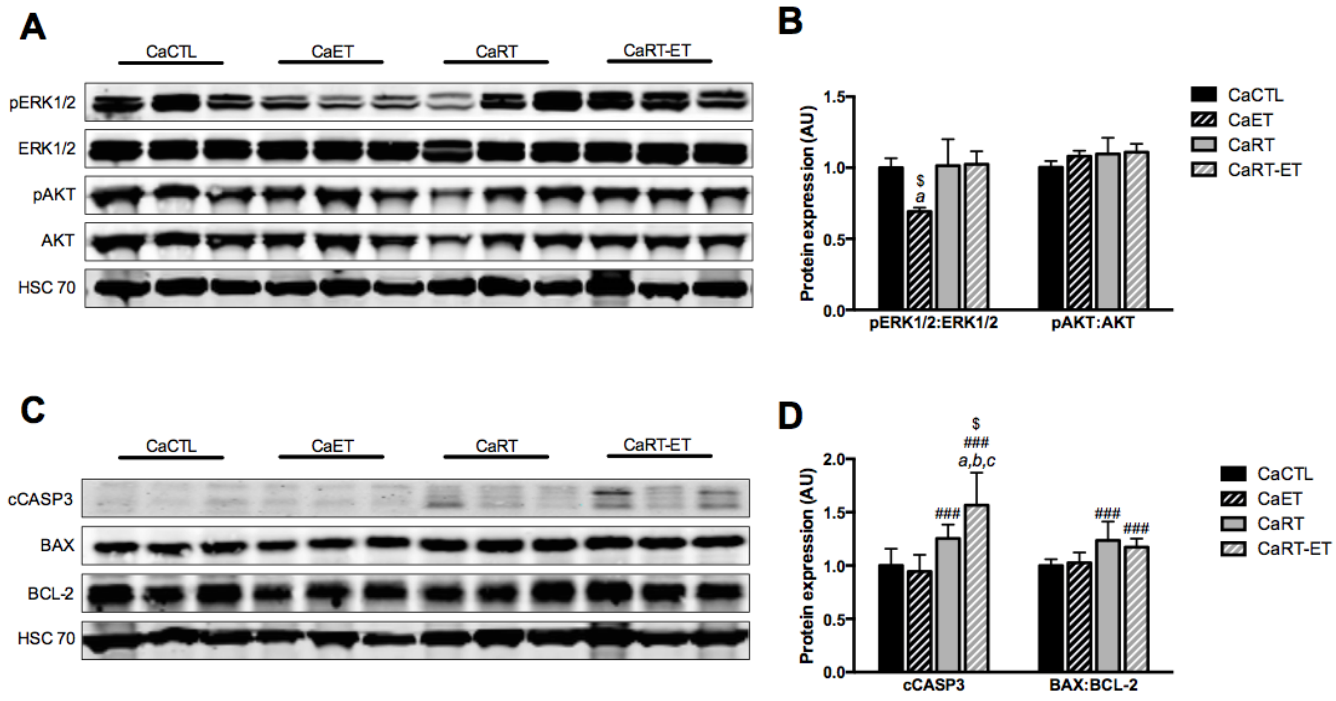

*Figure R-4: Western blots for cellular signaling in the tumor tissue. (A) representative blots and (B) blot quantification for tumoral pERK1/2, ERK1/2, pAKT, AKT, and HSC70 (C) Representative blots and (D) blot quantification for tumoral cleaved caspase-3 (cCASP3), BAX, BCL-2 and HSC70. Data are represented as means ± SEM. ET main effect is represented by * p<0.05, ** p<0.01, *** p<0.001; RT main effect is represented by # p<0.05, ## p<0.01, ### p<0.001; interaction is represented by $ p<0.05, $$ p<0.01, $$$ p<0.001; Tukey post hoc tests performed when an interaction was found is represented by letters: a compared to CaCTL, b compared to CaET, c compared to CaRT.*

At the molecular level, our findings indicate that AKT was activated in the prostate cancer tissue from CaCTL group and this activation was not modulated by the various treatments. The activation of ERK1/2 however significantly decreased in response to ET by 31% (Tukey post hoc p=0.0489), as previously observed by our team (26). Interestingly, RT did not alter the phosphorylation of ERK1/2, even when combined with ET (ET main effect p=0.3266; RT main

effect p=0.1309; interaction p=0.0126) (Figure R-4.A. and Figure R-4.B.). These results suggest that ET alone does not activate the same molecular mechanisms as when combined with RT. Overall, the decreased cell proliferation observed in response to ET might be a reflection of a reduced ERK1/2 activation, which could further explain, at least partly, the reduction in tumor growth observed with ET alone. RT also appears to decrease tumor cell proliferation, but in a ERK1/2-independent manner.

RT significantly up-regulated the apoptotic effector cleaved caspase-3 by 26% (RT main effect p<0.0001) while ET alone did not have any effect on this marker (ET main effect p=0.114; Tukey post hoc p=0.9483). Similarly ET did not impact BAX:BCL-2 protein ratio, while it was significantly increased with RT (ET main effect p=0.6522; ET main effect p<0.0001). Importantly, the up-regulation of cleaved caspase-3 expression was significantly higher (by 25%) when RT was combined with ET (interaction p=0.0243; Tukey post hoc p=0.0446). The combination of both strategies however did not potentiate the increase in BAX:BCL-2 protein ratio (interaction p=0.2766) (Figure R-4.C. and Figure R-4.D.).

Our data showed for the first time that ET potentiates RT response, through an increase in caspase-3 activation.

ET potentiates RT-induced NK cells infiltration within tumor tissue

In order to decipher the mechanisms explaining how ET could improve RT efficiency, several markers of the innate immune system were analyzed in the tumor tissue. RT has long been considered as immunosuppressive, but recent data has challenged this idea, suggesting that it could also be immunostimulatory, depending on the modalities of treatment delivery (dose, fractionation, and targeting site) (40, 41). The H&E staining performed here suggested that in both the peri- and intra-tumor sections, RT combined with ET appeared to show a greater immune cell infiltration (shown by arrows) (Figure R-5.A. and Figure R-5.B.).

NK cells infiltration within tumor tissue has been correlated with improved prognosis (42, 43) and their implication in exercise benefits against cancer has recently been highlighted (44). To investigate more precisely the implication of NK cells in the enhanced RT response with ET, immunohistological staining for NK1.1 was performed (red staining on the images). NK1.1 is an activating receptor expressed on NK cells, and can be used as a marker for this cell type. In our study, NK1.1 expression was not influenced by ET, but was significantly increased with RT

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

(RT main effect p<0.001) by approximately 300%. Importantly, this effect was potentiated when combined with ET (interaction p=0.0234), with an increase of 124% when compared with CaRT mice (Tukey post hoc p=0.0168) (Figure R-5.A. and Figure R-5.C.).

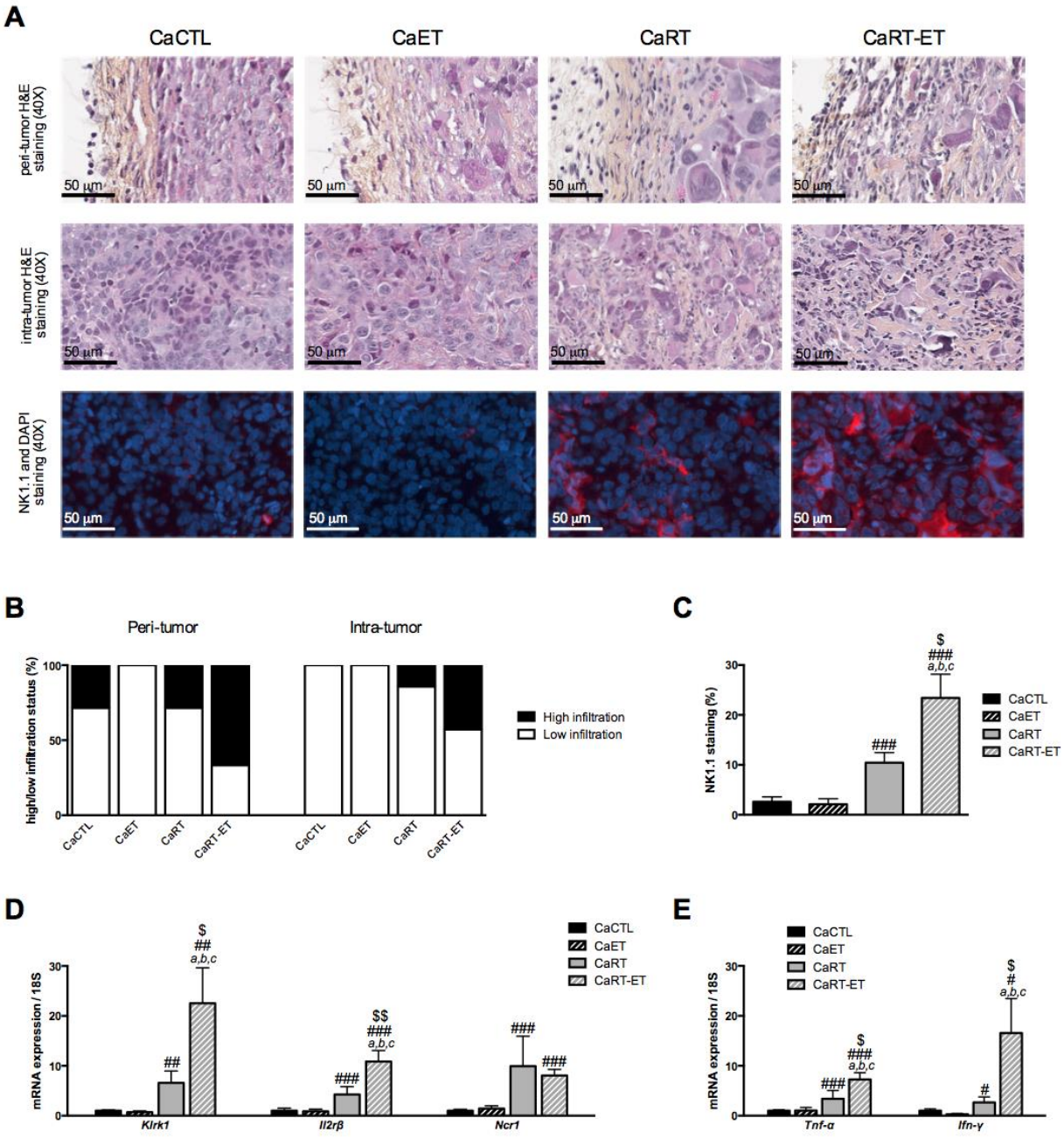

*Figure R-5: NK cell infiltration. (A) Representative H&E staining of the peri- and intra-tumor areas with immune cell infiltration evidenced by arrows; representative images for NK1.1 staining (B) Distribution of high and low immune cell infiltration status across experimental groups in the peri- and intra-tumor areas (C) NK1.1 staining quantification (D) mRNA expression for NK cell markers Klrk1, Il2rβ, Ncr1 relative to 18S (E) Tnf-α and Ifn-γ mRNA expression relative to 18S. Data are represented as means ± SEM. ET main effect is represented by * p<0.05, ** p<0.01, *** p<0.001; RT main effect is represented by # p<0.05, ## p<0.01, ### p<0.001; interaction is represented by $ p<0.05, $$ p<0.01, $$$ p<0.001; Tukey post hoc tests performed when an interaction was found is represented by letters: a compared to CaCTL, b compared to CaET, c compared to CaRT.*

At the transcriptional level, similar results were observed for *Klrk1* and *Il2rβ*, two genes coding for NK cell activating receptors. Both of these genes were not affected by ET alone while RT significantly increased their expression by 6.6-fold (RT main effect p=0.0026) and 4.3-fold (RT main effect p<0.0001) respectively. The mice combining RT and ET showed an even higher expression of these genes, with a 3.4-fold increase in *Klrk1* (interaction p=0.0495; Tukey post hoc p=0.0358) and a 3.4-fold increase in *Il2rβ* (interaction p=0.0078; Tukey post hoc p=0.0053) when compared to the CaRT group. *Ncr1* gene expression was also up-regulated with RT (RT main effect p<0.0001) but was however not further increased when combined with ET (interaction p=0.4489) (Figure R-5.D.).

NK cells can induce cell death directly by the release of granzymes and perforin but also indirectly through cytokine secretion, and notably TNF-α and IFN-ɣ (45, 46). While TNF-α expression can be modulated by numerous molecular pathways (47), IFN-ɣ is exclusively produced by immune cells, and notably by NK cells (48). Both *Tnf-α* and *Ifn-ɣ* gene expression within tumor tissue were not modified with ET alone, but were significantly higher in response to RT (RT main effect p<0.001 and p=0.0175 for *Tnf-α* and *Ifn-ɣ* respectively), this increase being significantly superior when RT was combined with ET (interaction p=0.0279 and Tukey post hoc p=0.0154 for *Tnf-α* ; interaction p=0.0458 and Tukey post hoc p=0.0451 for *Ifn-ɣ*) (Figure R-5.E.).

Overall, these results suggest that ET could enhance RT immunostimulatory effects by potentiating NK cell activation, resulting in enhanced cancer cell apoptosis thereby decreasing tumor growth.

DISCUSSION

Our study shows for the first time that, *in vivo*, ET can sensitize PCa cells to RT, resulting in improved RT efficiency. Both ET and RT performed alone decreased tumor growth, and their combination resulted in a potentiating effect at the molecular level rather than an additive one.

The forced treadmill running program used in our study did not induce an energy expenditure imbalance, as it was not associated with neither weight loss nor weight gain. Furthermore, the ET program appeared efficient, as it led to improved time to exhaustion in the endurance

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

test performed at the end of the protocol despite a relative short duration (two weeks). Overall, these results are supportive of the feasibility of exercise in the oncologic setting and are in line with clinical trials showing that engaging in physical activity while undergoing RT is safe and achievable. Furthermore, patients exercising regularly had improved quality of life and physical functioning (12, 14, 15) which could be related to higher aerobic capacity.

In our study, ET was able to significantly decrease prostate tumor volume after two weeks of forced treadmill running. While the majority of pre-clinical studies have found similar beneficial effects of regular physical activity and/or ET on tumor growth in several cancer types (49), inconsistencies remain. In PCa more specifically, regular exercise was reported to slow tumor growth in five different studies (21–24, 26), this effect being borderline significant in one other study (25), while it did not decrease tumor volume in three other ones (27, 29, 50). McCullough et al. even observed a faster tumor growth in immunodeficient rats after five weeks of treadmill running in comparison to their sedentary counterparts (50). These conflicting findings may be explained by differences in cancer type (cell line injected; implantation process), murine model (rat or mice; transgenic, immunocompetent or immunodeficient) and exercise modalities (initiation; frequency, intensity, duration, type) (49).

Despite this divergence, exercise is increasingly recognized as a strategy able to modulate several major signaling pathways involved in carcinogenesis and cancer growth. In light of this increasing evidence, the potential for exercise to impact cancer treatment efficiency has emerged (31). In line with this questioning, our study showed for the first time that *in vivo*, ET was able to improve RT efficiency. Interestingly, while both ET alone and in combination with RT reduced tumor growth, the underlying molecular pathways mediating these effects differ. Fractionated RT influenced key morphological features of the tumor tissue and notably reduced necrotic areas, which are associated with cancer aggressiveness and poor prognosis in PCa (29). Furthermore, fractionated RT resulted in higher intra-tumor angiogenesis, an effect previously observed in both pre-clinical and clinical studies (51–54). However, ET did not impact angiogenesis. These results are in contrast with the findings from Jones *et al.* where voluntary wheel running improved tumor vascularization in an orthotopic model of PCa, while decreasing the expression of plasmatic angiogenic cytokines (27). The same group also showed that voluntary wheel running increased angiogenesis in breast tumors implants (34). This effect was however not negative as it improved chemotherapy efficiency through

vascular normalization and reduced intra-tumor hypoxia. Consequently, it has been hypothesized that modulation of angiogenesis by exercise could improve the RT response (31). In our model, ET did increase tumor sensitivity to RT, but this effect was not mediated by changes in intra-tumor angiogenesis. Differences in tumor model and/or exercise modality may explain these inconsistencies.

All three experimental groups (CaET, CaRT and CaRT-ET) decreased cancer cell proliferation compared to the CaCTL mice. We hypothesized that ET alone induced this effect, at least partly, through decreased activation of the ERK-MAPK pathway. These results are in line with previous studies showing that exercise could slow PCa growth by reducing cell proliferation (22, 24, 26) and lowering ERK activation (26). Jones *et al.* however found contrasting results and observed that 38 days of voluntary wheel running increased the phosphorylated expression of ERK1/2 and did not result in reduced prostate tumor growth (27). These inconsistent and conflicting results highlight the necessity to clearly determine if variations in exercise modalities (e.g. exercise type and initiation) and/or cancer models could have different effects on prostate tumor growth.

The mechanisms responsible for the reduced phosphorylation of ERK1/2 in response to ET alone was not investigated in our study, but several hypotheses can be proposed. Growth factors (such as EGF and IGF-1) and tyrosine kinase receptors (such as EGFR or IGF-1R) are known to activate the ERK cascade when bound (55, 56). Interestingly, ET has been shown to decrease systemic levels of IGF-1 in both pre-clinical and clinical breast cancer studies (57–59) as well as in PCa patients (60), which could inhibit the ERK-MAPK pathway. Another plausible mediating this effect is the modulation of myokines by ET. It has been demonstrated that myokines such as irisin, OSM and SPARC have the ability to inhibit cancer growth *in vitro* and/or *in vivo* (61–63) and could possibly modulate ERK activation. Finally, micro-RNAs (miRNA) represent another hypothetical pathway through which ET counteracts ERK activation. Several miRNAs, such as miR-20a, miR-874 and miR-133a have been shown to regulate the phosphorylation of ERK (64–66). Interestingly, ET has the ability to modulate miRNA expression in the muscle (67), the blood (68) and the tumor (69), which could impact ERK activation in cancer cells.

The decrease in tumor growth observed in both CaRT and CaRT-ET groups may rather be a consequence of increased apoptosis. Interestingly, ET associated with RT showed higher levels of apoptosis compared to RT alone. This potentiation did not appear to be mediated by changes in the intrinsic apoptotic pathway but was rather the result of an increase in intra-tumor NK cell infiltration. Interestingly, ET alone did not enhance tumor cell apoptosis, which is in contrast with previously published findings in PCa (22, 24) and did not induce NK cell infiltration in the tumor tissue. Acute and chronic exercise are well known for influencing both the innate and adaptive branches of our immune system. As early as in 1893, Schulte reported a recruitment of lymphocytes within the blood stream in response to exercise (70). Since then, a general model for the regulation of circulating NK cells, T cells, and to a lower degree B cells, in response to exercise have been established (71, 72). The involvement of the immune system in exercise-mediated benefits against cancer has been suggested for more than 20 years (73) but was only recently confirmed. In an *in vivo* experiment, Pedersen *et al.* showed that voluntary wheel running inhibited tumor initiation and growth through the regulation of NK cell trafficking, mediated by epinephrine and IL-6 secretions (44). Several other mechanisms may also be implicated in this exercise response, as reviewed elsewhere (74). Modifications in other circulating factors (such as myokines) as well as improvements in the tumor vascularization with ET observed in several pre-clinical studies (27, 27, 28, 34, 50, 75) could increase NK cell infiltration and cytotoxicity (74, 31). Additionally, the increase in body temperature generated by an acute bout of exercise may also trigger NK cell anti-tumor activity as hyperthermia has been shown to enhance NK cell cytotoxicity against tumor cells (76) and improve RT efficiency (77). In our study, forced treadmill running alone did not provide such effects, while the association of ET with RT potentiated the NK cell infiltration induced by RT. These results could hence suggest that ET can potentiate the abscopal effect generated by RT and enhance treatment efficiency, as previously hypothesized (31).

Our study however contains several limitations, including the use of athymic mice, and ectotopic injection. Therefore, while engaging in regular exercise during RT may thus represent a strategy for improving RT outcomes, further studies are warranted to confirm these results.

CONCLUSIONS AND FUTURE DIRECTIONS

Overall, our study shows that (a) ET enhances RT effectiveness by potentiating NK cell activation and infiltration; and (b) while ET alone and ET combined with RT both reduce tumor growth, the mechanisms mediating these effects are different.

Therefore, despite our results suggesting a beneficial impact of ET on RT and thereby supporting the promotion of exercise for PCa patients, caution should be warranted. The current rationale for introducing exercise in cancer patients' health care is based on studies examining the impact of acute exercise or ET alone. However, some of the exercise-induced benefits against cancer may be lost when combined with treatment, while exercise can also impact treatment efficiency (here in a positive way) through biological pathways which do not respond to exercise alone. Therefore, additional studies investigating the potential interactions between exercise and treatments are warranted, notably in immunocompetent and orthotopic mouse models, in order to help healthcare practitioners in making the appropriate decisions when promoting exercise to cancer patients.

AUTHORS CONTRIBUTION

A. Rebillard and F. Paris designed research; S. Dufresne, J. Guéritat, A. Rebillard, S. Chiavassa, C. Noblet, M. Assi and L. Lefeuvre-Orfila performed research; S. Dufresne, J. Guéritat, A. Rebillard, N. Rioux-Leclercq and F. Rannou-Bekono analyzed data; S. Dufresne and A. Rebillard wrote the paper.

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II – Voluntary wheel running does not enhance radiotherapy efficiency in mouse bearing PC-3 tumors: towards a tailored approach to physical activity?

To provide a more in depth understanding of the relationship between physical activity and RT in PCa, we conducted a second study aiming to investigate whether voluntary wheel running could improve RT efficiency in mice bearing PC-3 prostate tumors.

Our team and others have published studies showing that physical activity may improve RT and/or immunotherapy efficiency through modulation of the immune system (Pedersen et al. 2016; Dufresne et al. 2020). Indeed, in the above-mentioned study, our group showed that exercise training was able to increase NK cell infiltration to the tumor tissue, resulting in higher apoptosis and enhanced RT response in a preclinical PCa model (Dufresne et al. 2020). Furthermore, in mice bearing 4T1 mammary tumors, Wennerberg *et al*. showed that exercise training led to slower tumor growth, and improved the response to PD-1 blockade combined with RT. This improvement was mediated by increased NK and T cell activation along with the offset of cancer-induced increase in immunosuppressive MDSC, resulting in a more favorable immune profile (Wennerberg et al. 2020). Interestingly, these two studies used treadmill running (TR) as the exercise training model and while this modality has advantages such as control of exercise timing, duration, and intensity, it also possesses weaknesses, notably by inducing high levels of stress to the animals (Moraska et al. 2000; Svensson et al. 2016). Voluntary wheel running (VWR) on the other hand, is a less stressful activity where rodents usually run in spurts with short intense running bouts interspersed by short breaks, in accordance with their circadian rhythms (De Bono et al. 2006). However, there is a lack of control over the duration and intensity of the running behavior, and subsequent heterogeneity in the total amount of physical activity achieved by the animals. Interestingly, the physiological impact induced by these two modalities may differ. For instance, a recent study compared TR (a classic protocol of 8 weeks) and VWR modalities in mice, and suggested differences in total energy expenditure as well as disparities in muscular adaptations (i.e., PGC-1α expression) (Kim et al. 2020). Furthermore, VWR might resemble, to some extent, to unsupervised physical activity in cancer patients. Hence, evaluating the impact of VWR, rather than TR, on radiotherapy efficiency may provide valuable information to better understand how physical activity can impact the RT treatment response. In addition, preclinical studies

have shown that VWR was able to slow down tumor growth in several cancer types, including PCa (Ashcraft et al. 2016). VWR even led to improved tumor vascularization and enhanced response to chemotherapy in a murine model of mammary carcinoma (Betof et al. 2015). Increased tumor perfusion in response to physical activity has indeed been proposed as a potential mechanism modulating the response to standard anticancer treatments, including RT (Ashcraft et al. 2019; Schumacher et al. 2020). The present study hence aimed to investigate whether VWR was able to improve tumor vascularization in a preclinical model of PCa cancer and enhance the RT response.

Mice were inoculated with PC-3 prostate cancer cells, and either remained sedentary or were submitted to VWR after tumor establishment. VWR did not impact tumor growth but significantly altered tumor vascularization. Indeed, tumor echography revealed that the divergence of flux was increased with VWR without modifying flux amplitude were observed. We subsequently evaluated whether these modifications induced by VWR were enough to improve the RT response in a similar cancer model. However, no effect of VWR was observed on tumor growth, even when combined with RT. These results are in contrasts with our previously published results showing slower tumor growth and improved RT efficiency in response to TR in mice bearing PPC-1 prostate tumors (Dufresne et al. 2020). These differences may be explained by differences in the physical activity paradigm (VWR *vs*. TR) or in the cancer model used (PC-3 *vs*. PPC-1 cancer cells). Hence, we further investigated whether a different physical activity paradigm could have resulted in different outcomes. Mice were inoculated with PC-3 prostate cancer cells and either (1) remained sedentary, (2) had access to a running wheel, or (3) completed a TR program. The results obtained suggest that neither TR did not significantly slower tumor growth. VWR, similar to what was observed in experiments #1 and #2, did not alter tumor growth. Additionally, we evaluated *in vitro* whether or not physical activity affected different prostate cancer cell lines differently. Conditioned media obtained from either sedentary men, or men playing golf regularly was used to treat cancer cell lines PC-3 and LNCaP cells *in vitro*. The results obtained showed that PC-3 remained unaltered by physical activity while LNCaP proliferation was inhibited when treated with serum from active men compared to sedentary men.

Overall, this study shows that VWR initiated after tumor formation did not alter PC-3 tumor growth *in vivo* despite an increase in the divergence of tumor flux and did not improve RT efficiency. We further showed in the same model that TR, similar to VWR, was not able to slow

down tumor growth. Interestingly, the evaluation of cancer cell proliferation in response to physical activity conditioned media *in vitro* showed that PC-3 cells were not responsive to physical activity, contrary to PPC-1 and LNCaP cells. Hence, the anti-cancer effects of physical activity in PCa appear to be dependent on both the physical activity paradigm used and the cancer cell line. Consequently, some exercise programs may be more or less beneficial for PCa patients depending on their modalities (e.g., more or less intense), and physical activity may lead to small or large decrease in mortality rates depending on the PCa subtypes.

Additional experiments will be conducted before submitting this article to a peer review journal.

Voluntary wheel running does not enhance radiotherapy efficiency in a PC-3 preclinical model: towards a tailored approach to physical activity?

Suzanne Dufresne[1], Richard Cindy[1], Luz Orfila[1], Gregory Delpont[2], Sophie Chiavassa[2], Jordan Guéritat[1], Brice Martin[1], François Paris[2], Jean-Michel Escoffre[3], Baudouin Denis de Senneville[4], Nathalie Rioux-Leclercq[5], Vincent Potiron[2], Amélie Rébillard[1]

1. M2S lab, EA 1274, Université Rennes 2, ENS Rennes, 35170 Bruz, France
2. CRCINA, INSERM, Université de Nantes, Université d'Angers, 44000 Nantes, France; Institut de Cancérologie de l'Ouest Centre René Gauducheau, 44805 Saint Herblain, France
3. UMR 1253, iBrain, INSERM, Université de Tours, Tours, France
4. UMR 5251, IMB Bordeaux, CNRS, Université de Bordeaux, Bordeaux, France
5. Department of Pathological Anatomy and Cytology, Université Rennes 1, 35000 Rennes, France

ABSTRACT

Improved tumor vascularization has been hypothesized to enhance the radiotherapy (RT) response in prostate cancer (PCa). Several preclinical models have shown that physical activity has the ability to modulate tumor blood flow but its impact on RT efficiency remains largely unknown. In our study, athymic mice were injected with PC-3 cells and either remained inactive or were housed with voluntary running wheels. Echography of the tumor revealed that despite no observed effect on tumor growth, voluntary wheel running (VWR) enhanced tumor perfusion. We thus evaluated whether VWR could increase RT efficiency in a similar PC-3 cancer model. Contrary to our hypothesis, no effect on the RT response was observed. These findings contrast with previously published results by our team and prompted us to evaluate (1) if a different physical activity modality (treadmill running) could slow down tumor growth and (2) whether the potential anti-neoplastic effects of physical activity are dependent on PCa type. Treadmill running did not significantly slow down tumor growth in mice bearing PC-3 tumors, similar to what was observed for VWR. Interestingly however, *in vitro* experiments suggested that while PC-3 cells were not responsive to chronic exercise-conditioned media, LNCaP had a significant reduction in their proliferation. Hence, the potential anti-cancer effects of physical activity may be dependent on PCa type. A better understanding of which PCa cell lines are sensitive to the anti-cancer effects of physical activity *in vitro* and *in vivo* may help to develop a personalized approach to physical activity in the clinical setting.

Key words: radiation therapy; physical activity; exercise training; prostate cancer; treatment

INTRODUCTION

Over the 20 years, the relationship between physical activity and survival in cancer patients has raised considerable interest (1). In a recent systematic review and meta-analysis, Friendereich *et al*. highlighted that engaging in regular physical activity resulted in lower all-cause and cancer-specific mortality across several cancer types (2). For instance, it has been estimated that prostate cancer (PCa) patients engaging in vigorous activity more than 3 hours per week reduced the risk of PCa death by 61% (3). In line with this, numerous preclinical studies investigated the potential anti-cancer effect of physical activity across a wide variety of cancer types, with the majority reporting reduced tumor initiation or multiplicity and slower tumor growth in active versus sedentary rodents (4). Importantly, these effects are likely to be dependent on the cancer type or subtype and the physical activity modality used (4). Nevertheless, several plausible biological mechanisms underpinning the benefits of physical activity on tumor growth have been identified and can be grouped into four main categories: (a) vascularization and blood perfusion, (b) immune function, (c) tumor metabolism and (d) muscle-to-cancer cross-talk (5). The identification of these mechanisms, as well as the gathering of epidemiological evidence showing improved survival rates in active *versus* inactive cancer patients, provides a rationale for the potential of physical activity to improve cancer treatment efficiency (6). In particular, exercise may enhance the delivery of various treatments through increased tumor perfusion and reduced hypoxia. Indeed, two preclinical studies reported that improved tumor vascularization in response to regular physical activity sensitized tumor cells to chemotherapeutic agents in models of breast cancer (7) and pancreatic ductal adenocarcinoma (8). Furthermore, physical activity has been shown to improve immunotherapy and/or radiotherapy (RT) efficiency through modulation of the immune system in preclinical models of breast and prostate cancers (9,10). However, the potential implication of tumor vascularization in these effects remains unknown. In this study, we primarily evaluated whether voluntary wheel running (VWR) could improve tumor perfusion and modulate radiotherapy efficiency in a murine model of PCa. We secondly assessed whether physical activity benefits of PCa growth were dependent on PCa subtype and physical activity modality.

MATERIALS AND METHODS

In vivo experiments

Animals and tumor model

The use and care of animals followed the guidelines of the French Agriculture Ministry. Mice were maintained on a 12:12 hours dark-light cycle at the animal facility of the M2S laboratory (n°A3504734). Experiments were conducted in accordance with the ethical standards of the European Community (directive 86/609/EEC). A total of 110 male nude athymic mice (Janvier Labs, Le Genest-Saint-Isle, France) were housed in standard laboratory conditions, with a temperature- and humidity-controlled environment. Their body weight was monitored throughout the entire study. 101 of these mice received a subcutaneous injection of $2.5x10^6$ human PCa PC-3 cells and the 9 remaining mice were injected with PBS. The PC-3 cells were either purchased from Sigma-Aldrich (Sigma Aldrich, St Louis, MO, USA) experiments #1 and #2) for experiments #1 and #2 or from ATCC (ATCC, Manassas, Virginia, USA) for experiments #3. The PC-3 cancer cells were maintained in a humidified atmosphere at 37°C; 5% CO2 and cultured in either Coons Modified Ham's F12 or RPMI 1640 medium supplemented with 10% FBS and 0.1% penicillin/streptomycin (Gibco, Invitrogen, Waltham, MA, USA).

Either 25 days (experiments #1 and experiments #2) or 14 days (experiments #3) after injection, mice were randomized into the different experimental groups described below. Approximately 5% of the mice injected with PC-3 cells did not develop tumors or had uncharacteristic tumor size/shape and were withdrawn from the study. Tumor volume was assessed regularly throughout the protocol with sliding calipers. The formula used to calculate tumor volume was: length x width x height x 0.5236 (in mm^3).

At the end of the protocols, mice were anesthetized by intraperitoneal injection of ketamine (50mg/kg) and xylazine (4mg/kg) before being euthanized.

Several tissues including tumor and muscles were retrieved and washed in PBS. Muscles were snap frozen in liquid nitrogen or embedded in OCT before being immersed in pre-cooled isopentane. Tumors were separated in two in their center, with half being frozen in liquid nitrogen and the other half being embedded in OCT prior to being immersed in cooled isopentane. All of these samples were stored at -80°C.

Experimental groups in experiments #1: influence of VWR on tumor perfusion and tumor growth in a PC-3 murine model

In this experiment, mice bearing PC-3 tumors were randomized to either inactive control (CTL mice) or to the active group (VWR mice) 25 days after tumor cell injection. Additionally, one group of healthy mice was included and remained inactive. Healthy mice (n=9) and mice in the CTL group (n=8) did not follow any specific intervention while mice in the VWR group (n=7) had access to a running wheel all day, every day. All mice were housed individually. VWR activity was recorded daily and non-running mice were excluded from the study. 50 days after PC-3 inoculation, all the mice were euthanized.

Experimental groups in experiments #2: influence of VWR on RT efficiency in a PC-3 murine model

In this set of experiments, mice with PC-3 prostate tumors were divided into four experimental groups 25 days after tumor cell inoculation: inactive control mice (CTL group); active mice (VWR group); mice treated with RT (RT group); and mice combining RT treatment with VWR (RT-VWR group). Similar to experiment #1, all mice were housed individually. CTL mice (n=9) did not follow any specific intervention and mice in the VWR group (n=8) had access to a running wheel all day every day of the week (similar to experiments #1). Mice in the RT group (n=17) received a total of 20 Grays (Gy) localized to the tumor site and split into four doses of 5 Gy (XRAD 225Cx, PXI, CRCNA, Nantes, France) as previously described (10). These doses were distributed on days 30, 34, 37 and 41. Finally, mice in the RT-VWR group (n=17) had access to a VWR all day, every day, and received the same RT treatment as the RT mice. VWR activity was recorded daily and non-running mice were excluded from the study. 50 days after PC-3 inoculation (Sacrifice T1), all the mice from the CTL and VWR groups were euthanized. 9 mice and 9 mice from the RT and RT-VWR groups, respectively, were also euthanized. 8 RT mice and 8 RT-VWR mice were kept for a longer time until their tumors reached ~600 mm^3 after the RT treatment. This allowed for the mice to run for a longer period of time and to assess whether VWR could influence the tumor growth delay (TGD) following RT (TGD sacrifices).

Experimental groups in experiments #3: influence of VWR versus TR in PC-3 xenografts

Mice with PC-3 tumors were randomized into three different experimental groups 14 days after tumor cell injection: inactive control mice (CTL group), active mice (VWR group) and exercise-trained mice (TR). Similar to experiments #1 and #2, all mice were housed individually. CTL mice (n=9) did not follow any specific intervention and mice in the VWR group (n=7) had access to a running wheel all day every day of the week (similar to experiments #1 and #2). TR mice (n=9) underwent TR five days per week, with duration and intensity gradually increased to reach at 18m/min for 60 minutes with a 10% incline on day 22. For the final 9 sessions, duration was reduced to 45 minutes per day as the tumor grew larger. VWR activity was recorded daily and non-running mice were excluded from the study. 65 days after PC-3 inoculation, all the mice were euthanized.

Assessment of tumor perfusion

Four representative mice in the CTL and VWR were selected for the assessment of tumor perfusion. Under anesthesia, a 3D B-mode ultrasound imaging was performed to determine tumor volume. Contrasting agents were then injected intravenously and video clips were recorded during 36 s at a 10 images/s frequency in order to evaluate tumor perfusion par contrast-enhanced ultrasound. Following image acquisition, tumor perfusion was quantitatively analyzed with the Vevo CQ software based on the raw data.

Western blot analysis

Tissue samples were lysed in buffer (50 mM Tris-HCl, 10 mM EDTA, 1% SDS) and protein concentration was determined using a Lowry protein assay. Proteins (50 µg) were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After being blocked with 5% BSA or nonfat dry milk in TBS-Tween (0.05%), membranes were incubated overnight at 4°C with the appropriate primary antibodies (Table R-3). After being washed three times with TBS-Tween (0.05%), membranes were incubated with secondary antibodies for 1 hour at room temperature. Immunoreactive bands were visualized with Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA). Protein loading was normalized to HSC70 expression.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

Table R-3: List of western blot antibodies

Protein	Molecular weight	Reference	Dilution	Source
Cleaved caspase-3	17, 19 kDa	Cell signaling 9661	1/1000	Rabbit
BAX	20 kDa	Cell signaling 2772	1/1000	Rabbit
P21	21 kDa	Cell Signaling 2947	1/1000	Rabbit
P27	27 kDa	Cell Signaling 2552	1/1000	Rabbit
BCL-2	28 kDa	Abcam ab7973	1/1000	Rabbit
p-ERK1/2	42, 44 kDa	Cell signaling 4376	1/1000	Rabbit
ERK1/2	42, 44 kDa	Santa Cruz sc-514302	1/1000	Rabbit
p-AKT	60 kDa	Cell signaling 9271	1/1000	Rabbit
AKT	60 kDa	Cell signaling 9272	1/1000	Rabbit
HSC70	70 kDa	Santa Cruz sc-7298	1/5000	Mouse

Immunohistological analysis

Half of each tumor was embedded in OCT prior to being immersed in cooled isopentane and stored at -80°C. Tissue sections of 8µm thickness were obtained using the LEICA CM3050S cryostat and mounted on glass slides.

Tumor sections were stained with Hematoxylin and Eosin (H&E) which colors the nucleus in dark purple and the cytoplasm in a lighter pink shade. This enables for the evaluation of necrotic areas. Necrotic areas were defined on the H&E slides by the absence of tumor cell nuclei and were expressed as necrotic area relative to total tumor area.

Immunohistological staining for Ki67 were performed on the Discovery XT Automated IHC stainer using the Ventana detection kit (Ventana Medical Systems, Tucson, AZ, USA). Antigen retrieval was performed with a Tris-based buffer solution pH8 (Ventana Medical Systems, Tucson, AZ, USA) at 95-100°C for 40 minutes. Endogen peroxidase was blocked with 3% H_2O_2 for 12 minutes. After rinsing, slides were incubated at 37°C for 60 minutes with the primary antibody for Ki67 (NB600-1252, Novus Biologicals, Centennial, CO, USA; dilution at 1/400). Signal enhancement for Ki67 was performed using a goat anti-rabbit at 37°C for 16 minutes and Discovery Rhodamine Kit (542-568 nm) for 8 minutes (Ventana Medical Systems, Tucson, AZ, USA). To visualize the nucleus, DAPI staining was added and cover-slipped. Ki67 was analyzed with ImageJ software and reported as the percentage of Ki67 positive cells (in red) relative to the total number of cells (DAPI staining, in blue).

In vitro experiments (experiments #4):

Volunteer recruitment

Ten volunteer men older than 50 years of age were enrolled in these experiments after signing an informed consent sheet. They were asked to complete the physical activity questionnaire for the elderly developed by Voorrips *et al*. (11), and were divided into two groups according to their answers: inactive (n=5) and active (n=5). Fasting blood samples were drawn from the participants in the morning. Half of each blood sample was sent to a medical analysis laboratory, and the other half was centrifuged after clotting to obtain serum and stored at -80°C.

Cell culture

LNCaP and PC-3 cells (ATCC, Manassas, Virginia, USA) were grown in RPMI 1640 medium with phenol red, supplemented with 10% FBS, 1 mM sodium pyruvate and 4 mM L-glutamine (Gibco, Invitrogen, Waltham, MA, USA) in humidified incubator maintained at 37°C in 5% CO_2. Cells were collected using 0.25% trypsin-EDTA (Gibco, Invitrogen, Waltham, MA, USA).

Proliferation assay

Methylene Blue assays were performed in 96-well culture plates: LNCaP and PC3 cells ($3x10^3$) were plated in each well containing 100 µl of RPMI 1640 supplemented with 10% FBS. After 48h, medium was replaced either by with serum-free RPMI 1640 supplemented either with 10% FBS or 10% of serum obtained from volunteer men. 48h after, culture medium was removed, and cells were fixed with 70% ethanol. The number of cells was determined by adding 100 µl of Methylene Blue per well. The plates were rinsed three times in water and air-dried overnight. The cells were then incubated for 15 min at room temperature with 100µl of eluting solution (0.1 N HCl) per well in order to release the stain from the cells. Following 10 min of low shaking, the absorbance at 620 nm was determined using a microwell plate reader Synergy HT (BioTeck).

Statistical analyses

Tumor growth was analyzed with statistical parametring mapping using the open-source SPM1D package (v.M0.4.www.spm1d.org) in Matlab (R2018b, Mathworks Inc., Natick, MA, USA). All other statistical analyses were performed in Prism6 (GraphPad softwares, San Diego, CA, USA). After testing for normality, the results for experiments #1 were analyzed using either t-test or Mann-Whitney test when two groups were compared. When three groups were compared, a one-way ANOVA or Kruskal-Wallis test was used, depending on whether the data followed a normal distribution or not, followed by Tukey or Dunn's post hoc tests, respectively. In experiments #2, the data was analyzed using a two-way ANOVA with the two main variables being (a) ET and (b) RT. If the two-way ANOVA showed a significant interaction, the main effects were analyzed, and Tukey post hoc tests were performed. In the experiments #3 and #4, the results were analyzed either with one-way ANOVA or Kruskal-Wallis test, depending on whether the data followed a normal distribution or not. If the one-way ANOVA or the Kruskal-Wallis test were significant, Tukey or Dunn's post hoc tests were performed, respectively. Comparisons were considered significant for p values below 0.05. Data are represented as means ± SEM.

RESULTS

Experiments #1: influence of VWR on tumor perfusion and tumor growth in a PC-3 murine model

The study design for these experiments in depicted in Figure R-6.A.

VWR activity and variations in body weight

In this experiment, PC-3 mice ran on average 3.13±0.68 km/day (Figure R-6.B.). This VWR activity did not result in weight loss nor weight gain compared to PC-3 inactive control mice. However, both inactive and active PC-3 bearing mice exhibit lower body weight compared to healthy mice (Figure R-6.C. and Figure R-6.D.)

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

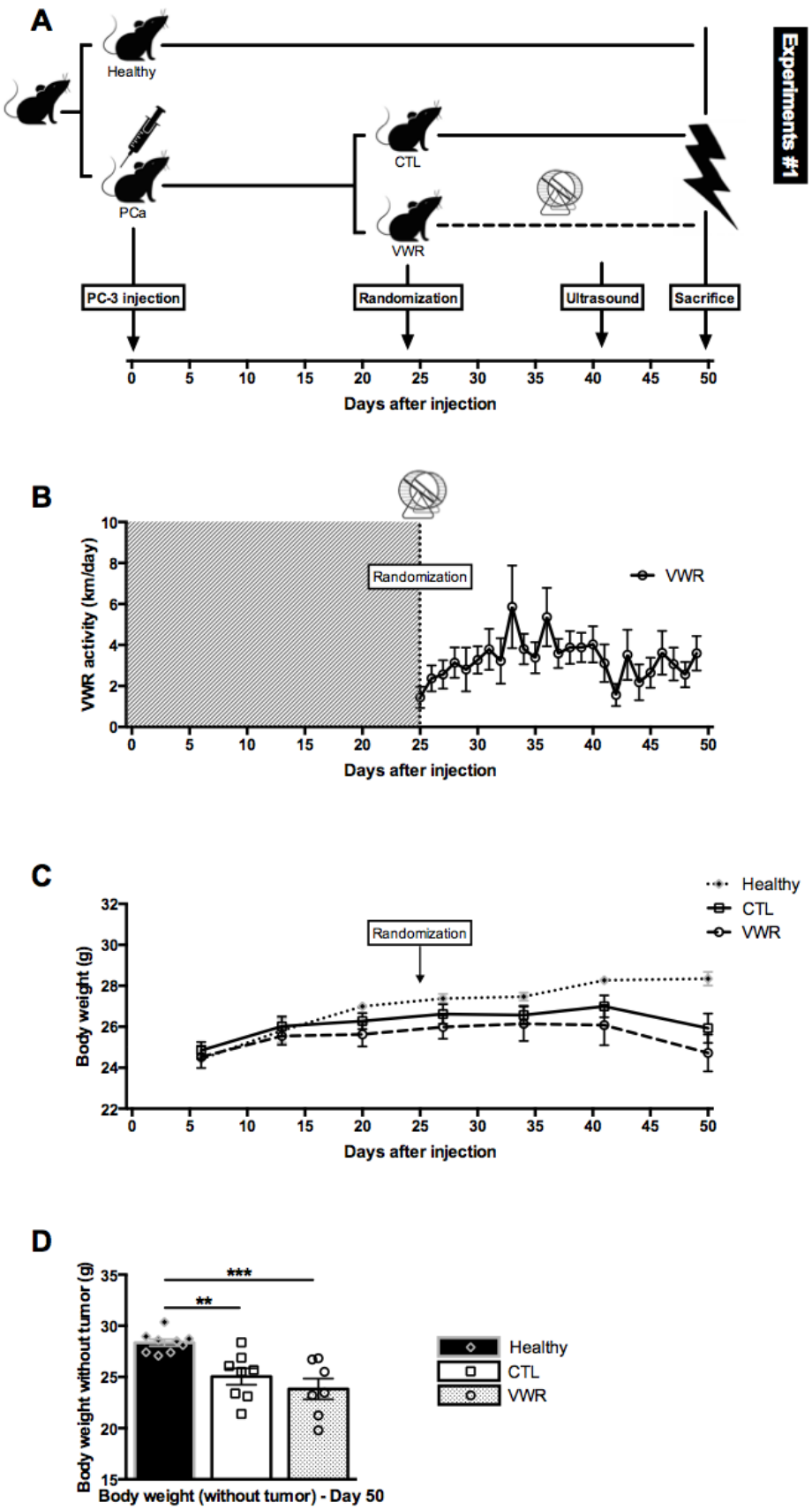

*Figure R-6: Study design and characteristics - experiments #1 A. Study design B. VWR activity over time from day 24 (randomization) onwards; C. Body weight evolution; D. Body weight without the tumor weight on the last day of the experiment (day 50). Data are represented as means ± SEM. ** p<0.01; ***p<0.001*

VWR improves tumor perfusion

Tumor perfusion was assessed through dynamic contrast agent enhanced ultrasound 41 days after tumor cell injection with gas-filled microbubble contrast agents used as intravascular flow tracers. Both flux amplitude and flux divergence were analyzed. Flux amplitude corresponds to tumor blood flow velocity, while flux divergence (i.e., sources, sinks) represents the directions of tumor blood flow (centrifugal and centripetal trajectories). The results obtained showed that flux amplitude was unaffected by VWR (Figure R-7.A.) while flux divergence was significantly increased with VWR ($p<0.05$; Figure R-7.B.).

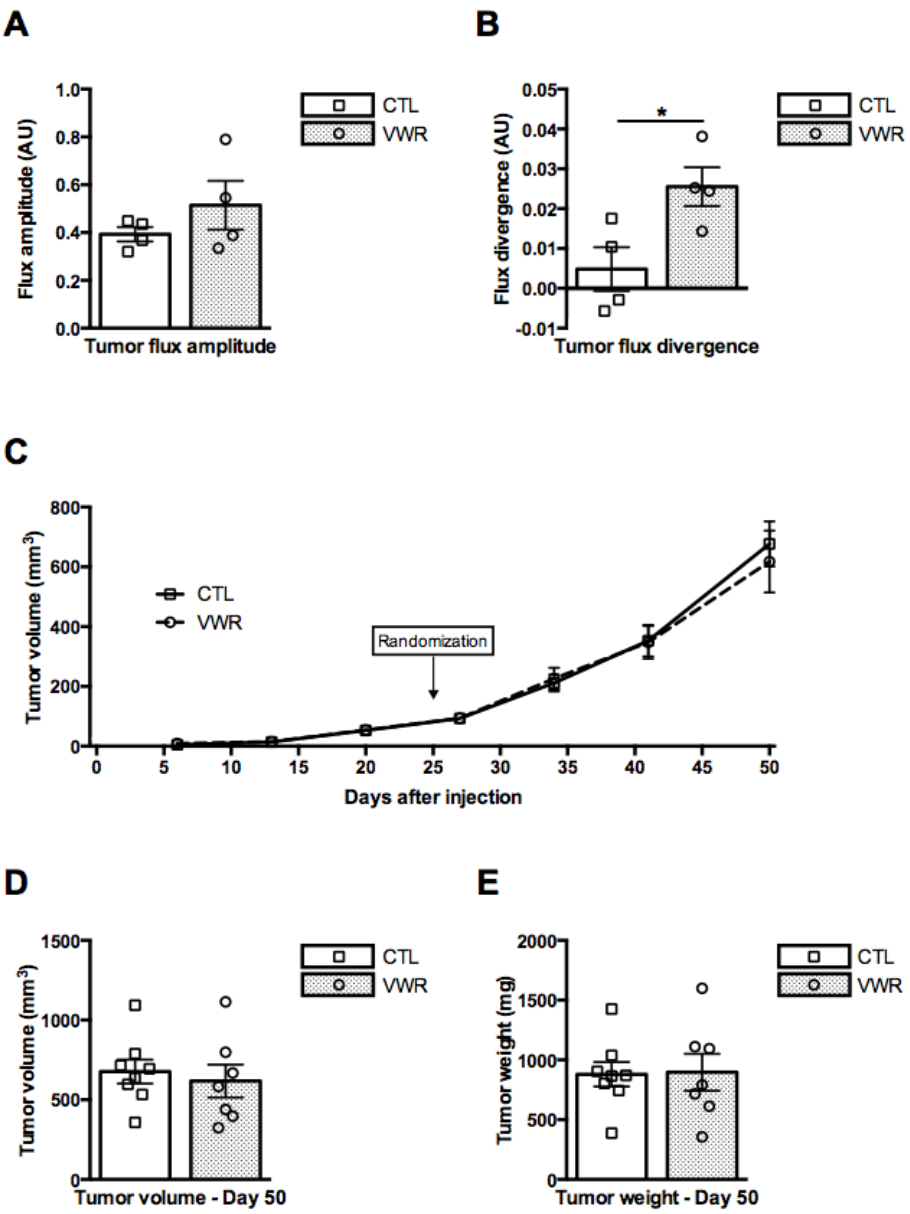

*Figure R-7: Tumor perfusion and tumor growth. A. Tumor flux amplitude and B. tumor flux divergence measured by dynamic contrast agent ultrasound 41 days after tumor cell injection; C. Tumor volume evolution; D. tumor volume and E. tumor weight measured on day 50 at sacrifice. Data are represented as means ± SEM. * p<0.05*

VWR does not impact tumor growth in PC-3 tumor xenografts

Enhanced tumor perfusion in response to physical activity has been suggested to slow down tumor growth (Zhang et al. 2019). In our study, VWR was not able to slow down tumor growth (Figure R-7.C.). On the last day of the experiment (day 50) average tumor volume was 677.20±75.31mm^3 in CTL mice and their tumors weighed 879.8±102.6 mg. VWR had tumors measuring 620.30±89.69mm^3 and weighing 870.9±135.9 mg on average, with no notable difference compared to the CTL group (Figure R-7.D. and Figure R-7.E.).

Experiments #2: influence of VWR on RT efficiency in a PC-3 murine model

Enhanced tumor perfusion by physical activity has been hypothesized to enhance RT efficiency (12) and this second set of experiments aimed to evaluate whether VWR could provide such effects. The study design for experiments #2 is depicted in Figure R-8.A.

VWR does not affect RT efficiency

Similar to experiment #1, VWR alone did not impact tumor growth, while RT, as expected, decreased it (VWR main effect $p>0.05$; RT main effect $p<0.001$; Figure R-8.B.). The combination of RT with VWR did not influence RT efficiency, even when mice kept the running wheel for an additional 23-51 days until tumor volume reached ~600 mm^3. In line with this, tumor volume and tumor weight on day 50 was similar between CTL and VWR mice, with tumors measuring 691.2±111.0 mm^3 and weighing 860.3±115.5 mg on average in the CTL group and 684.0±76.70 mm^3 and 801.7±63.44 mg in the VWR group. RT and RT-VWR mice had significantly smaller and lighter tumors on day 50 compared to CTL mice with tumors averaging 171.6±19.03 mm^3 and 214.1±25.01 mg in the RT group and 177.4±26.51mm^3 and 204.7±28.75mg in the RT-VWR (VWR main effect $p>0.05$; RT main effect $p<0.001$ for both tumor volume and tumor weight; Figure R-8.C., Figure R-8.D., and Figure R-8.E.). In mice kept for an extra 23-51 days to observe the tumor relapse, the time for the tumor to reach 600 mm^3 after RT cessation (tumor growth delay) was similar between RT and RT-VWR mice, both averaging ~93 days after injection (Figure R-8.F.). Overall, VWR had no effect on tumor growth whether performed alone or when combined with RT.

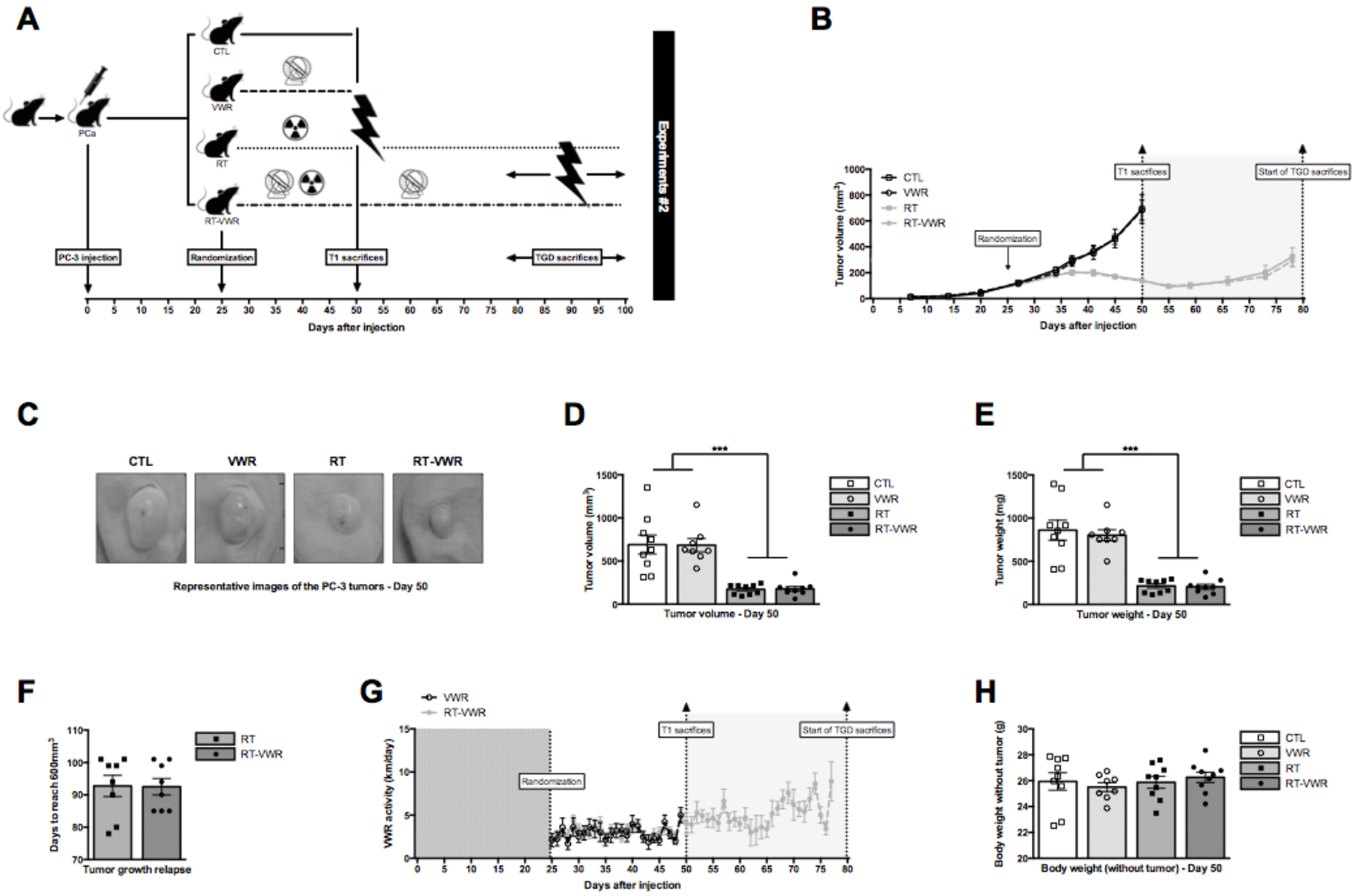

*Figure R-8: Study design and characteristics - experiments #2. A. Study design; B. Evolution of tumor volume over time; C. Representative pictures of the tumors in mice euthanized on day 50; D. Tumor volume and E. tumor weight of mice euthanized day 50; F. Days for tumors to reach ~600 mm3 (tumor growth delay) in RT and RT-VWR mice not sacrificed on day 50; G. VWR activity of VWR and RT-VWR over time; H. Body weight of mice sacrificed on day 50. Data are represented as means ± SEM. ***p<0.001*

VWR activity is not affected by RT and does not result in weight loss

To evaluate whether the lack of effect of VWR on RT efficiency could be attributable to lower VWR activity in RT mice, the distance run by each mouse was recorded daily. On average, VWR mice ran 2.92±0.55km/day and RT-VWR mice 3.32±0.40km/day (Figure R-8.G.), with no significant differences observed between these two groups (Figure R-8.G.). Hence RT did not impact VWR activity. Furthermore, neither VWR (as observed in experiments #1) nor RT resulted in weight loss or weight gain compared to inactive CTL mice (Figure R-8.H.).

VWR does not affect tumor cell proliferation and necrosis; RT lowers the number of proliferative cells and reduces necrotic areas in the tumor tissue, regardless of VWR

Even though prostate tumors did not differ in volume or weight, we evaluated whether VWR alone or combined with RT could alter tumor physiology and signaling pathways. H&E staining and Ki67/Dapi co-staining were performed on tumor sections of mice sacrificed at T1. The images revealed that VWR did not affect Ki67 index while it was reduced in both RT and RT-VWR mice by 39% and 51%, respectively, with no additional effect of VWR observed (VWR main effect >0.05; RT main effect $p<0.001$; Figure R-9.A. and Figure R-9.B.). Furthermore, the percentage of tumor necrotic areas was similar between CTL and VWR mice (representing 4.44% and 3.75% of the total tumor area in CTL VWR mice respectively), while they were completely non-existent in both RT and RT-VWR groups (ET main effect $p>0.05$; RT main effect $p<0.05$; Figure R-9.A. and Figure R-9.C.). Hence, only RT reduced cancer cell proliferation and decreased necrosis in tumors, regardless of VWR.

RT but not VWR is effective at remodeling tumor vasculature

Tumor vascularization is dependent on the functionality but also on the morphology of tumor vessels. In experiments #1, we observed improved tumor vascularization with VWR, but tumor morphology was not evaluated. To evaluate whether VWR, RT and their combination could remodel the tumor vasculature, immunostaining of pericyte markers α-smooth-muscle-actin (α-SMA) and desmin were performed in the tumor tissue, and vessel density was measured. Neither of these parameters were affected by VWR, while RT resulted in a significant increase in α-SMA (RT main effect $p<0.05$; Figure R-9.D.) and a borderline increase in desmin (RT main effect $p=0.0508$; Figure R-9.E.). Vessel density was similar across all groups (Figure R-9.F.).

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

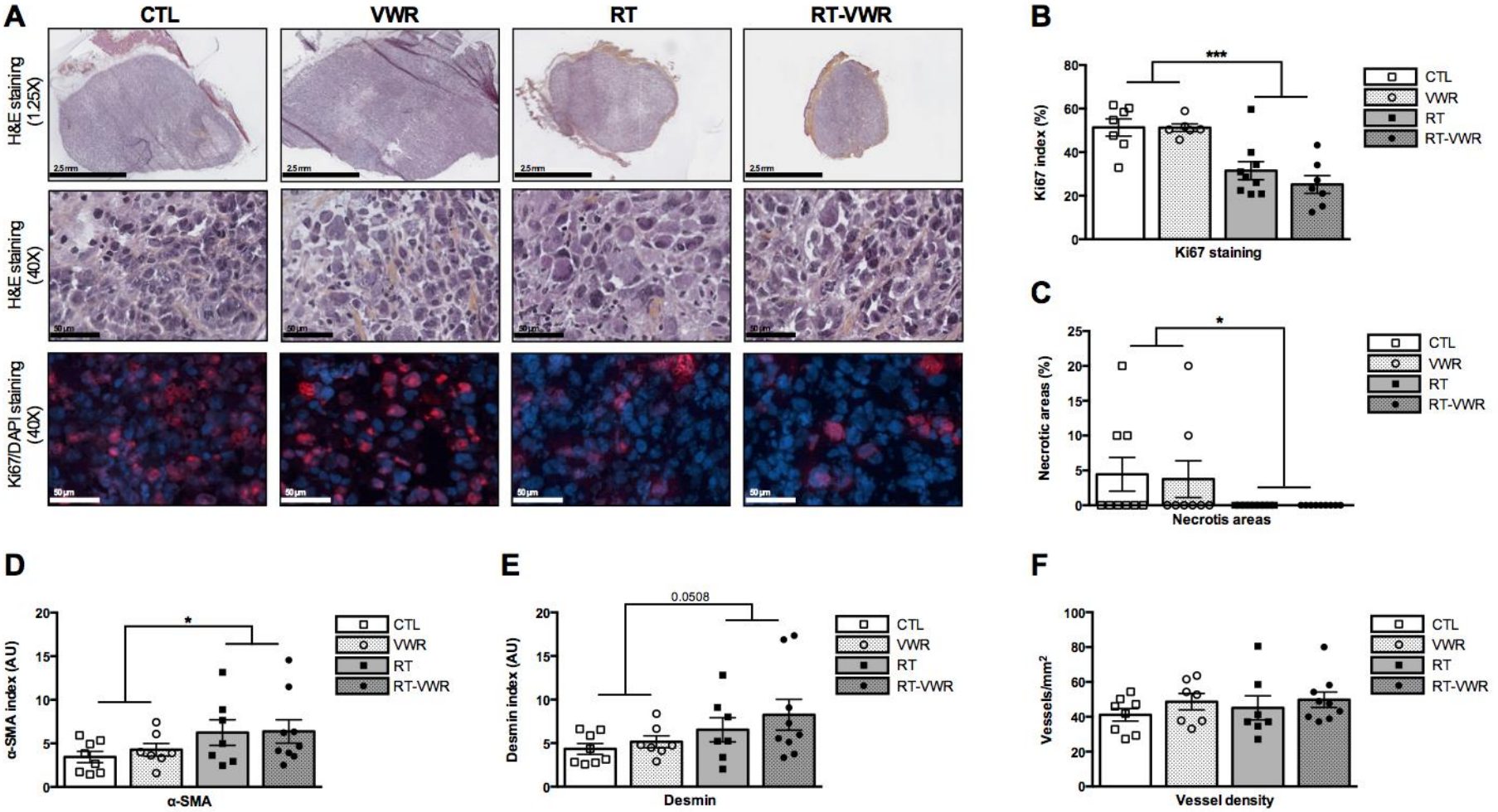

*Figure R-9: Tumor immunohistological analyses. A. Representative immunohistological images of H&E and Ki67 staining; B. Quantification of C. Ki67 index; D. α-SMA index; E. Desmin index and F. Vessel density Data are represented as means ± SEM. * p<0.05; ***p<0.001*

Hence, only RT appeared to remodel tumor vasculature by increasing tumor pericyte coverage, while VWR did not provide additional effects. The number of vessels remained unchanged in response to either VWR or RT. Hence, while RT remodeled tumor vasculature, VWR did not. The improved tumor vascularization measured in experiments #1 are therefore likely a result of improvements in tumor vessel functionality rather than changes in their morphology.

pERK1/2:ERK1/2 protein ratio remained unaltered across all three intervention groups while pAKT:AKT protein ratio was significantly decreased with RT

At the molecular level, activation of ERK1/2 and AKT, key players in proliferative and survival pathways respectively, were evaluated. pERK1/2:ERK1/2 protein ratio was similar across all experimental groups. The pAKT:AKT protein ratio remained unchanged with VWR but was significantly reduced by 42% in both RT and RT-VWR mice. VWR combined with RT did not provide any additional effect compared to RT alone (VWR main effect $p>0.05$; RT main effect $p<0.001$; Figure R-10.A and Figure R-10.B.). Hence, neither VWR nor RT was able to modify the activation of ERK1/2, but RT significantly decreased AKT activation.

VWR alone or in combination with RT does not affect apoptosis in the tumor tissue; RT increases apoptotic markers cCASP3 and BAX:BCL-2 protein ratio

Apoptotic effector cCASP3 protein expression remained unchanged with VWR but was significantly increased with RT by 40%. VWR combined with RT resulted in a similar increase (34%) compared to RT alone (VWR main effect $p>0.05$; RT main effect $p<0.01$). Similarly, BAX:BCL-2 protein ratio was not affected by VWR but increased in both RT groups (by 96% and 100% in RT and RT-VWR mice, respectively), with no added benefits from VWR (VWR main effect $p>0.05$; RT main effect $p<0.001$; Figure R-10.C. and Figure R-10.D.). Hence, VWR did not result in increased apoptosis, and did not enhance RT-induced apoptosis.

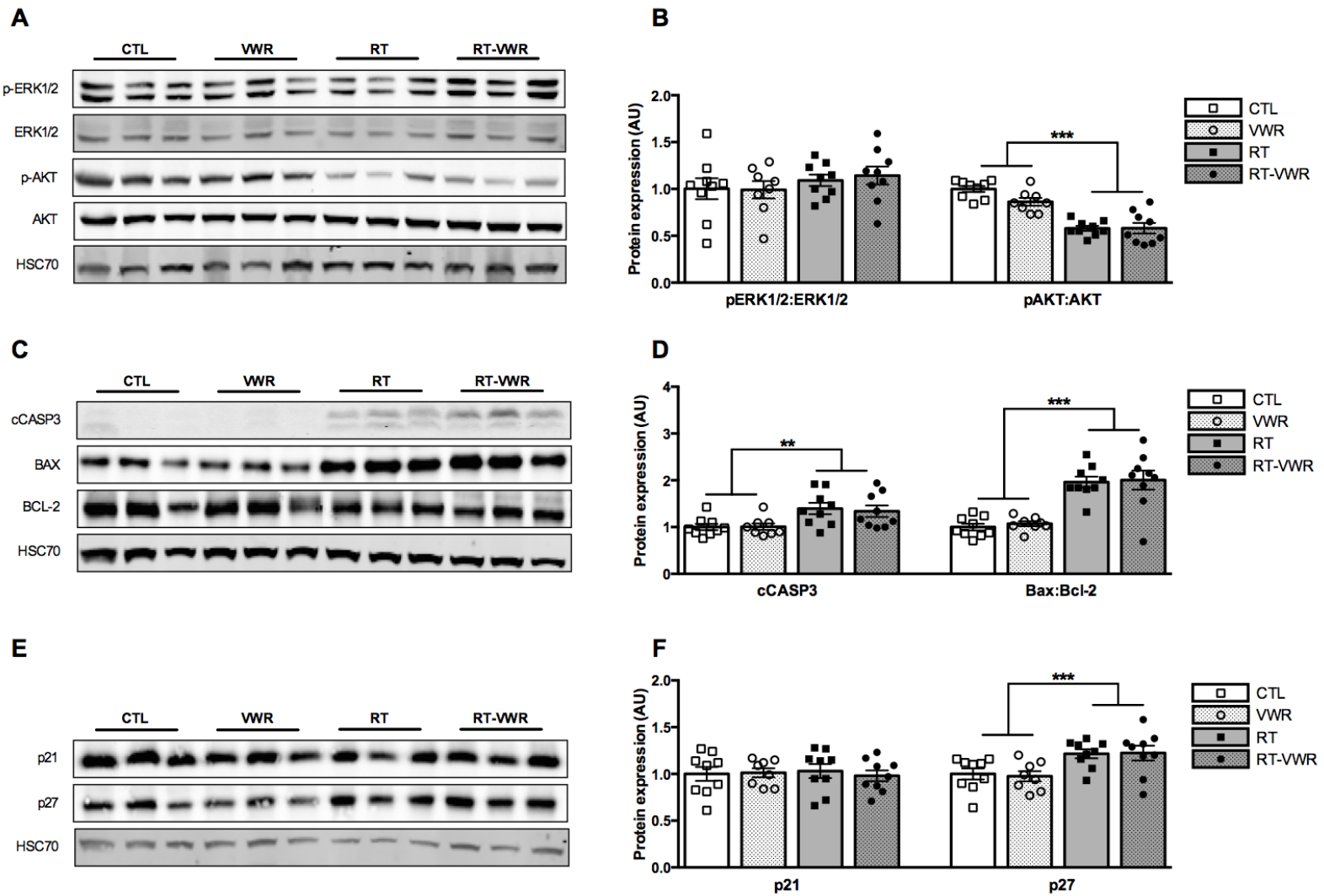

*Figure R-10: Western blot analysis. A. Representative images and B. quantification of western blots for pERK1/2:ERK1/2 and pAKT:AKT; C. Representative images and D. quantification of western blots for cCASP3, and BAX:BCL-2; E. Representative images and F. quantification of western blots for p21 and p27. Data are represented as means ± SEM. ** p<0.01; ***p<0.001*

VWR does not impact cell cycle regulators p21 and p27; increases p27 protein expression but not p21, with no additional effect when combined with VWR

The expression of key cell cycle regulators p21 and p27 was assessed in the tumor tissue. None of the interventions affected p21 expression (VWR main effect $p>0.05$, RT main effect $p>0.05$). p27 was not affected by VWR but increased by 22% in both RT and RT-VWR (VWR main effect $p>0.05$; RT main effect $p<0.001$; Figure R-10.E. and Figure R-10.F.). Therefore, only RT was able to modulate cell cycle regulators.

Hence, VWR did not impact tumor growth nor RT efficiency, and did not appear to modulate major tumor signaling pathways. This contrasts with previous findings published by our team (10). Importantly, two major factors differ between the two studies: the physical activity modality (treadmill running (TR) versus VWR) and the cancer cell line used (PPC-1 versus PC-3). To evaluate the importance of these two factors on prostate tumor growth and provide a better understanding of the relationship between physical activity and PCa, two additional experiments were conducted.

Experiments #3: influence of VWR versus TR in PC-3 xenografts

Since we had previously observed enhanced RT efficiency in response to treadmill running in mice bearing PPC-1 prostate tumors (10), we investigated whether the lack of effect of VWR in PC-3 xenografts could be attributed to differences in physical activity modalities (VWR versus TR). The study design is depicted in Figure R-11.A.

VWR activity led to greater daily and weekly distances run than TR in our preclinical model

VWR mice had access to their wheels every day of the week and ran an average of 3.93±0.57km/day, and hence ran approximately 27.5km/week. TR mice on the other hand ran five days/week for up to ~1.08km/day (18m/min with 10% incline for one hour) corresponding to 5.4km/week. Hence, VWR ran approximately five times longer weekly distances than TR mice (Figure R-11.B).

Neither VWR nor TR slowed down tumor growth

Similar to the other experiments, tumor volume was regularly assessed throughout the experiment to evaluate PCa growth. Neither VWR (as observed in the other experiments) nor

TR had a significant effect on tumor growth (Figure R-11.C.). In line with these findings, tumor volume and tumor weight were similar across all groups at sacrifice on day 65 (Figure R-11.D. and Figure R-11.E.). Hence, the choice of physical activity modality does not appear to explain the difference between the present results and our previous findings (10).

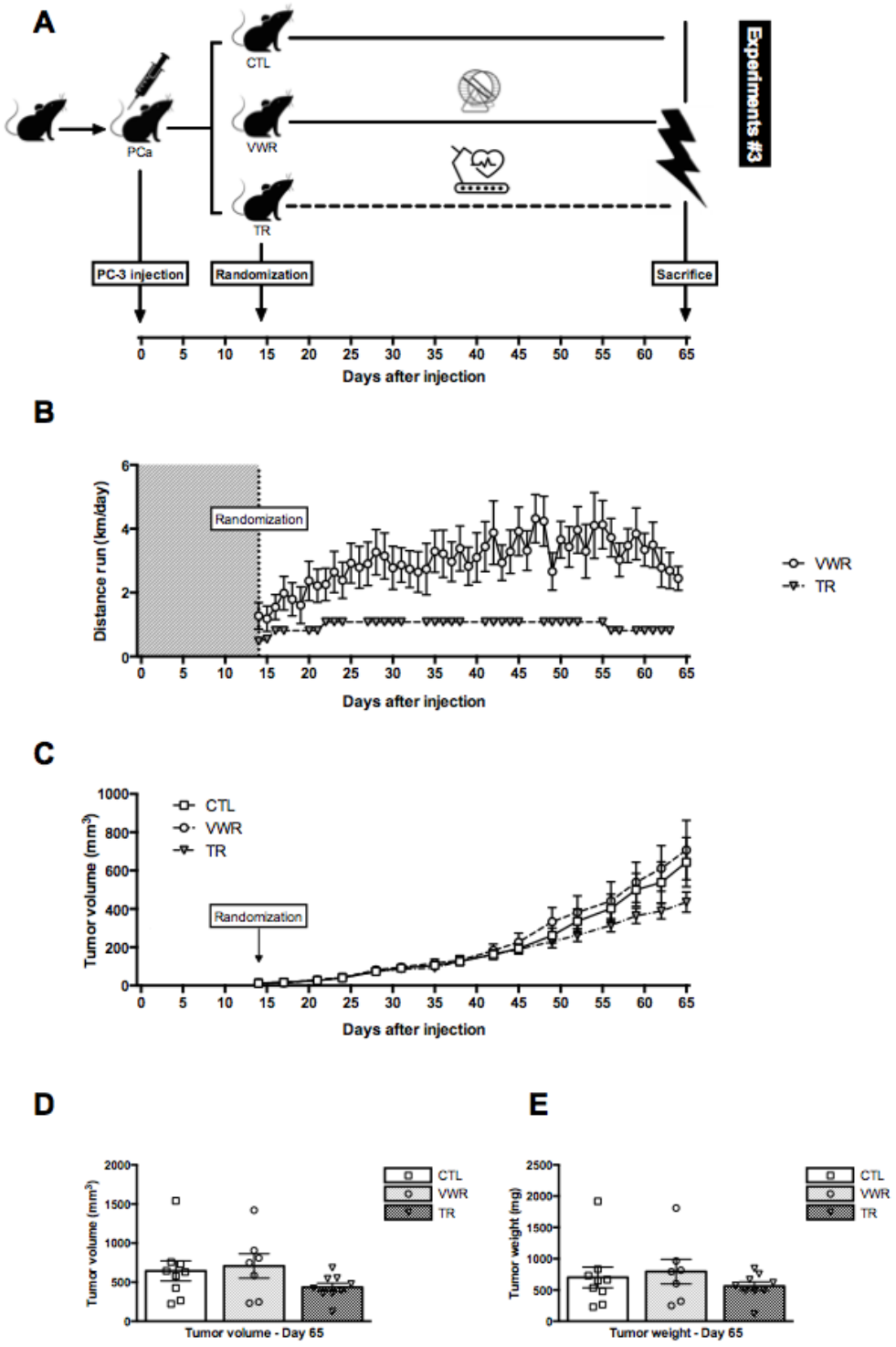

Figure R-11: Study design and characteristics - Experiments #3. A. Study design; B. Evolution of the distance run by VWR and TR mice over time; C. Tumor volume evolution over time; D. Tumor volume and E. tumor weight measured on day 65 (sacrifice). Data are represented as means ± SEM.

Experiments #4: sensitivity of PC-3 and LNCaP PCa cell lines to exercise-conditioned media in vitro

To investigate whether the anti-cancer effects of physical activity may be dependent on the PCa cell line, we conducted *in vitro* experiments. Fasting serum was collected from 10 men over 50 years considered inactive (n=5) and active (n=5) according to the physical activity questionnaire for the elderly developed by Voorrips *et al*. (11). All men included had PSA values within the normal range (PSA < 4.0 ng/dL) and had comparable body mass indexes (BMIs).

Serum from active men reduced LNCaP proliferation but had no effect on PC-3 cells

The serum samples were used to treat PC-3 and LNCaP cells *in vitro*. When performing a methylene blue assay to assess cancer cell proliferation, we found that the treatment of LNCaP cells with serum from active men led to a significant reduction in cell proliferation compared to when treated with serum from inactive men ($p<0.05$; Figure R-12). However, when PC-3 was treated with serum from active and inactive men, no significant effect was found. Hence, these results show that the ability for chronic physical activity to inhibit PCa cell proliferation *in vitro* is dependent on the PCa cell line.

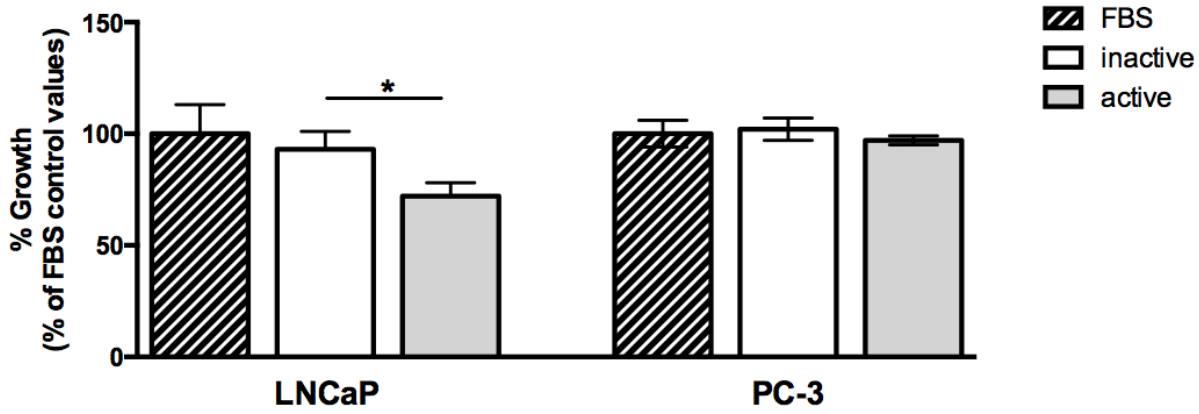

*Figure R-12: Growth proliferation assay in LNCaP and PC-3 cells treated with serum from inactive or active men. Data are represented as means ± SEM. *p<0.05*

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

DISCUSSION

Our study is the first to investigate the impact of VWR on the RT response. In our model, PC-3 tumor xenografts were used and VWR was initiated after tumor establishment (1-week post-injection). The main findings obtained are that (1) VWR activity was not affected by the RT treatment, showing that mice can stay active even while undergoing treatment; (2) VWR improved tumor perfusion but influenced neither tumor growth nor RT efficiency; (3) the lack of effects observed on tumor growth does not appear to be attributable to the choice of physical activity modality, as TR – similar to VWR – did not slow down prostate tumor growth; and (4) the potential anti-cancer effects of physical activity may be dependent on the PCa cell line, as LNCaP, but not PC-3, showed reduced proliferation when treated with serum from active versus inactive men.

VWR activity is not affected by the RT treatment

VWR as a physical activity model possess several advantages over TR: it does not require stressful stimuli (such as electric shocks) and allows the animals to engage in physical activity in accordance with their circadian rhythms and close to their natural running pattern (13). In our study, VWR activity was recorded daily and the data collected revealed that VWR remained unaffected by RT throughout the experiment. Indeed, VWR and RT-VWR mice ran comparable distances throughout the experiment. This was surprising as adjuvant therapy for cancer has been shown to cause cardiotoxicity in preclinical models (14) but also in the clinical setting (15), significantly altering cardiorespiratory fitness. For instance, Segal *et al*. reported that men with PCa had a significant decline in their VO_{2peak} following RT treatment. Interestingly, these authors also showed that supervised exercise training was able to counteract this decline (16). Nevertheless, our results support the safety and feasibility of physical activity in PCa patients undergoing RT, even though caution should be warranted when extrapolating data from preclinical studies to the clinical setting.

VWR improves tumor perfusion but does not influence neither tumor growth nor RT efficiency

In our study, VWR did not impact tumor growth. These results are in contrast with studies previously published in the literature. Indeed, the majority of preclinical studies investigating

the effect of physical activity on tumor growth reported slower tumor growth in active rodents (4). Nevertheless, there are large disparities between studies with some reporting no effect or even increased tumor growth in response to physical activity. In PCa more specifically, some studies reported slower tumor growth with physical activity (10,17–23) while others found no effect (24–29). McCullough et al. even reported that while TR had no effect on tumor weight in immunocompetent Copenhagen rats, it significantly increased tumor weight in nude rats (30). These discrepancies may be attributed to differences in the physical activity models used (i.e., modality, dose, timing) or in the cancer models employed (i.e., host, tumor induction, tumor type and subtype, site of tumor development). Interestingly, Zheng *et al*. used a similar cancer model to ours (PC-3 xenograft) and showed that VWR activity was able to reduce PCa growth (17). Importantly however, VWR was initiated one week prior to tumor cell injection while in our study, VWR started 14 days post-injection at the earliest. The timing of VWR may be important as engaging in regular physical activity before tumor cell inoculation could notably prepare the tissue microenvironment, and more broadly the host, against future tumor formation. However, in our study we chose to focus on physical activity initiated after tumor establishment as it may be partially representative of PCa patients previously sedentary and engaging in regular physical activity after diagnosis as part of their health care.

Nevertheless, despite no effect on tumor growth being observed, VWR was able to improve tumor perfusion in our study. Indeed, we observed that tumor flux divergence was enhanced with VWR, suggesting enhanced blood flow distribution. These results are not surprising as there is accumulating evidence showing that physical activity is able to improve tumor vascularization, as reviewed and discussed elsewhere (12,31). In PCa more specifically, both acute and chronic physical activity have been shown to modulate tumor perfusion/vascularization. Indeed, an acute bout of treadmill running resulted in increased tumor blood flow (by approximately 180-200%) and resulted in a higher number of patent vessels compared to what was measured at rest in rats bearing orthotopic prostate tumors (32,33). Chronic physical activity following cancer transplant has also been shown to improve prostate tumor vascularization in two preclinical studies (24,30), which is in line with our findings.

Tumor growth relies partly on angiogenesis, by which new blood vessels are created to grant oxygen and nutrients to the tumor. However, aberrant angiogenic signaling leads to chaotic and immature vessel structure. As a result, a discrepancy between the oxygen supply and

demand occurs, thereby resulting in hypoxia (34). In PCa, hypoxia has been shown to correlate with increased tumor aggressiveness, higher metastastic potential, and poor prognosis (35). Hence, improving tumor perfusion with VWR may result in tumor reoxygenation and improve PCa outcomes. However, in our study, there was no difference in tumor growth between sedentary and VWR mice. Nevertheless, improved tumor perfusion associated with VWR may limit the establishment of hypoxia, known to be associated with radioresistance. Indeed, low oxygen availability reduces the amount of reactive oxygen species induced by radiotherapy and a hypoxic microenvironment leads to the selection of radioresistant cancer cells (36). Hence, higher tumor blood flow in response to physical activity may improve tumor oxygenation and enhance treatment response (6,12), similar to what can be observed with hyperthermia (37). Thus, in our model, we hypothesized that despite no effect of VWR alone on tumor growth, the combination of VWR with RT could improve RT efficiency through improved tumor perfusion. Contrary to our hypothesis, VWR did not improve RT efficiency as no difference in tumor growth rate, tumor volume, tumor weight nor tumor growth delay was found. Furthermore, the molecular analysis performed in the tumor tissue revealed that VWR did not impact tumor cell proliferation nor cancer cell death. Hence, even though VWR appeared safe, it did not alter major tumor characteristics. Importantly, even though tumor flux divergence was enhanced by VWR, no difference in tumor flux amplitude, in tumor pericyte coverage nor in vessel density was found. RT on the other hand led to increased pericyte coverage, suggesting a remodeling of tumor vasculature with RT, as previously reported (38), and VWR did not provide additional effects. Hence, physical activity in our model may have only led to a moderate effect on tumor vascularization. This might notably be attributed to the use of ectopic tumors rather than orthotopic, as the tumor vascularization in response to acute exercise has been shown to be highly dependent on inoculation site. Garcia *et al*. indeed reported that while tumor blood flow was increased by ~180% in rats bearing orthotopic prostate tumors, it was reduced by ~25% in rats with ectopic tumors (33). Differences in tumor blood flow between ectopic and orthotopic tumors in response to chronic physical activity were however not investigated. Hence, the impact of improved tumor vascularization with physical activity on RT efficiency remains to be evaluated in an orthotopic PCa model.

Two previous preclinical studies have reported improved RT response with physical activity. Wennerberg *et al*. found enhanced treatment response with TR in mice treated with RT

combined with immunotherapy in a murine model of 4T1 mammary carcinoma (9). Furthermore, our team recently showed improved RT efficiency with TR in a PCa xenograft model (10), a model relatively similar to the present study. The two main differences were (1) the PCa cell line injected (PPC-1 *vs*. PC-3 cells) and (2) the physical activity modalities (TR *vs*. VWR). Hence, we conducted additional experiments to better understand whether the cancer subtype and the physical activity model may be major determinants in the effect of physical activity on PCa.

TR, similar to VWR, does not affect tumor growth: the modality of physical activity does not explain the absence of benefit on PC-3 prostate tumor growth

To assess the importance of physical activity modalities on prostate tumor growth, we evaluated whether TR rather than VWR could alter tumor growth *in vivo*. TR did no significantly slow down tumor growth and led to the development of tumors with a similar volume and weight to the ones measured in CTL and VWR mice. These results are in line with a recent study showing no effect of TR on the prostate tumor weight in rats bearing orthotopic PC-3 tumors (28). Hence, these results suggest that PC-3 tumor growth is not sensitive to physical activity (initiated after tumor inoculation) regardless of the type of modality.

Physical activity anti-cancer effects may be dependent on the PCa cancer cell line

In addition to physical activity modalities, the cancer type and subtype may be determinant for the effects of physical activity on tumor growth. Interestingly, we found that serum from active men led reduced LNCaP cells proliferation compared to serum from inactive men, but that this was not the case for PC-3 cells. Numerous studies have shown that LNCaP are sensitive to conditioned exercise media, alone (39–41) or in combination with dietary interventions (39,42–45) including when using conditioned media from cancer patients (46). Similar experiments conducted with PC-3 cancer cells have provided more conflicting results, with some reporting reduced proliferation with exercise-conditioned media (28,45) and one other study reporting no effect (42). Interestingly, Leung *et al*. reported that LNCaP but no LN-56 prostate cancer cells were sensitive to exercise-conditioned serum (40), supporting the idea that physical activity may impact prostate cancer growth differently according to the cancer type. Furthermore, the authors suggested that the difference in p53 functional status

between LNCaP and LN-56, respectively p53 functional and non-functional, may be responsible for the differential effect of exercise on cancer cell proliferation. Since PC-3 cancer cells are deficient in p53, the effects of exercise on this cancer cell line might also be limited. However, we have previously shown that exercise training in mice bearing PPC-1 tumors (p53 null) resulted in slower tumor growth (10), suggesting that p53 status may not be the only determining factor for the anti-cancer effects associated with physical activity.

Interestingly, a preclinical study showed that in three distinct mouse models of triple negative breast cancer, the same treadmill running program either inhibited, had no effect or accelerated tumor growth compared with sedentary controls, depending on the cancer model (47). Similar results may be obtained in different PCa models as our *in vitro* data supports that the effects of physical activity are highly dependent on the PCa cell line used. Therefore, additional studies are warranted to determine which PCa subtypes may obtain the most benefits from physical activity in order to personalize and improve PCa patient health care.

Limitations

It is necessary to discuss that there are some limitations to the present study. Notably, the animals used in our experiments were immunodeficient, in order to evaluate the impact of physical activity on tumors of human origin. Since immunodeficient mice do not benefit from the adaptive immune response, some of the effects of physical activity might be masked.

Furthermore, tumors were injected subcutaneously, which may not provide the same microenvironment as the one from the tissue of origin (i.e., the prostate).

CONCLUSION AND FUTURE DIRECTIONS

Overall, our study showed that VWR was able to alter some parameters related to tumor perfusion (e.g., tumor flux divergence) in a preclinical model of PCa, but surprisingly influenced neither tumor growth nor RT efficiency. In line with this, no differences were found at the molecular level. We further showed that physical activity might however provide benefits in PCa but that these effects appeared to be rather dependent on the cancer subtype than on physical activity modality. Indeed, we found that (1) TR did not slow down tumor growth in mice bearing PC-3 tumors, similar to what was observed for VWR and (2) that the impact of physical activity on PCa cell viability *in vitro* appeared to be dependent of the cell

line used. Thus, this study highlights the necessity to better understand the relationship between physical activity and PCa. More specifically, while physical activity may lead to more favorable outcomes in some cancer patients, it may have little to no effect in others. Hence, our study highlights the necessity to conduct additional studies investigating the effect of different exercise modalities on different PCa subtypes, with or without treatment, in order to better tailor exercise programs in cancer patients.

AUTHORS CONTRIBUTION

A. Rebillard, F. Paris and V. Potiron designed research; S. Dufresne, C. Richard, L. Orfila, A. Rebillard, S. Chiavassa, G. Delpont, J. Gueritat, B. Martin, JM. Escoffre and V. Potiron performed research, S. Dufresne, A. Rébillard, V. Potiron, B. Denis de Senneville and N. Rioux-Leclercq analyzed data; S. Dufresne and A. Rebillard wrote the paper.

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III – A review of physical activity and circulating miRNA expression: implications in cancer risk and progression

A secondary aim of the PhD was to evaluate the possibility for epigenetic mechanisms to mediate some of the anti-cancer effects of exercise in cancer.
This work is secondary as it was initiated before the start of the PhD and did not represent the core of the work and reflections that occurred during the PhD.

The first study responding to this objective and included in this thesis is a review discussing how alterations in the expression of circulating miRNAs in response to chronic physical activity and acute exercise in the healthy population could hypothetically affect tumor growth.

As previously mentioned, several biological processes mediating the benefits of physical activity on tumor growth have been identified (Pedersen, Christensen, and Hojman 2015). Nevertheless, there is still not a clear understanding of the exact mechanisms underlying the effects of physical activity in cancer.
Skeletal muscle is the most abundant tissue in the human body and acts as an endocrine organ. Indeed, it can impact other tissues in the body through either autocrine, paracrine, or endocrine signals (Pedersen and Febbraio 2012; Severinsen and Pedersen 2020). Muscles are highly solicited during exercise and their contraction may be implicated in the anti-cancer effects of physical activity. Several studies have unraveled a link between cytokines released by the muscle (known as "myokines") and tumor growth. Notably, Aoi *et al*. identified the myokine SPARC as able to increase cancer cell apoptosis and inhibit colon tumorigenesis (Aoi et al. 2013). Oncostatin M (OSM) and IL-6 have also been suggested as myokines mediating some of the anti-cancer effects of physical activity in an *in vitro* model of breast cancer cells and in a mouse model of B16F10 melanoma, respectively (Hojman et al. 2011; Pedersen et al. 2016).
In addition to myokines, skeletal muscles also release miRNAs. miRNAs are small non-coding RNAs involved in the post-transcriptional regulation of gene expression. They can be highly and specifically enriched in specific tissues (Sood et al. 2006) and skeletal muscle enriched miRNAs are known as myomiRs. Importantly, upon stimulation or injury, miRNAs can be released from one organ to other tissues or fluids in an active (secretion) or passive

(membrane leaking) manner (Chen et al. 2012). Circulating miRNAs (c-miRNAs) present in the bloodstream are currently being extensively studied in the oncology field as their expression has been found to be modified in numerous cancers, including PCa (Bryant et al. 2012). They appear not only as promising biomarkers but also as therapeutic tools (Kosaka, Iguchi, and Ochiya 2010), based on the evidence that extracellular miRNAs can have direct biologic effects on other cells (Mittelbrunn et al. 2011; Fabbri et al. 2012). Extracellular vesicles, such as exosomes, can transport miRNAs to neighboring cells or even distant organs. Thus, miRNAs may be involved in cell-to-cell communication and in organ crosstalk (Guay and Regazzi 2017). However, the role of miRNAs in the anti-cancer effects of physical activity remain largely unknown.

In this review, we gathered available data on circulating miRNA expression in response to either chronic or acute physical activity in healthy individuals and discussed their potential anti-cancer effects in the context of cancer.

A total of 16 studies were included, with 14 investigating c-miRNA expression in response to a single bout of exercise and six evaluating the impact of chronic physical activity. Both acute and chronic physical activity led to significant differences in c-miRNA expression, which may be dependent on exercise modalities (Banzet et al. 2013) and/or individual characteristics. Interestingly, several c-miRNAs modulated by physical activity have been shown to have their expression altered in cancers. This led us to hypothesize that changes in miRNA expression in response to physical activity may play an important role in cancer risk and progression, perhaps via c-miRNA intercellular communication. For example, miR-133 has been shown to act as a tumor suppressor but has also been identified as a myomiR modulated in both the muscles (Nielsen et al. 2010; Russell et al. 2013) and the blood circulation (Banzet et al. 2013; Uhlemann et al. 2014; Mooren et al. 2014; Nielsen et al. 2014; Baggish et al. 2014; Gomes et al. 2014; Clauss et al. 2016; Min et al. 2016) in response to physical activity. We hence hypothesized that miRNA may translocate from the muscles to the blood (e.g. via exosome release) and eventually to the tumor tissue where they can be expressed.

Interestingly, two preclinical studies showed that exercise training in a model of breast cancer led to a decrease in tumor miR-21 expression and was associated with slower tumor growth (Khori et al. 2015; Isanejad et al. 2016). Furthermore, miR-206 and let-7 were also reported to be upregulated in the tumor tissue in response to exercise training (Isanejad et al. 2016). These two studies provide evidence that physical activity has the ability to modulate miRNA

within the tumor tissue. Since these miRNAs are known to be involved in carcinogenesis, the alteration of their expression by physical activity may counteract cancer initiation and growth. Furthermore, it is interesting to note that these three miRNAs (miR-21, miR-206 and let-7) are modulated in the blood circulation in response to exercise (as reviewed in our study) but also in response to high intensity interval training (HIIT) in women with breast cancer as reported in a recent clinical study (Alizadeh et al. 2019). Additionally, studies have reported that miR-206 and miR-21 expression could be altered by exercise in the muscle tissue (Drummond et al. 2008; Nielsen et al. 2010; Fernandes et al. 2012). Hence, these results support the plausibility of a crosstalk between the muscle and the tumor mediated by miRNA trafficking.

Overall, despite limited data available, modulation of miRNA might represent a biological mechanism mediating some of the physical activity benefits in cancer. miRNA trafficking could be involved in the muscle-to-cancer crosstalk, and additional studies are necessary to investigate this hypothesis.

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A review of physical activity and circulating miRNA expression: implications in cancer risk and progression

Suzanne Dufresne[1], Amélie Rebillard[1], Paolo Muti[2], Christine M Friedenreich[3,4,5], Darren R Brenner[3,4,5]

1. M2S lab, EA 1274, Université Rennes 2, ENS Rennes, 35170 Bruz, France
2. Department of oncology, McMaster University, Hamilton Ontario, Canada
3. Department of Cancer Epidemiology and Prevention Research, CancerControl Alberta, Alberta Health Serbice, Calgary, Alberta, Canada
4. Department of oncology, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada
5. Department of Community Health Sciences, Cumming School of Medicine, University of Calgary, Calgary, Alberta, Canada

ABSTRACT

The role of circulating miRNAs (c-miRNAs) in carcinogenesis has garnered considerable scientific interest. miRNAs may con- tribute actively to cancer development and progression, making them potential targets for cancer prevention and therapy. Lifestyle factors such as physical activity (PA) have been shown to alter c-miRNA expression, but the subsequent impact on cancer risk and prognosis is unknown. To provide a better understanding of how PA reduces the risk of cancer incidence and improves patient outcomes, we conducted a review of the impact of PA on c-miRNA expression, which includes a comprehensive synthesis of studies examining the impacts of acute

and chronic exercise on expression of c-miRNAs. While the variability in methods used to assess miRNA expression creates challenges in comparing and/or synthesizing the literature, results to date suggest that the circulating form of several miRNAs known for playing a role in cancer (c-miR-133, c-miR-221/222, c-miR-126, and c-let-7) are altered by both acute and chronic PA. Additional research should develop standardized procedures for assessing both c-miRNA and PA measurement to improve the comparability of research results regarding the direction and amplitude of changes in c-miRNAs in response to PA.

Key words: microRNAs; c-miRNA; physical activity; exercise; cancer

INTRODUCTION

miRNAs are implicated in the etiology of various diseases, including cancer (1), and understanding their biologic characteristics has led to novel insights for cancer diagnosis, treatment, and prognosis (2). miRNAs are considered to be stable in healthy individuals, but external factors including lifestyle can impact their expression. Several studies have reported that physical activity (PA) can modulate miRNA expression (3–5). The impact of PA on reducing cancer risk and improving cancer progression has been well documented, but the exact mechanisms underlying these relations are not yet completely understood (6, 7). A better understanding of altered expression and function of miRNAs in response to various forms of PA could assist in clarifying the role of PA in cancer prevention and prognosis.

miRNAs and cancer

miRNAs are a class of small noncoding RNAs that regulate gene expression post-transcriptionally, either by mRNA cleavage, mRNA destabilization, or inhibition of translation (8). Since the first miRNA was discovered by Lee and colleagues in 1993, more than 2,500 human miRNA sequences have been identified (miRBase; ref. 9). This rapid expansion of discovery can be partially explained by the broad importance of miRNAs in regular bodily functions. miRNAs are predicted to modulate more than 60% of protein-coding genes (10) and are involved in numerous integral cellular processes, including development, proliferation, metabolism, and signal transduction (11, 12). Given their regulatory importance, it is not surprising that miRNAs are widely implicated in carcinogenesis and progression. The mechanistic role of miRNAs in cancer was first discussed by Johnson and colleagues in 2005 (13) and the number of studies has been rapidly expanding ever since. In both solid and hematologic tumors, numerous miRNAs have been found to be altered, and their expression is associated with the severity and stage at diagnosis (14). In breast cancer, for example, several miRNAs have been observed as very early biomarkers of this disease (15).

The role of miRNAs in carcinogenesis and cancer progression is twofold. Both in vivo and in vitro studies have demonstrated they target either tumor suppressors or oncogenes (16). Expression of particular miRNAs can downregulate various oncogenes or re-express tumor suppressor genes, leading to tumor suppression, whereas upregulation of other miRNAs, also

referred to as oncomiRs, inhibits tumor suppressor genes or overexpress oncogenes, thereby promoting tumor cell proliferation and metastasis (16).

miRNAs are found in tissues and organs and are also released into the circulation in a remarkably stable form (17). The expression of those c-miRNAs has been found to be modified in numerous cancers including, but not limited to, colorectal, prostate, breast, hepatocellular, gastric, and head and neck cancers (1, 18–24). Altered c-miRNA expression was first observed in B-cell lymphoma patients by Lawrie and colleagues in 2008 (25) and it is now established that, on average, the expression of 100 c-miRNAs are altered in each cancer type. More importantly, the discovery of c-miRNAs in body fluids resulted in the implication of new possible pathways through which miRNAs may impact tumor development and progression and led to novel insights for their use as therapeutic tools for cancer (26, 27).

miRNAs and potential mediators of the association between physical activity and cancer

PA has been shown to reduce cancer risk and progression (6, 28–31). Multiple hypothesized biologic mechanisms have been proposed to explain these benefits (6, 29, 32) including reductions in chronic inflammation, regulation of metabolic factors, changes in insulin resistance, enhanced immune system function, and levels of circulating sex hormones, myokines, or adipokines (6, 29, 32, 33). The exact mechanisms, however, whereby PA alters cancer risk and progression at various cancer sites are not fully understood and additional pathways are likely. As miRNAs are now well recognized for playing important roles in carcinogenesis and cancer outcomes, they could be involved in the PA-related benefits toward cancer.

The impact of PA on miRNAs has been investigated by several research groups, but mainly in the context of physical performance, muscle pathogenesis, and muscular dysfunction related to aging, as well as in muscle disorders associated with diabetes, cancer, and inflammation (3). The majority of the miRNAs shown to be altered by either acute or chronic exercise in these studies are muscle-specific miRNAs, also called myomiRs. miRNAs are differentially expressed within various organs, and if a miRNA is expressed more than 20-fold in a specific tissue compared with the mean of its expression in other tissues, it is thus considered as a tissue-specific miRNA (34). The expression of a variety of miRNAs, and particularly myomiRs, is modulated by PA within muscle tissues (4, 35) as well as in plasma (5). Zacharewicz and

colleagues (4) have published a detailed review on miRNA expression within muscles in response to PA. Here we restrict our review to miRNA expression in blood following acute or chronic PA.

c-miRNAs are currently being extensively studied due to several advantages from a clinical and etiologic perspective including a remarkable stability in the circulation, resistance to RNase activity (36), and preservation through multiple freeze–thaw cycles (37). Consequently, c-miRNAs can be easily evaluated by standard and relatively inexpensive qRT-PCR techniques, even in low concentrations (38), and their measurement is a noninvasive procedure (39). This is in stark contrast to the analysis of miRNA expression within specific tissues, which require biopsies. On the basis of these differences, c-miRNAs measured from stored blood samples will prove advantageous in the conduct of large epidemiologic studies of PA-related cancer etiology. For these reasons, in this review, we focused on the role of c-miRNAs as an underlying mechanism mediating PA's benefits against cancer through the exploration of potential links between PA-induced c-miRNA modulation and cancer-related c-miRNA expression.

The majority of c-miRNAs are derived from leukocytes and endothelial cells (40) but they can also originate from organs exposed to high blood flow (3). When exercising, the blood flow is significantly elevated, with up to 80-fold blood flow increase in times of high physical output (41, 42), leading to expression changes in miRNAs originating from various organs (43–45), and in particular from skeletal muscles. The release of miRNAs in the blood circulation can originate from destroyed tissues (46, 47), especially if the exercise load is great (48). However, increases in the expression of c-miRNAs have also been reported in the absence of cell damage markers in plasma (49). miRNAs can, in fact, be exported by active transport systems, either encapsulated in extracellular vesicles (such as exosomes; refs. 46, 50, 51), or associated with protein or lipid- based complexes [e.g., Argonaute2 (AGO2), high-density lipoproteins (HDL), and low-density lipoproteins (LDL); refs. 36, 52–54]. Importantly, increasing evidence shows that miRNAs, through their circulating form, can be transported from donor cells to recipient cells, where they exert their functions (18, 54). c-miRNAs are thus being recognized as important components in intercellular communication (53, 55, 56), and have notably been demonstrated to play key roles in crucial cellular processes, such as apoptosis, proliferation, metastasis, and immunity (56, 57). For example, exosomal-miR-105 secreted by breast cancer cells and transferred to endothelial cells have been shown to promote metastasis (58).

MATERIALS AND METHODS

We conducted a literature search up to March of 2016 in PubMed using the following search strategy: "miRNAs AND exercise" or "miRNAs AND physical activity" or "microRNAs AND exercise" or "microRNAs AND physical activity." Only studies conducted in humans containing data measuring c-miRNAs were included. We did not employ a minimum sample size threshold and studies with small numbers of participants were also included, as the total number of studies meeting our inclusion criteria was relatively small. Studies examining populations with disease were excluded, and all studies included in the review presented measurements among healthy subjects as underlying diseases may impact c-miRNAs. To our knowledge, there are no data on the effect of PA on c-miRNA expression in patients with cancer. One study has investigated the impact of PA on miRNA expression within tumor tissue based on an animal model and is included in the Discussion section. We also analyzed reference lists of the identified studies for additional relevant articles. The title and abstract were examined, and full text was obtained if the article seemed eligible. No period or language restrictions were applied. We compiled the results from studies investigating c-miRNA expression in response to a single bout of PA (acute PA) in either normally active individuals (Table R-4) or trained subjects (Table R-5) as well as the impact of a training period and/or regular PA (chronic PA) on basal c-miRNA expression (Table R-6).

Table R-4 and Table R-5 include details on study (design, sample size, timing of blood draws), participant (age, gender, PA level), and protocol characteristics [training program if any, type of exercise (resistance or endurance exercise)]. Included in these tables are descriptions of the number of miRNAs screened, number of miRNAs altered by PA, miRNAs measured but not altered, and undetectable or unreliable miRNAs.

With regards to the type of exercise, we considered predominantly endurance exercises as exercises involving the whole body and increasing oxidative capacity and aerobic endurance (e.g., a marathon run), while resistance exercises were defined as exercises using machines, weights, or even individuals' body weight to stimulate muscle hypertrophy and increase muscular strength or power (e.g., leg press, squats, pull-ups). We highlight the distinctions between normally active and "trained" participants, based on the information provided by the studies. We considered the subjects trained if: (i) the subjects underwent a specific training program described in the study; (ii) a large bout of exercise, such as a marathon run, was

included in the protocol as undergoing such an effort implies previous training (even though not always specified in the reviewed studies); (iii) the authors stated that the participants were "trained individuals" and/or part of a competitive program. We considered the subjects "untrained" or "normally active" if the participants engaged in recreational activity 4 hours per week and/or if specified in the study. In total, we included 5 studies examining normally active individuals and 10 studies investigating trained individuals, according to our criteria.

To facilitate comparisons across studies, we present the data for c-miRNAs that were examined in multiple studies. We first synthesize and discuss the results obtained for c-miRNA expression following acute PA, followed by a discussion and synthesis of results examining basal miRNA expression in response to chronic PA.

PA has also been shown to modulate miRNA expression within peripheral blood mononuclear cells (PBMC). PBMCs are leukocytes with a round nucleus, and comprise lymphocytes (T cells, B cells, and natural killer cells), monocytes, and dendritic cells (59). Similar to c-miRNAs, PBMC miRNAs can easily be detected by standard qRT-PCR techniques, but it remains controversial whether their expression is similar or not to whole blood miRNAs (60, 61). Thus, even though two different research groups found an impact of acute PA in circulating leukocytes (62–65), those results are not discussed in this review.

RESULTS

We have identified a total number of 16 studies in our review, which are displayed in Table R-4, Table R-5 and Table R-6. Among these studies, 14 investigated c-miRNA expression in response to a single bout of exercise (48, 49, 65–76), and six (49, 66, 71, 75, 77, 78) evaluated the impact of chronic exercise on c-miRNA expression.

Impact of acute physical activity on c-miRNA expression

Acute PA refers to a single isolated PA session. It is clear that a single bout of PA alters expression of several c-miRNAs shortly after its completion (Table R-4 and Table R-5). However, the modalities of PA [frequency, intensity, timing and type (FITT)] and the individual's physical fitness levels (trained or untrained) may impact those changes. Modifications in several c-miRNAs are not always observed immediately after PA but are seen

only after 2–3 hours of rest. For example, Banzet and colleagues reported no changes in miRNA expression immediately after the completion of a single bout of eccentric exercise (30 minutes downhill walking) but observed an increase in miR-1, miR-133a, miR-133b, miR-499-5p, and miR-208b 6 hours post bouts of PA (68). These c-miRNAs then returned to baseline expression after periods of inactivity, which has been reported to be relatively slow in some studies (66–68, 70, 72). For example, decreased expression of c-miR-221 was still observed by Sawada and colleagues 3 days postcompletion of a resistance exercise session (67).

Muscle- or cardiac-specific miRNAs

In healthy individuals, muscle- or cardiac specific miR-1, miR-133, miR-206, miR-499, and miR-208 are expressed at low levels in the circulation, whereas miR-486 is generally found in higher concentrations (74, 79). In response to PA, several studies have shown an increase in the circulating forms of miR-1, miR-133, miR-206, miR-208, and miR-499 immediately after the completion of various PA modalities (FITT; refs. 48, 68, 70, 72, 73, 75, 76), and a decrease in miR- 486 (66). It can be noted that specific exercise modalities appear to impact changes in miRNA levels. Banzet and colleagues found different c-miRNA expression patterns in normally active participants who underwent a downhill walk versus others who completed an uphill walk (68). Downhill walking is considered an eccentric exercise as the muscles actively lengthen during this effort, whereas uphill walking induces active shortening of the muscles and is thus considered a concentric exercise. Interestingly, Banzet and colleagues found significantly higher muscle/cardiac specific c-miRNA expression for participants who walked down- hill compared with subjects walking uphill (68). Thus, these data suggest that the type of muscle contraction involved in the exercises impacts c-miRNA expression. Furthermore, data from various studies also suggests that muscle/cardiac c-miRNAs can be differentially expressed depending on PA's intensity. For example, when looking at c-miR-133, Uhlemann and colleagues showed that modifications in c-miR-133 expression in response to PA were correlated with phosphocreatine kinase (CPK) activity, a marker for muscle damages: the more damaging the exercise was, the most altered c-miR-133 expression was (48).

Modulations in muscle/cardiac c-miRNA expression postexercise appear to be temporary, yet the exact delay before a return to baseline values is unclear. When examining measures taken one day postexercise, most studies showed that muscle and cardiac specific c-miRNAs had

returned to their basal value. Others, however, reported a decrease in expression of those c-miRNAs compared to immediately after exercise, but still significantly elevated compared to baseline values. Focusing on c-miR-1 for example, four studies found similar expression of this c-miRNA between preexercise and 24 hours postexercise (49, 68, 75, 76), while one other observed significantly higher expression com- pared with baseline value (but attenuated from expression measured immediately after exercise; ref. 70).

Interestingly, high interindividual variation in c-miR-499 expression has been reported in response to an acute bout of endurance exercise in two different studies (68, 72) which suggests that c-miR-499 is highly dependent on participants' characteristics, notably their training status. miR-499 is a cardiac-specific miRNA and studies have shown its circulating form may represent a marker of myocardial injury (80–84). Strenuous exercises such as marathon running can induce transient myocardial injuries (85–88), this risk being higher in less trained individuals (87, 89). Therefore, the interindividual variation observed in c-miR-499 expression following a marathon run may reflect variation in training among participants, and/or cardiac muscle exhaustion.

Other circulating miRNAs

Non muscle- or cardiac-specific c-miRNAs have also been reported to be impacted by acute PA, but the exact direction and magnitude of those changes remain unclear. Two different research groups observed an upregulation of c-miR-126, an endothelial-specific miRNA involved in angiogenesis (90), immediately after a single bout of PA (48, 72), while no statistical differences in c-miR-126 expression pre- and post-PA have also been reported (48, 69). Interestingly, Uhlemann and colleagues measured c-miR-126 expression before and after an acute bout of PA: subjects were either trained or untrained and the exercises which had to be completed varied among protocols in type, duration, and intensity (48). They observed an upregulation in c-miR-126 in response to a single maximal symptom–limited test performed by healthy individuals, as well as in trained men who underwent 4 hours of bicycling at 70% of their anaerobic threshold, and in trained runners who completed a marathon. However, Uhlemann and colleagues also reported no significant difference in c-miR-126 expression in trained subjects who per- formed a resistance training session compared with basal values (48). As miR-126 is an endothelial-specific miRNA, the authors explained the increase in c-miR-

126 expression following acute exercise observed in three of their protocols by exercise-induced endothelial damages, while they suggested that the resistance exercise session, which resulted in unaltered c-miR-126 expression, did not cause such damages (48).

c-miR-146a, a miRNA known for playing a role in inflammation and immunity (91, 92) has also been found to be altered by acute PA; however, the effects appear to be highly dependent on the type of activity intervention. Two studies have reported an upregulation of this c-miRNA in response to a single bout of endurance exercise in trained individuals (49, 72), but Nielsen and colleagues observed opposite results with a decrease in c-miR-146a in response to a similar type of PA in normally active subjects (71). In contrast, Van Craenenbroeck and colleagues reported no change in c-miR-146a expression in response to a single maximal symptom–limited test, while Sawada and colleagues measured no alteration in c-miR-146a expression in healthy men immediately after they performed resistance exercise session but found a decrease in its expression three days postexercise (67). Interestingly, in trained athletes, Baggish and colleagues found an upregulation miR-146a in response to an acute bout of PA both before and after a training program, the magnitude of the elevation being higher after completion of the training program (49).

Similarly, changes in the circulating expression of miR-221 and miR-222, important players in vascular biology (93), have been reported, but high variations can be found between the studies (49, 67, 71). In response to a 60-minute cycle ergometer exercise bout below anaerobic threshold, c-miR-221 has been found to be downregulated in normally active participants by Nielsen and colleagues (71), as opposed to Baggish and colleagues who showed an upregulation of c-miR-221 expression in trained individuals following an acute exhaustive cycling exercise (49). When assessing the impact of a short exhaustive bout of PA on the c-miRNA expression, Sawada and colleagues did not report any changes immediately after PA completion but found a decrease three days later (67). c-miR-222 expression in response to an acute bout of PA has only been investigated by two different studies: one of them showed an upregulation of this c-miRNA in trained men undergoing an exhaustive bout of exercise (49), while the other one reported no change in its expression between before and after a resistance exercise session (67). The differences observed between studies are likely attributable to differences in protocols, suggesting that alterations of c-miRNAs in response to acute PA are dependent on the dose and type of exercise (FIIT) as well as participants'

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

characteristics (e.g., age, gender, fitness level, health status, personal history, diet, smoking habits, etc.).

Impact of chronic physical activity on c-miRNA expression

Chronic PA is defined as regular PA done over an extended time period (a minimum of several weeks). Interestingly, contrary to what is observed for c-miRNA expression following an acute bout of PA (Table R-4 and Table R-5), there is a general trend for a down- regulation of c-miRNAs expression in response to chronic PA (Table R-6). Among the six different studies investigating the impact of chronic PA on resting expression of c-miRNAs we included in our review, four were intervention trials (49, 66, 71, 75) and two were observational studies (77, 78). The blood samples analyzed in those research projects were taken at rest, at least 12 hours after the last training session.

Muscle- or cardiac-specific miRNAs

Cardiac- or muscle specific c-miRNA expression changes in response to chronic PA are hard to measure because of their low concentrations in humans at rest, consequently, there are limited results thus far. For c-miR-1, one study reported no change between its resting expression before and after a 10-week marathon training period in either trained or untrained individuals (75), whereas two other research groups found its expression too low to interpret its measurement (66, 78). A trend towards a decrease in c-miR-133 expression in response to a supervised training on a cycle ergometer was found by Nielsen and colleagues (71), while other studies reported no changes (49, 75) or nondetectable amounts for the quantification of c-miR-133 (66, 78). Several studies measured the resting expression of c-miR-206 (66, 78), c-miR-208 (66), and c-miR-499 (66, 78) before and after a training period, but their expressions were too low to be interpreted. Finally, Aoi and colleagues found that a 4-week cycling program downregulates c-miR-486 basal expression (66).

Other circulating miRNAs

When evaluating c-let-7d expression, a miRNA that has a crucial role in cell division and differentiation (94, 95), Nielsen and colleagues showed a downregulation of its baseline expression after a 12-week training period (71) while Bye and colleagues' observational study

(77) showed that subjects with high maximal oxygen uptake (VO_{2max}; 145.2 ± 20.7 mL/kg$^{0.75}$/min) had a lower c-let-7d expression compared with individuals engaging in similar activity levels but with lower VO_{2max} (101.1 ± 18.0 mL/ kg$^{0.75}$/min). For c-miR-21, Nielsen and colleagues reported that baseline expression was decreased after chronic PA (71), while the results obtained from Bye and colleagues' observational study shows that independently of activity levels, individuals with high VO_{2max} have decreased c-miR-21 basal expression compared with subjects with lower VO_{2max} (77). However, Baggish and colleagues found an upregulation of c-miR-21 after 13 weeks of rowing training, while Wardle and colleagues reported no alteration in expression between athletes (endurance or resistance trained) and controls but showed a significant increase in resistance trained individuals when compared with endurance athletes (72, 78).

c-miR-221 and c-miR-222 expression in response to chronic PA remains unclear. Baggish and colleagues reported increased expression of c-miR-222 after a 13-week training program (72), in line with Wardle and colleagues' findings when comparing endurance trained athletes with control individuals (78). However, Wardle and colleagues found a downregulation of c-miR-222 in resistance trained athletes, and Bye and colleagues reported a decrease in c-miR-222 expression in individuals with high VO_{2max} when compared with subjects with a lower VO_{2max} (77, 78). Similarly, various results have been found for the changes of c-miR-221 expression in response to chronic PA: Baggish and colleagues reported an increase in its baseline expression after a training period (72), whereas Wardle and colleagues found no statistical differences between trained athletes (endurance or resistance) and controls, but a downregulation in resistance-trained athletes when compared with endurance- trained athletes (78).

DISCUSSION

Potential impact of PA-induced c-miRNA changes on cancer risk, progression, and treatment

Overall, the current results available from the scientific literature suggest that several c-miRNAs are impacted by acute PA (miR-1, miR-133, miR-206, miR-208, miR-499, miR-486, miR-126, miR-146, miR-221, and miR-222) and/or by chronic PA (miR-133, miR-486, let-7d, miR-21, miR-222, and miR-221). Importantly both acute PA and chronic PA appear to induce changes in c-miRNA expression depending on exercise modalities and individuals' fitness status. While results to date are promising and suggest a clear impact of PA on miRNA

expression, comparisons between studies are challenging and efforts should be devoted toward standardized protocols and procedures for miRNA collection, analysis, and reporting. The studies included in this review suggest that PA modulates c-miRNA expression in healthy individuals. Several studies have also observed that PA can alter c-miRNA expression within disease populations such as patients with chronic kidney diseases (69) and prediabetic individuals (96). To our knowledge, however, no data exist regarding the impact of PA on miRNA in patients with cancer. PA also appears to be able to influence miRNA expression within organs other than skeletal muscles (97, 98). Taken together, these elements suggest that PA may influence c-miRNA expression in cancer patients, perhaps via c-miRNA intercellular communication. This hypothesized mechanism is illustrated in Figure R-13.

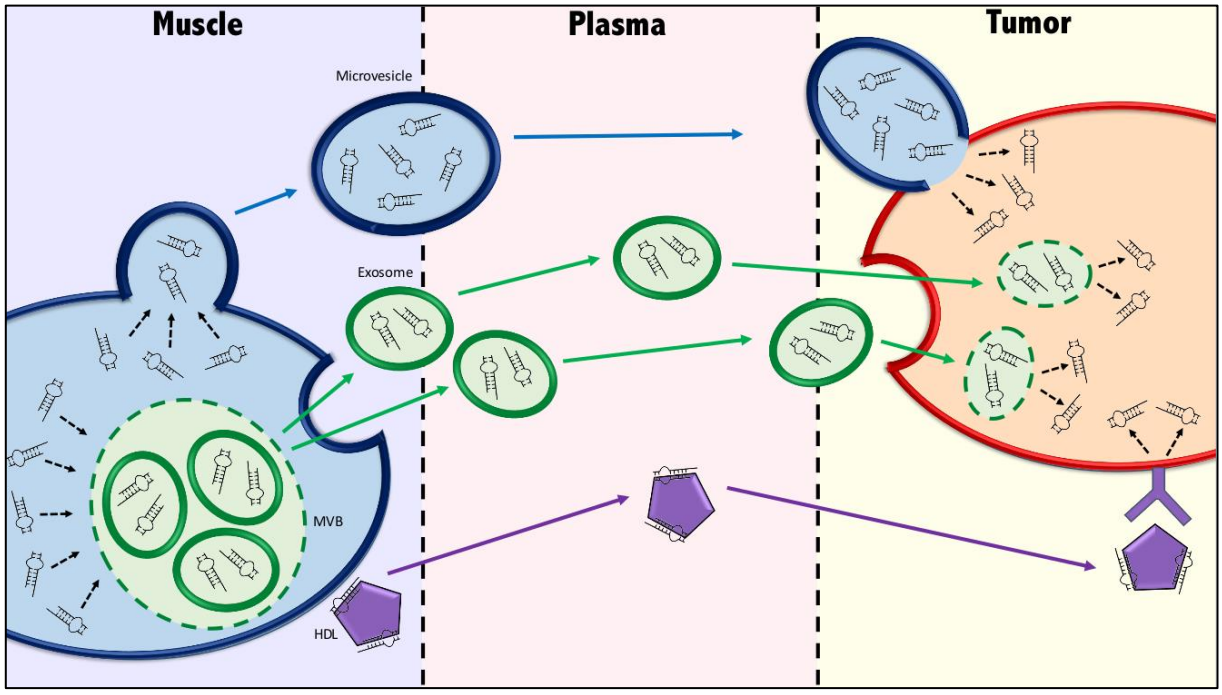

Figure R-13: Hypothesized pathways of miRNA transport from muscles to tumor cells via the circulation. miRNAs can be exported from one cell to another via plasma through various active transport systems including: exosomes developed within multivesicular bodies (MVB); released into the blood circulation after fusion of the MVBs with the plasma membrane; transport through protein or lipid-based complexes, such as HDL, and delivered to recipient cells. Abbreviations: MVB, multivesicular body.

Throughout this section, we discuss how modulation of specific c-miRNA expression by PA might impact cancer risk, progression, and treatments, and provide some examples of promising early findings. Figure R-14 presents hypothesized examples of how PA could impact cancer via c-miRNA modulation using c-miR-133, c-miR-221/222, c-miR-126, and c-let-7.

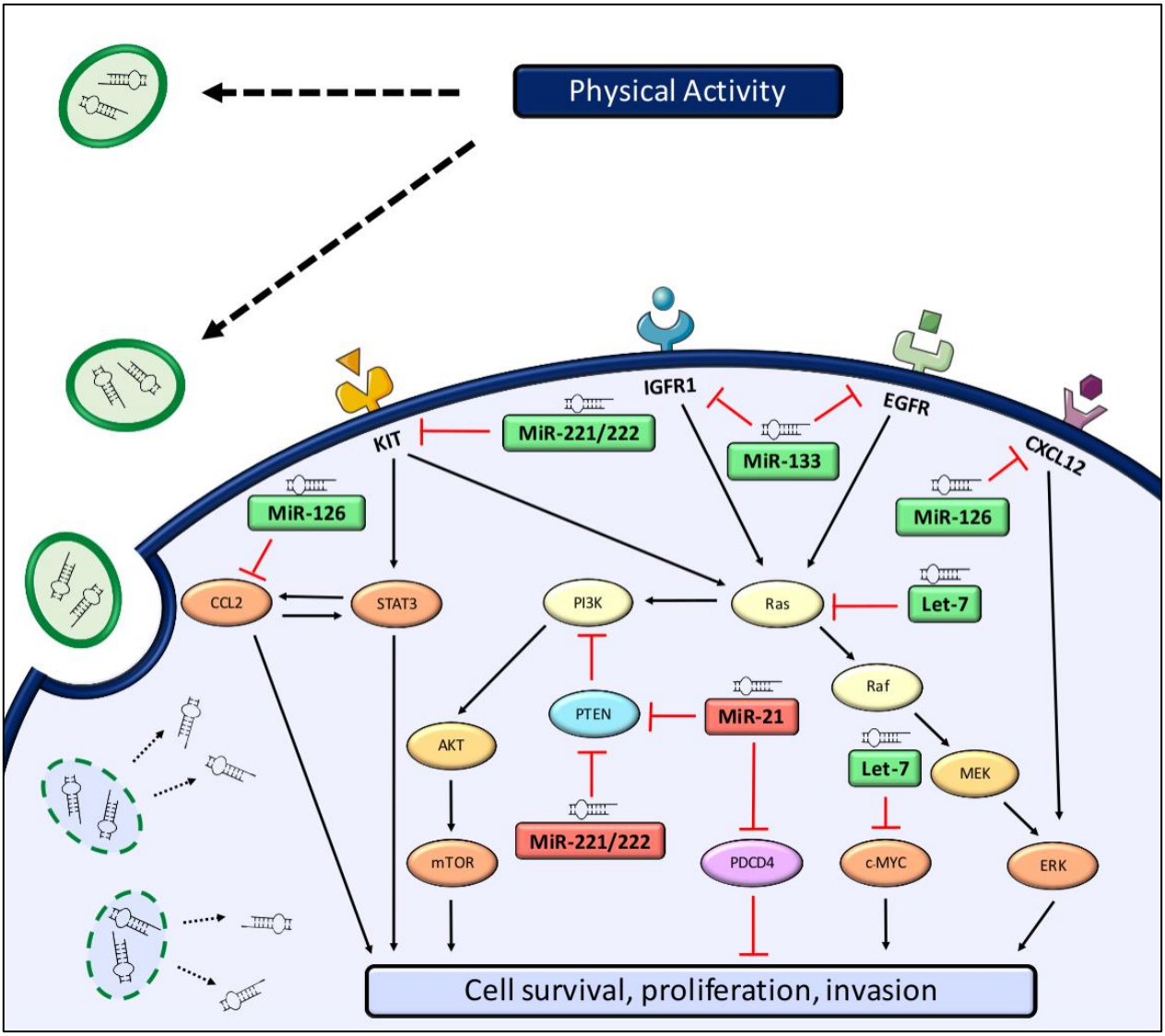

Figure R-14: Hypothesized mechanisms of action of PA on cancer risk and progression through c-miRNA modulation. Multiple c-miRNAs (miR-126, miR-221/222, miR-133 and Let-7) that impact pathways involved in cancer risk and progression have been shown to be altered by PA.

Impact of miRNAs on cancer risk and development

Multiple miRNAs are involved in DNA repair, checkpoint functions, tumor suppression, etc. (99), and their modulation by PA might play an important role in cancer risk and progression. For example, PA can alter c-miR-133 expression (48, 68, 70–73, 75, 76, 100), a well-known myoMiR participating in myoblast differentiation, which has also been identified as a tumor suppressor (101) in several cancers, including ovarian, colorectal, bladder, breast, prostate, and gastric cancers (100, 102–107). miR-133 has also been shown to be modified within muscle tissues in response to acute and chronic PA. More specifically, acute bouts of exercise seem to increase muscular miR-133 expression (108, 109), while training tends to decrease

expression (108). These data suggest that miR-133 can translocate from muscle tissue to blood vessels, and that this miRNA can impact cancer progression as depicted in Figure R-13. Several studies suggest that miR-133 targets several oncogenes, such as the EGFR (100) and the insulin-like growth factor 1 receptor (IGF1R; refs. 107, 110). When activated, those oncogenes stimulate various pathways causing deregulation in several cell processes, eventually leading to carcinogenesis. For example, activation of EGFR pathway leads to the stimulation of intracellular signaling cascades such as the MAPK/ERK pathway, which plays a role in cell-cycle progression, differentiation, proliferation, and apoptosis (111, 112). When activated, the EGFR pathways also stimulate the PI3K/AKT cascade, known for its crucial role in regulation of apoptosis and protein synthesis (113, 114). An increase in c-miR-133 by PA originating from muscle tissue, may therefore impact tumor cells and regulate target oncogenes and associated pathways.

Similarly, let-7 is a tumor suppressor and proapoptotic miRNA (115) whose expression is decreased in numerous cancers (116), and reported to be modulated in plasma by exercise (71, 77). While no direct evidence was identified in humans, in an animal model, breast tumor-bearing mice who underwent 5 weeks of interval exercise training (treadmill running) had increased let-7 expression within the tumor itself when compared with breast-tumor sedentary mice (117). Let-7 is known for inhibiting mRNA translation of well-known oncogenes, including the RAS family (HRAS, KRAS, and NRAS; ref. 13) and c-MYC (refs. 118, 119). When activated, Ras stimulates several signaling pathways, including MAPK cascade and PI3K/AKT (120), thereby influencing many cellular functions.

We must note the complex and often paradoxical role of miRNAs, with several miRNAs exerting stimulatory as well as inhibitory effects depending on cancer type or stage of the disease (121–123). miR-221 and miR-222 for example have a dual role: they can either act as tumor suppressors as oncogenes. On one hand, miR-221 and miR-222 have been found to relent cancer progression in several cancer types, and their expression in the circulation have been found to be altered by PA in several studies (49, 71, 72, 77, 78). In gastrointestinal stromal tumors, for example, miR-221 and miR-222 are thought to have prophylactic effects by negatively regulating the stem cell factor receptor KIT, and are found underexpressed in tumors when compared with healthy tissues (124–126). KIT is a receptor tyrosine-kinase (RTK), and its activation leads to the stimulation of several intracellular signaling pathways, including the STAT3, PI3K, phospholipase C (PLC), and the MAPK cascade (127–129). KIT

promotes cell survival, proliferation, and motility (130), and its inhibition by miR-221 and miR-222 therefore contributes to carcinogenesis suppression. Modulation of those miRNAs by PA could thereby inhibit KIT activation, consequently lowering the risk of developing cancer.

On the other hand, miR-222 and miR-221 have been shown to act as oncomiRs in other cancer types. In lung and liver cancer for example, these miRNAs have been shown to inhibit the action of the PTEN, known to inhibit MAPK/ERK pathway, and the tissue inhibitor of metalloproteinases-3 (TIMP3), thereby enhancing cell proliferation and migration through PI3K/AKT pathway (131). The dual role played by miR-222 and miR-221 suggest that clarifying the influence of exercise modalities (endurance vs. resistance, short vs. long duration, regular vs. irregular training, etc.), tissue and/or cancer site of interest will be extremely important in subsequent research.

Impact of miRNAs on cancer invasion and metastasis

PA also modulates c-miRNAs involved in cell proliferation, invasion and metastasis (Figure R-14). For example, miR-21 is altered by resistance and endurance training (71, 72, 78) and is also known for participating in tumor invasion. In fact, in vitro studies have shown that in several cancer types, miR-21 knockdown mice displayed suppression of cell proliferation and tumor growth (132), as well as reduced invasion and metastasis (132–134). Furthermore miR-21 negatively regulates tumor suppressor programmed cell death 4 (PDCD4; ref. 135) and downstream signaling targets (136, 137) in colorectal cell lines. The results in this review (Table R-4, Table R-5 and Table R-6) suggest that acute exercise can transiently upregulate c-miR-21 expression (65, 49), while chronic exercise (which reflects physiologic adaptations) can also lead to alterations in miR-21 expression within circulation (71, 77); however, the influence of chronic PA is less clear. By lowering miR-21 expression within cancer cells, PA may restore PDCD4 and PTEN activation and limit cancer proliferation. This is supported by literature that suggests PA is able to modulate miR-21 within multiple tissues/fluids, for example muscle tissue in mice (138) as well as in plasma in healthy human subjects (49, 65, 71, 77, 78), as well as in tumors of breast tumor-bearing mice (Figure R-13; ref. 117).

The upregulation of c-miR-126 by acute PA (48, 72) might also represent a pathway through which PA impacts cancer progression. In breast cancer, miR-126 regulates the tumor microenvironment composition by directly inhibiting stromal cell–derived factor-1 alpha

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

(CXCL12) expression and indirectly suppressing chemokine ligand 2 (CCL2) expression in cancer cells; ref. 139). These two chemokines play a role in recruiting stromal cells to the primary tumor microenvironment (140, 141), thereby leading to cancer cell invasion and metastasis. Therefore, the increase in c-miR-126 expression in response to acute PA followed by active transport into tumor cells could lead to inhibition of CXCL12 and CCL2, consequently suppressing cancer expansion by modifying the tumor microenvironment composition (48).

Overall, PA-induced c-miRNA expression changes could have the potential to impact tumorigenesis and cancer development. Importantly, several studies suggest that reexpression of tumor suppressor miRNAs within tumor tissues can inhibit tumor growth, thereby representing a promising therapeutic tool against cancer (18, 142–144). For example, reexpression of let-7 within various tumor types have been proven to slow cancer growth (94, 145–147) and is thus considered a promising tool against cancers underexpressing let-7 family members (145, 148). Additional research, particularly large intervention studies in populations of cancer patients, is needed to determine the exact impact of PA on miRNA expression and potential roles in cancer therapy.

Limitations of the research to date

Investigating c-miRNAs in response to PA presents several challenges. First, to date, there are few studies that have compared similar c-miRNAs using comparable methods to enable direct comparisons across studies. Although some consistency has been observed for several c-miRNAs such as miR-133 (48, 70, 72, 73, 75, 76), contradictory results have often been observed. This inconsistency can largely be attributed to discrepancy in methods across studies, including: (i) differences in sample collection; (ii) postprocessing of samples, and (iii) the use of serum or plasma for miRNA extraction.

Different normalization strategies have been used between the reviewed studies. The current results available on c-miRNA expression changes in response to PA should therefore be carefully interpreted. For example, Nielsen and colleagues used a stable expressed c-miRNA to account for the biological variation between samples, whereas Baggish and colleagues used a synthetic spike in approach (71, 72). Some studies also included a hemolysis control phase in the study design, as it has been shown that hemolysis occurring during blood collection has substantial impact on the miRNA content in plasma/serum (149). This issue occurs because

erythrocytes contain numerous miRNAs that unavoidably will contaminate a plasma sample if the erythrocyte bursts during sampling (150).

Another methodologic factor likely to impact the reliability of the results obtained from the various studies reviewed here is the fact that miRNA concentrations vary between serum and corresponding plasma samples (151). Analyzing and comparing miRNA expression from serum and plasma within or between studies should be done carefully.

To date, miRNA expression in response to PA has mainly been measured in the circulation or muscles, but it would also be interesting to study the changes in miRNA expression in other tissues. More specifically, investigating miRNA expression after PA in target tissues, such as tumor tissues, would provide novel data of how PA can impact cancer through miRNA modulation. Tissue-specific approaches would also aid in a better understanding of the role of c-miRNAs in specific cancer sites that may result in the development of targeted novel therapies.

CONCLUSION AND FUTURE DIRECTIONS

PA represents a lifestyle behavior that influences the expression of several c-miRNAs, some of which have been associated with carcinogenesis and cancer progression. Our results suggest that alteration of miRNAs within the circulation is dependent on the type/modality/frequency of exercise as well as on the participants' characteristics. Furthermore, the evidence to date on c- miRNA expression in response to PA is limited by small sample sizes without standardized measures of miRNA or physical activity. miRNAs appear to have a meaningful impact on cancer risk and progression; however, the effect varies between cancer types. Therefore, it appears essential to provide a better under- standing of how various types of PA in a specific population could impact c-miRNA expression: it could represent a useful tool for healthcare practioners in establishing and monitoring PA programs for patients with cancer.

Future research should focus on large epidemiologic studies with standardized blood storage and collection as well as standardized measures of c-miRNA expression and objective measures of PA. Additional consistency is needed to provide more meaningful conclusions as well as standardization of PA measurement. Further investigation into the effects of PA on miRNAs is necessary and could have implication both for cancer prevention, treatment, and survival outcomes.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

Table R-4: Effect of acute physical activity on the expression of c-miRNAs in healthy and normally active subjects

Reference	Subjects	Type of acute PA	Plasma/serum base	No of miRNAs screened	miRNAs altered by PA (compared to basal values)			Unaltered miRNAs among the measured ones		miRNAs not detectable or unreliable	
					Immediately post-ex	During recovery					
Acute endurance exercise											
Aoi et al., 2013 (66)	10 males 21.5 ± 4.5 yrs	60-minute cycling exercise (70% $\dot{V}O_2$max)	Serum	—	miR-486↓	Post 3h: miR-486↓		NONE		miR-1 miR-133a miR-133b	miR-206 miR-208b miR-499
Banzet et al., 2013 (68)	9 males 27 to 36 yrs	30-minute uphill walking exercise (concentric)	Plasma	—	miR-181b↑ miR-214↑ miR-499-5p↑	Post 2h and 6h: miR-133a↑ miR-133b↑ Post 2h, 6h, and 1 day: miR-499-5p↑	 miR-1↑ miR-208b↑	 miR-181a			miR-206 miR-208a
Banzet et al., 2013 (68)	9 males 27 to 36 yrs	30-minute downhill walking exercise (eccentric)	Plasma	—	NONE	Post 2h and 6h: miR-133a↑ miR-133b↑ Post 2h, 6h, and 1 day: miR-499-5p↑ Post 6h: miR-208b↑	 miR-1↑ miR-214	 miR-499-5p miR-181b	 miR-181a miR-208a	 miR-206	
Uhlemann et al., 2014 (48)	7 males and 6 females 30.4 ± 2.0 yrs	Maximal cycle ergometry test	Plasma	—	miRNA-126↑	No recovery blood draw time points		miR-133			
Van Craenenbroeck et al., 2015 (69)	7 males and 5 females 43.4 ± 4.7 yrs	Maximal cycle ergometry test	Plasma	—	miR-150↑	No recovery blood draw time points		miR-146a miR-21	miR-126 miR-210		
Acute resistance exercise											
Sawada et al., 2013 (67)	3 males 29.9 ± 1.2 yrs	Bench press and leg press (5 sets of 10 reps with 1 min rest between sets)	Serum	1,458	NONE	Post 1 day: miR-149*↑ Post 3 days: miR-146a↓ miR-221↓		miR-20a miR-21 miR-133a miR-210	miR-222 miR-328 miR-1908	miR-299-5p	

Abbreviations: ex, exercise; wk, week; yrs, years.
Results of a systematic review of studies investigating the impact of acute PA on c-miRNA expression in healthy and "normal" activity levels in human subjects either immediately after acute exercise completion and/or several hours up to a couple days after.

Table R-5: Effect of acute physical activity on the expression of c-miRNAs at various time points in healhy, trained subjects

Reference	Subjects	Training program	Type of acute PA	Plasma/ Serum base	No of miRNAs screened	miRNAs altered by PA (compared to basal values)			Unaltered miRNAs among the measured ones		miRNAs not detectable or unreliable
						Immediately post-ex		During recovery			
Acute endurance exercise											
Baggish et al., 2011 (49)	10 males 19.1 ± 0.6 yrs	13 weeks rowing training	Maximal cycle ergometry test	Plasma	—	miR-146a↑ miR-222↑	miR-21↑ miR-221↑	No recovery blood draw time points	miR-20a miR-328	miR-210 miR-133a	
Tonevitsky et al., 2013 (65)	8 males 21.7 ± 2.6 yrs	Regular ski training	30-minute run on a treadmill (80% $\dot{V}O_2$max)	Serum	200	miR-24-2-5p↑ miR-27a-5p↑ miR-181a-5p↑		Post 1/2h and post 1h: miR-24-2p↓ miR-27a-5p↓ miR-181-5p↓ Post 1h: miR-21-5p↑			
Mooren et al., 2014 (70)	14 males 42.8 ± 6.0 yrs	Regular endurance training	Marathon run	Plasma	—	miR-1↑ miR-133a↑ miR-206↑	miR-208b↑ miR-499↑	Post 1 day: miR-1↑ miR-133a↑	miR-206↑	miR-21 miR-155	
Nielsen et al., 2014 (71)	13 males 28 ± 8 yrs	12 weeks endurance training (cycle ergometry)	60-minute cycle ergometry exercise (65% Pmax)	Plasma	188	miR-221↓ miR-30b↓ let7i↓ miR-652↓	miR-106a↓ miR-146a↓ miR-151-3p↓ miR-151-5p↓	Post 1h: miR-338-3p↑ miR-330-3p↑ miR-223↑ miR-139-5p↑ Post 3h: miR-1↑ miR-133a↑‡	miR-143↑ miR-145↑‡ miR-424↑‡ miR-133b↑‡		
Baggish et al., 2014 (72)	21 males 51.8 ± 1.4 yrs	Regular endurance training	Marathon run	Plasma	—	miR-126↑ miR-1↑ miR-133a↑ miR-208a↑	miR-499-5p↑ miR-208a↑ miR-134↑ miR-146a↑	Post 1 day: miR-499-5p↑			
Uhlemann et al., 2014 (48)	13 males 32.4 ± 2.3 yrs	Regular endurance training	4 h cycle ergometry exercise (70% anaerobic threshold)	Plasma	—	miR-126↑		Post 1h: NONE Post 1 day: NONE		miR-133	
Uhlemann et al., 2014 (48)	22 males 56.8 ± 5.2 yrs	Regular endurance training	Marathon run	Plasma	—	miR-126↑ miR-133↑		No recovery blood draw time points			
Gomes et al., 2014 (73)	5 males 31.6 ± 4.39 yrs	Regular endurance training	Half-marathon run	Plasma	—	miR-1↑ miR-133a↑ miR-206↑		No recovery blood draw time points			
Cui et al., 2015 (74)	18 males 20.23 ± 0.97 yrs	Regular exercise training (activity type not specified)	Cycle ergometry sprint intervals (2 sets of 30s all out sprints with 4 min rest between sets)	Plasma	—	miR-1↓ miR-133b↓ miR-133a↓	miR-122↓ miR-16↓	No recovery blood draw time points		miR-206 miR-499	
Clauss et al., 2016 (75)	15 males 40.1 ± 1.4 yrs	Marathon training ≤40 km/wk for 10 wk	Marathon run	Plasma	—	miR-1↑ miR-133a↑		Post 1 day: miR-133a↑		miR-30a miR-26a miR-29b	
Clauss et al., 2016 (75)	15 males 40.0 ± 1.7 yrs	Marathon training ≥55 km/wk for 10 wk	Marathon run	Plasma	—	miR-1↑ miR-133a↑ miR-30a↑		Post 1 day: NONE		miR-26a miR-29b	
Min et al., 2016 (76)	20 males and 8 females 53.0 ± 6.5 yrs	Regular endurance training	Marathon run	Plasma	—	miR-1↑ miR-133a↑ miR-206↑		Post 1 day: miR-499-5p↑			
Acute resistance exercise											
Uhlemann et al., 2014 (48)	4 males and 7 females 37 ± 2 yrs	Nonsupervised regular training (activity type not specified)	Lat pulldown, leg press and butterfly (3 sets of 15 reps with 1 min rest between sets)	Plasma	—	miR-133↑		Post 1h: NONE		miR-126	

Abbreviations: ex, exercise; no, number; reps, repetitions; wk, week; yrs, years.

‡Borderline significant miRNAs.

Results of a systematic review of studies investigating the impact of acute PA on c-miRNA expression in healthy trained human subjects either immediately after acute exercise completion and/or several hours up to a couple days after.

Table R-6: Effect of chronic/regular/long-term physical activity on c-miRNA levels in healthy subjects

References	Type of study	Participants characteristics	Training program	Blood draw timing and plasma /serum base	No of miRNAs screened	miRNAs altered by chronic PA		miRNAs measured but not altered by chronic PA		miRNAs not detectable/unreliable	
Chronic endurance exercise											
Baggish et al., 2011 (49)	Intervention	10 males 19.1 ± 0.6 yrs Very trained	13 weeks rowing training	At least 12 h post exercise -plasma	—	miR-146a↑ miR-222↑ miR-21↑	miR-221↑ miR-20a↑	miR-328 miR-210	miR-133a		
Aoi et al., 2013 (66)	Intervention	10 males 21.5 ± 4.5 yrs Normally active	4 weeks cycling training	2 days post training period -serum	—	miR-486↓				miR-1 miR-133a miR-133b miR-206	miR-208b miR-486 miR-499
Nielsen et al., 2014 (71)	Intervention	7 males 28 ± 5 yrs Normally active	12 weeks of supervised training on cycle ergometer	3 days and 5 days post training period -plasma	188	miR-342-3p↓ let-7d↓ miR-103↑ miR-107↑ miR-766↓ miR-25↓ miR-148a↓	miR-185↓ miR-21↓ miR-148b↓‡ miR-133a↓‡ miR-92a↓‡ miR-29b↓‡				
Wardle et al., 2015 (78)	Observational	20 males 22.6 ± 3.7 yrs Trained (endurance athletes) vs. control (not active)	Trained: 13 h of training per week	At least 12 h post PA -plasma	—	miR-222↑		miR-16-2 miR-20a-1 miR-103a miR-192	miR-451 miR-21 miR-146a miR-221	miR-1 miR-133a miR-206 miR-499	
Clauss et al., 2016 (75)	Intervention	15 males 40.1 ± 1.4 yrs Normally active	Marathon training: Running of ≤40 km/ week for 10 weeks	After the training period -plasma	—	NONE		miR-1 miR-133a miR-30a	miR-26a miR-29b		
Clauss et al., 2016 (75)	Intervention	15 males 40.0 ± 1.7 yrs Trained	Marathon training: Running of ≥55 km/ week for 10 weeks	After the training period -plasma	—	NONE		miR-1 miR-133a miR-30a	miR-26a miR-29b		
Chronic resistance exercise											
Wardle et al., 2015 (78)	Observational	20 males 22.2 ± 2.1 yrs Trained (resistance athletes) vs. Control (not active)	Trained: 13 h of training per week	At least 12 h post PA -plasma	—	miR-222↓		miR-16-2 miR-20a-1 miR-103a miR-192	miR-451 miR-21 miR-146a miR-221	miR-1 miR-133a miR-206 miR-499	
Unspecified chronic exercise (endurance and/or resistance exercise)											
Bye et al., 2013 (77)	Observational	miRNAscreening: 12 males 12 and females 40–45 yrs miRNAvalidation: 38 males 38 and females 40–45 yrs Active vs. nonactive based on $\dot{V}O_2$max	Training included in lifestyle	Before the start of the exercise test measuring the subjects' $\dot{V}O_2$max -serum	720	Screening: Males and females: miR-210↓ miR-125a ↓ miR-652 ↑ Males only: miR-151↑ miR-29a↓ let-7d↓ Validation cohort: Males and females: miR-222↓ miR-210↓ Males only: miR-21↓	Women only: miR-210↓ miR-125a↓ miR-21↓				miR-125 in the validation cohort

Abbreviations: no, number; yrs, years.
‡Borderline significant miRNAs
Results of a literature review of intervention and observational studies investigating the impact of chronic PA on c-miRNA expression in humans.

AUTHORS CONTRIBUTION

D. Brenner and C. Friedenreich designed the study; S. Dufresne collected the data; S. Dufresne, A. Rébillard, D. Brenner, P. Muti, C. Friedenreich analyzed the data, S. Dufresne, D. Brenner and A. Rébillard wrote the manuscript.

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IV – Exercise training as a modulator of epigenetic events in prostate tumors

As hypothesized in the review presented above, modulation of c-miRNAs by physical activity may reflect a larger miRNA trafficking between different organs, including the tumor. Nevertheless, the impact of physical activity on tumor miRNAs and more broadly on the tumor epigenetic profile remains largely unknown. Epigenetics represents a growing focus of research, as it is recognized for playing a crucial role in cancer initiation and growth. It involves modification in gene expression without changing the DNA sequence and is comprised of three main processes: (1) DNA methylation, (2) histone modifications, and (3) miRNA expression (Sharma, Kelly, and Jones 2010). Importantly, these mechanisms can be influenced by lifestyle factors such as diet, chemical exposure, or physical activity (Alegría-Torres, Baccarelli, and Bollati 2011). Indeed, physical activity is able to modify epigenetic processes in several tissues and fluids (Ling and Rönn 2014), but its ability to impact the epigenetic profile of the tumor is largely unknown.

In this study, we aimed to evaluate whether physical activity could alter tumor epigenetic mechanisms in a preclinical model of prostate cancer. Male Copenhagen rats were implanted with prostate tumors and randomized to either sedentary control or exercise training as described in a previously published study (Gueritat et al. 2014). The tumor tissue was collected at the end of the experiment for molecular analysis.

Firstly, we observed that exercise training was able to impact tumor DNA methylation, which corresponds to the covalent addition of a methyl group on the 5-carbon on cytosine residues (5mC). Cancer cells are characterized by genome-wide DNA hypomethylation as well as hypermethylation of specific CpG islands (Ehrlich 2002). These features increase genomic instability and participate in the inactivation of tumor suppressor genes while enhancing oncogene expression (Herman and Baylin 2003; Ehrlich 2009). Interestingly, we found that global DNA methylation levels of the tumor were significantly higher in exercising rats compared to the sedentary controls. Furthermore, exercise training was associated with lower gene expression of DNA methyltransferase (DNMTs), a class of enzymes mediating DNA methylation. Hence, the offset of aberrant methylation patterns by exercise training may participate in the anti-cancer effects of exercise.

In contrast, no effect of exercise training was found on histone acetylation. Histone acetylation is dependent of the balance between two enzymes with opposite effects: histone acetyltransferase (HAT) and histone deacetylase (HDAC). HATs transfer acetyl groups of lysine residues on histones, and HDACs remove them. By loosening or tightening the chromatin structure, HATs and HDACs increase and repress gene expression, respectively (Iizuka and Smith 2003; Ropero and Esteller 2007). In prostate cancer, high HDAC activity is associated with increased levels of serum PSA (Weichert et al. 2008) and poor outcomes (Ellinger et al. 2010). In our study, there was no difference between sedentary and trained rats regarding HDAC activity, HDAC1 and HDAC2 protein expression, nor in the protein ratio between histone H3 and acetylated histone H3 measured in the tumor tissue. All these results suggest that exercise training did not alter histone acetylation in the prostate tumor tissue.

Finally, we evaluated the expression of 1,166 miRNAs which, as previously mentioned, regulate gene expression through post-transcriptional modifications. The results revealed 75 miRNAs were differently expressed between sedentary control and exercise rats. We further confirmed by RT-qPCR that exercise training increased miR-27-5p expression, a miRNA which has been shown to inhibit prostate cancer cell proliferation *in vitro* (Barros-Silva et al. 2018)

Overall, this study shows for the first time that physical activity is able to extensively alter the tumor epigenetic profile in a preclinical model of prostate cancer. More specifically, exercise training was able to modulate DNA methylation and miRNA expression in the tumor tissue, but not histone acetylation. This may represent a novel pathway mediating the tumor-suppressive role of physical activity and opens new horizons for the exercise oncology field.

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Exercise training as a modulator of epigenetic events in prostate tumors

Suzanne Dufresne[1], Jordan Guéritat[1], Carmen P Wong[2,3], Amin Isanejad[1], Emily Ho[2,3,4], Eva Serna[5], Marie-Carmen Gomez-Cabrera[5], Amélie Rébillard[1]

1. M2S Laboratory, EA 1274, Université Rennes 2, ENS Rennes, 35170 Bruz, France
2. School of Biological & Population Health Sciences, College of Public Health & Human Sciences, 211 Milam Hall, Oregon State University, Corvallis, OR 97331, USA
3. Linus Pauling Institute, Oregon State University, 307 Linus Pauling Science Center, Corvallis, OR 97331, USA
4. Moore Family Center for Whole Grain Foods, Nutrition & Preventive Health, Oregon State University, Corvallis, OR 97331, USA
5. Freshage Research Group-Dept. Physiology-University of Valencia, CIBERFES, INCLIVA, 46010 Valencia, Spain

ABSTRACT

Regular exercise is increasingly recognized as an effective strategy to improve cancer prevention and prognosis. Several biological mechanisms mediating these benefits have been proposed, but the role of epigenetics remains largely unknown. Since epigenetics is highly susceptible to lifestyle factors, we hypothesized that exercise training could affect epigenetic modifications in cancer tissues.

Two weeks after tumor implantation, Copenhagen rats bearing R3327 Dunning AT1 tumors were randomized to either inactive control or exercise training group. The exercise training program consisted of four weeks of treadmill running, and rats were sacrificed 48h after the last training session. DNA methylation, histone acetylation and microRNA expression were analyzed in the tumor tissue. Exercise training increased global DNA methylation and decreased DNA methyltransferases mRNA expression in the tumor tissue. Furthermore, tumor microRNA expression profile differed between sedentary and trained rats. However, no effect of exercise training was observed on histone acetylation markers. Therefore, exercise training may reverse some of the cancer-related epigenetic alterations in the tumor tissue.

Overall, our study is the first to show that exercise training is able to counteract DNA methylation and miRNA expression in the tumor tissue. These novel findings suggest the existence of an epigenetic link between exercise and cancer and may explain, to some extent, the decreased tumor growth induced by exercise observed in numerous preclinical models.

Key words: physical activity, exercise training, prostate cancer, epigenetics, DNA methylation, histone acetylation, microRNA

INTRODUCTION

Prostate cancer (PCa) is the second most common cancer in men worldwide and the sixth leading cause of cancer-related death. Besides genetic mutations, many epigenetic alterations are involved in the development and progression of PCa (1–3). Epigenetic corresponds to heritable changes in gene expression that are not attributable to alterations in the DNA sequence. It comprises three main processes: DNA methylation, histone modifications and microRNA (miRNA) expression. Global DNA hypomethylation is now a well-recognized cancer feature and is frequently observed in malignant prostate tissues (4,5). It can facilitate chromosome instability, aberrant transcription, reactivation of transposable elements and occurs mainly in advanced stages of PCa. Conversely, local gene-specific hypermethylation is one of the first aberrations observed in early lesions and persisting throughout disease progression (6,7). Concerning histone modifications, elevated histone deacetylase (HDAC) expression is associated with increased prostate specific antigen (PSA) levels, increased proliferative potential and poor outcome (8). In the same line, miRNA deregulation also contributes to prostate tumorigenesis and various miRNAs have been proposed as useful tools for diagnosis, prognosis and therapy in PCa (9,10). In regards with these encouraging results, epigenetic modulators addressing different targets have been tested in preclinical models and more recently in PCa patients for their anti-cancer properties (11,12). These include DNA methyltransferase (DNMT) inhibitors (azacytidine, decitabine) (13,14), HDAC inhibitors (vorinostat, romidpesin, belinostat, *etc*.) (15) and miRNA regulators (16,17). In addition, adjusting lifestyle factors could also represent a promising strategy. There is a growing interest in incorporating exercise in the oncology setting. Randomized clinical trials have shown that regular exercise led to better physical fitness (cardiorespiratory capacity, muscle strength), reduced fatigue and improved quality of life in PCa patients (18–21). Observational studies also suggested that being physically active after diagnosis reduces overall- and cancer-specific mortality (22–25). *In vivo*, nine studies, including two conducted by our team, have shown that exercise reduced tumor growth (26–34), while seven others found no effect (35–40). Recent evidence suggests that exercise could alter epigenetic marks in several tissues and fluids of the body, both in healthy (41) and cancer populations (42,43). However, less is known about its direct impact on the tumor itself. Our current exploratory study aims to evaluate the impact of exercise training on tumor epigenetic modifications using a PCa pre-clinical model.

MATERIALS AND METHODS

Animals and tumor model

The use and care of animals were conducted in accordance with the ethical standards of the European Community (directive 86/609/EEC) and followed the guidelines of the French Agriculture Ministry under the authorization to experiment on living animals n° R-2012-AR-01 and the site agreement n° A3523840. The tumor samples used and analyzed in this study had been collected in a previous project conducted by our team (29) Briefly, male Copenhagen rats (Charles River Laboratories, Saint Germain Nuelles, France) were implanted with R3327 Dunning AT1 tumors (Cell Line Service, Eppelheim, Germany) and were randomized into four experimental groups 15 days after the surgery. The present study includes only two of these groups: control sedentary (CTL; n=8); and exercise training (ET; n=8). Rats in the exercise training group ran on a treadmill for 40-60 min with a 10° incline at 20-25 m/min five days per week for a total of four weeks. 48 hours after the last exercise session, all animals were sacrificed. Tumor tissues were retrieved to perform biological analysis (29).

Global DNA methylation

Genomic DNA was isolated from tumor tissues by using DNeasy Blood and Tissue kit (Qiagen, Valencia, CA). Global methylation status was then assessed with the MethylFlash™ Methylated DNA Quantification Kit (Epigentek, Brooklyn, NY) according to manufacturer's instructions.

Western blot analysis

Five representative tumor samples of each group were lysed in buffer (50 mM Tris-HCl, 10 mM EDTA, 1% SDS) and protein concentration was determined using Lowry protein assay. Proteins (50µg) were separated by SDS-PAGE and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA, USA). After being blocked with 5% BSA or non-fat dry milk in TBS-Tween (0.05%), membranes were incubated overnight at 4°C with the appropriate primary antibodies (Table R-7). After being washed three times with TBS-Tween (0.05%), membranes were incubated with secondary antibodies for one hour at room temperature. Immunoreactive bands were visualized with Odyssey Infrared Imaging System (LI-COR Biosciences, Lincoln, NE, USA).

Table R-7: List of western blot antibodies

Protein	Molecular weight	Reference	Dilution
H3Ac	15.4 kDa	Biolegend 698401	1/1000
H3	17 kDa	Biolegend 601901	1/1000
cCASP3	17, 19 kDa	Cell signaling 9661	1/500
HDAC1	60 kDa	Santa Cruz sc-7872	1/1000
HDAC2	60 kDa	Santa Cruz sc-7899	1/1000
HSC70	70 kDa	Santa Cruz sc-7298	1/5000

RNA extraction and quantification by quantitative real-time PCR

The prostate cancer tissue was homogenized in Trizol reagent (Life Technologies, Carlsbad, USA). RNA quantity was measured on NanoDrop and RNA integrity was determined by agarose gel electrophoresis with ethidium bromide. Reverse transcription (RT) reaction was prepared from 1 µg RNA using the SuperScript III First-Strand Synthesis Supermix for qRT-PCR (Life Technologies). Gene expression was quantified by qPCR, and the list of primers used are listed in Table R-8.

Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) was used for qPCR reactions on 7900 HT Fast Real-Time PCR System (Applied Biosystems). PCR conditions were: 95°C for 20 s, followed by 40 cycles of denaturing at 95°C for 1 s, annealing and extension at 60°C (55°C for Dnmt3b) for 20 s, followed by a dissociation curve at 95°C for 15 s, 60°C for 15 s, and 95°C for 15 s. Results were determined using the standard curve method. A dilution series of template DNA served as internal standard for quantification. Data represent the copy number of the gene of interest normalized to the copy number of 18S housekeeping gene, and normalized fold-change relative to control.

Table R-8: List of primers used for RT-qPCR analysis

Gene	Forward (5' → 3')	Reverse (5' → 3')
Dnmt1	TACGCCAAGGTTTGAGTCCCC	CCCAGTCGGTAGACAACACC
Dnmt3a	GGCCCATTCGATCTGGTGA	CTTGGCTATTCTGCCGTGTTC
Dnmt3b	GTTAAAGAAAGTACAGACAATAACCAC	TCTGATGACTGGCACACTCC

HDAC activity assay

HDAC activity assays were performed by the Linus Pauling Institute Cancer Chemoprevention Program Core Laboratory (Oregon State University) as previously described (44). Substrates

and standards for the assay were custom synthesized by AAPPTec, LLC (Louisville, KY). Prostate protein lysates were prepared in RIPA buffer. HDAC activities were reported as pmol/min/mg protein and normalized to control group.

Expression profiling of small non-coding RNAs in prostate tumor tissue

Small non-coding RNA expression profiling was performed by using GeneChip miRNA 3.0 Array (Affymetrix) on four tumor samples/group of animals. The array compromised of 680 miRNAs and 486 pre-miRNAs specifically of Rattus norvegicus. Microarray experiments were conducted according to the manufacturer's instructions. Briefly, 500 ng total RNA was labeled with FlashTag Biotin HSR RNA Labeling Kit from Affymetrix. The labeling reaction was hybridized on the miRNA Array in Affymetrix Hybridization Oven 640 (Affymetrix) at 48°C for 17 h. The arrays were stained with Fluidics Station 450 using fluidics script FS450_0002 (Affymetrix), and then scanned on GeneChip Scanner 3000 7G (Affymetrix). GeneChip Command Console Software supplied by Affymetrix was used to perform gene expression analysis. miRNA probe outliers were defined as per the manufacturer's instructions (Affymetrix) and further analyzed for data summarization, normalization and quality control by using the web-based miRNA QC Tool software (www.affymetrix.com).

Data analysis of microarrays

Data were analyzed and statistically filtered using software Partek Genomic Suite 6.6 (Partek Inc.). Input files were normalized with the RMA algorithm. A one-way ANOVA was performed with the Partek Genomics Suite across all samples. Statistically significant miRNAs between different groups studied were identified using a model analysis of variance of P value ≤ 0.05. The imported data were analyzed by Principal Components Analysis to determine the significant sources of variability in the data. PCA reduces the complexity of high-dimensional data and simplifies the task of identifying patterns and sources of variability in a large data set. The samples (8 biological replicates, each hybridized to a separate Genechip) are represented by the spheres in the three-dimensional plot. The distance between any pair of points is related to the similarity between the two samples in high-dimensional space (in this case, each variable corresponds as one dimensional space). Samples that are near each other

in the plot are similar in a large number of variables. Conversely, samples that are far apart in the plot are different in a large number of variables.

MicroRNA quantification by quantitative real-time PCR

Five tumor samples per group were homogenized in 400 µL of Trizol reagent (Invitrogen). RNA quantity and quality were measured with a NanoDrop ND-1000 spectrometer and RNA integrity was assessed by standard denaturing agarose gel electrophoresis. Mature miRNA expression was quantified by quantitative real-time PCR using the miScript Primer assay for rn-miR27a* (Qiagen) and PCR Starter kit (Qiagen) according to the manufacturer's protocol. Reverse transcription was carried out on 1 µg of total RNA. qPCR reactions were performed with Taqman and the PCR conditions were: 40 cycles of 95°C for 15 s and 60°C for 1 min. Results were normalized according to RNU6-2 (housekeeping control for miRNAs, Qiagen). The threshold cycle (Ct) values was determined, and then the $2^{-\Delta\Delta Ct}$ method was used to analyze the results.

Statistics

Statistical analyses were performed in Prism6 (GraphPad softwares, San Diego, CA, USA). After testing for normality, two-tailed t-tests or Mann-Whitney tests were performed accordingly. Comparisons were considered significant for *p* values below 0.05. Data are represented as means ± SEM.

RESULTS

Exercise training increases global DNA methylation and down-regulates gene expression of Dnmts in the tumor tissue

As previously described by Guéritat et al., a 4-week treadmill training program slowed down prostate tumor growth in Copenhagen rats through decreased proliferation (29). Indeed, tumor volume 21 days after treadmill running initiation was reduced in trained rats compared to controls, averaging 6.05±0.68 cm3 in inactive rats compared to 3.61±0.81 cm^3 in trained rats (29). Furthermore, we found that trained rats displayed increased apoptosis (41.7%), revealed by cleaved caspase-3 (cCASP3) expression (p=0.0334) (Figure R-15.A. and Figure R-

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

15.B.). By using a genome-wide DNA methylation array in these samples, global DNA methylation was increased by 58.9% in prostate tumor in response to exercise training (Figure R-15.C.) (p=0.0149). In addition, lower *Dnmt* gene expression was observed in prostate tumor of trained rats compared to their control counterparts, with a 44.38%, 55.96% and 45.03% decrease in fold change for *Dnmt1*, *Dnmt3a* and *Dnmt3b* respectively (Figure R-15.D.) (p=0.0117; p=0.0003 and p=0.0199 for *Dnmt1*, *Dnmt3a* and *Dnmt3b*, respectively). These results suggest that exercise training is able to modulate DNA methylation processes in the tumor tissue.

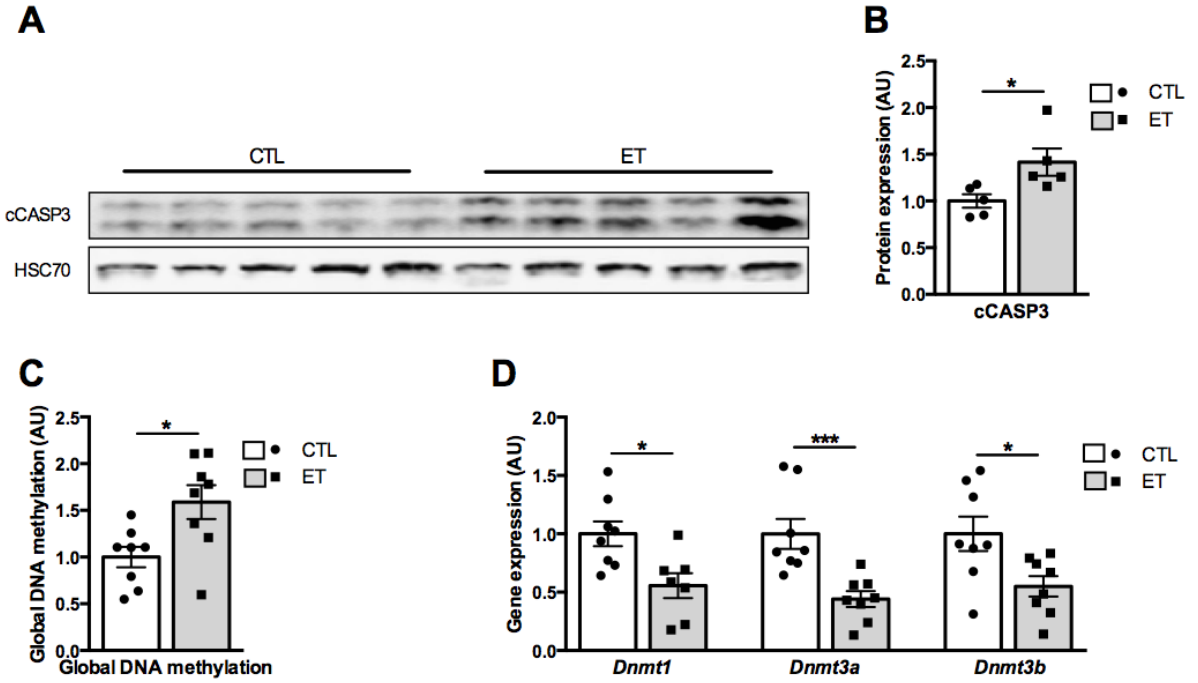

*Figure R-15: Apoptosis and DNA methylation (A) Western blot images for cCASP3 and HSC70 measured in the tumor tissue. (B) Quantification of tumor cCASP3 protein expression. (C) Global DNA methylation measured in the tumor tissue. (D) qPCR analysis of Dnmt1, Dnmt3a and Dnmt3b genes in the tumor tissue. Statistical significance was tested with unpaired t-test or Mann-Whitney test. Data are represented as means ± SEM. * p<0.05; *** p<0.001.*

Exercise training does not impact histone acetylation in the tumor tissue

To further investigate the impact of exercise training on histone modifications, an analysis of HDAC expression and activity were performed in prostate tumor from control and trained rats. Here, ET did not influence HDAC1, HDAC2 protein expression (p=0.6885 and p=0.5888 for HDAC1 and HDAC2, respectively) (Figure R-16.A. and Figure R-16.B.) and did not impact HDAC activity in the prostate tumor tissue (p=0.7152) (Figure R-16.C.). Furthermore, we looked at lysine acetylation of histone H3 (Lys9/14) more particularly, as this histone modification has

been correlated with prostate cancer prognosis (45). No effect of exercise training on this marker was however observed (p=0.5775) (Figure R-16.A. and Figure R-16.B.). Overall, our results show that in prostate cancer, exercise training does not impact histone acetylation processes. Other modifications, including histone methylation and histone phosphorylation, remain to be investigated.

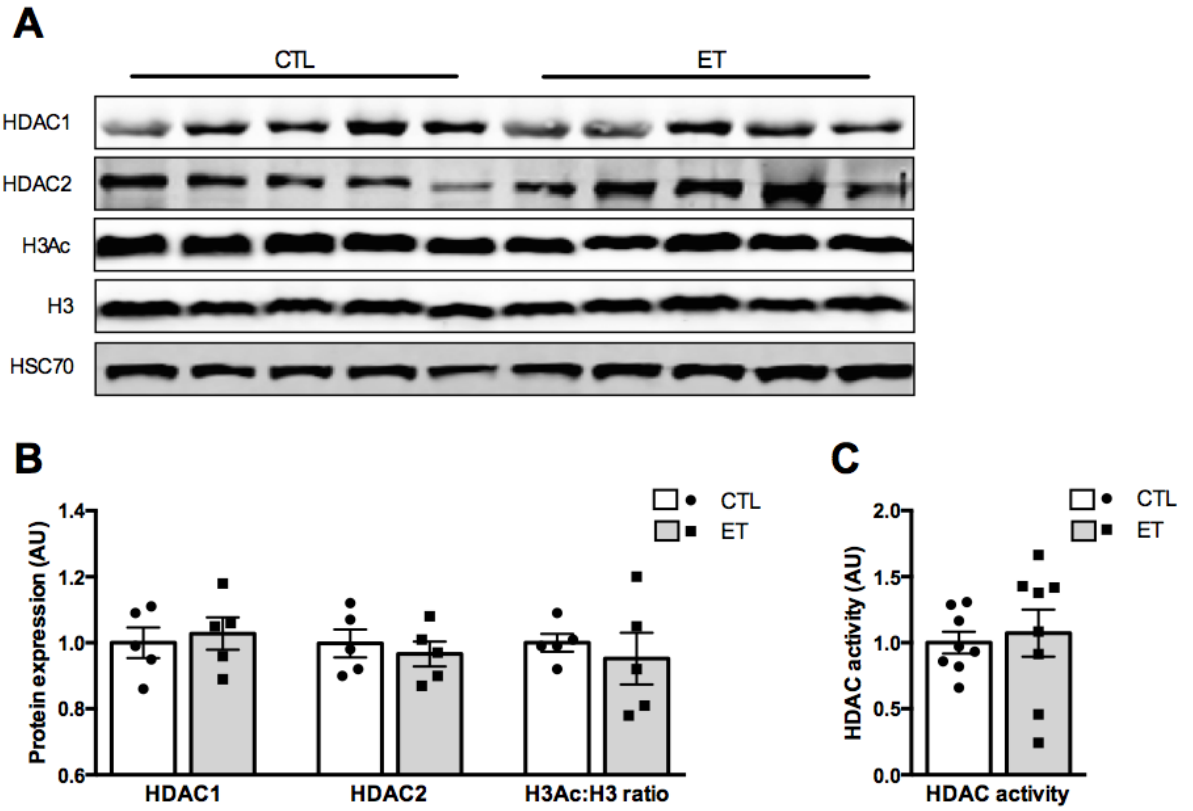

Figure R-16: Histone acetylation (A) Western blot images for HDAC1, HDAC2, H3Ac, H3 and HSC70 measured in the tumor tissue. (B) Quantification of tumor HDAC1, HDAC2, and H3Ac-to-H3 ratio. (C) HDAC activity measured in the tumor tissue. Statistical significance was tested with unpaired t-test. Data are represented as means ± SEM.

MicroRNA expression profile differs between sedentary and trained rats

The principal component analysis (PCA) obtained from the Genechip miRNA 3.0 Array showed a differential expression pattern of rat small non-coding RNA in the prostate tumors of trained rats compared with control rats (Figure R-17.A.). From 1,166 rat probes of miRNAs included in the microarray, we demonstrated that 75 were significantly differentially expressed miRNA candidates: 26 were down-regulated and 49 were up-regulated with exercise training (Figure R-17.B.). Among the most important expression changes, miR-27a-5p (fold change +2.33), miR-181a (+1.72) and miR-224 (+1.31) were overexpressed in the prostate tumor of the trained rats compared with the sedentary controls, whereas miR-301 (-1.53) was

downregulated. Since miR-27a-5p was the most significantly up-regulated miRNAs, a technical validation using RT-qPCR was performed, and a trend was confirmed (80.8% increase; p=0.0748; Figure R-17.C.).

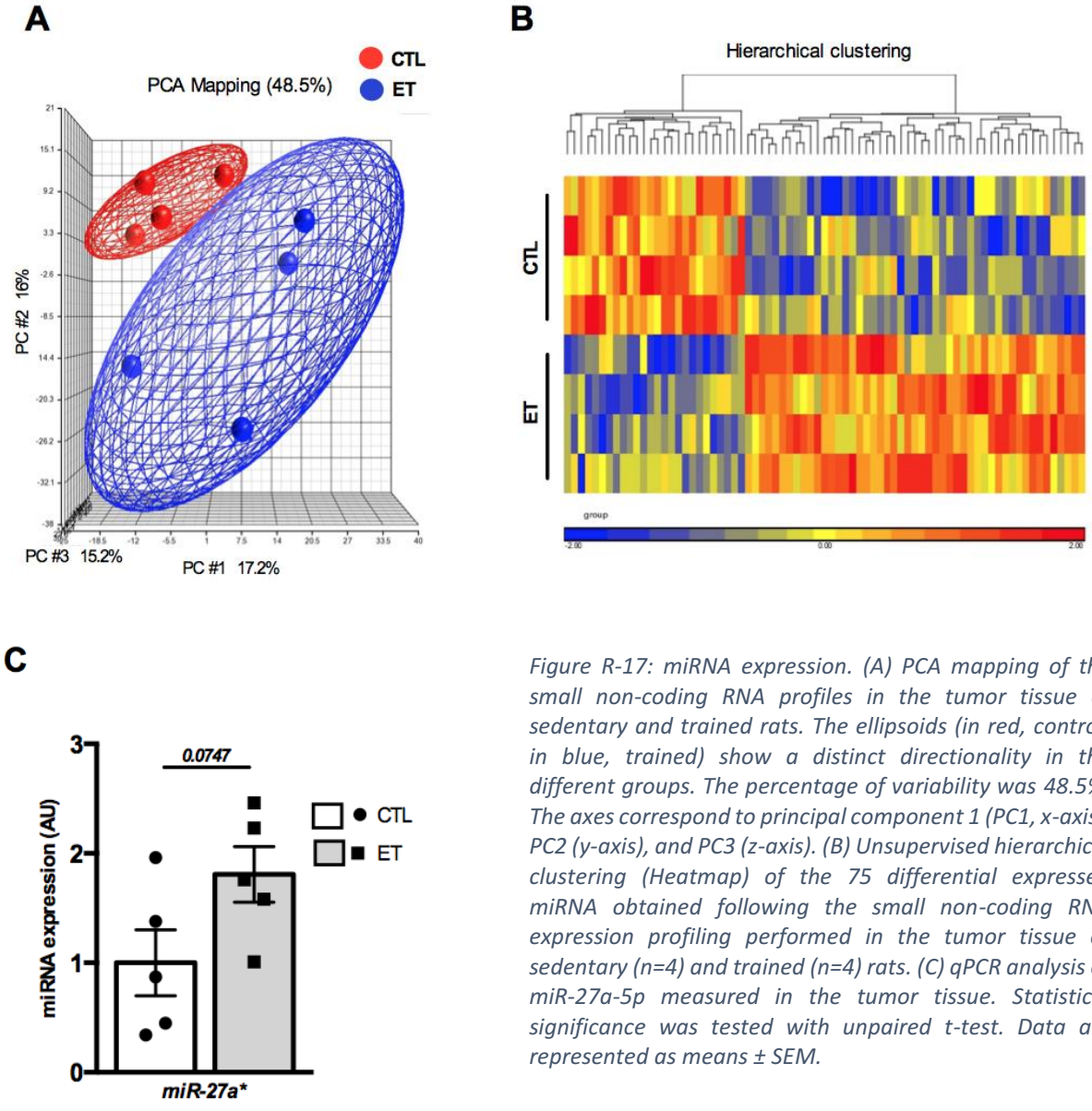

Figure R-17: miRNA expression. (A) PCA mapping of the small non-coding RNA profiles in the tumor tissue of sedentary and trained rats. The ellipsoids (in red, control; in blue, trained) show a distinct directionality in the different groups. The percentage of variability was 48.5%. The axes correspond to principal component 1 (PC1, x-axis), PC2 (y-axis), and PC3 (z-axis). (B) Unsupervised hierarchical clustering (Heatmap) of the 75 differential expressed miRNA obtained following the small non-coding RNA expression profiling performed in the tumor tissue of sedentary (n=4) and trained (n=4) rats. (C) qPCR analysis of miR-27a-5p measured in the tumor tissue. Statistical significance was tested with unpaired t-test. Data are represented as means ± SEM.

DISCUSSION

Our Epigenetic alterations are common events involved in prostate carcinogenesis arising in the earlier stages and persisting throughout cancer progression (46). Here, our results show for the first time that exercise training modulates some of these abnormalities.

Using a genome-wide DNA methylation array, we found that a moderate intensity aerobic exercise known to reduce prostate tumor growth (29) increased global DNA methylation within the tumor tissue and down-regulated the gene expression of *Dnmt*s. Therefore, it can be hypothesized that exercise training can re-establish standard methylation levels of promoter CpG islands by counteracting, to some extent, the cancer-associated genome-wide hypomethylation while decreasing *Dnmt*s' expression. The offset of these aberrant methylation patterns well known for triggering carcinogenesis (47) could thus represent a biological mechanism by which exercise training reduces cancer growth.

While our study is the first to investigate global DNA methylation of the tumor tissue in response to chronic exercise, previous findings from observational studies have found a significant association between physical activity levels prior to diagnosis and methylation of genes associated with cancer in the tumor tissue of gastric (48) and prostate (49) cancer patients. The modalities of physical activity (intensity, duration) seem to play a role in these modulations. In an interventional study, (50) demonstrated that a 6 months moderate-intensity aerobic exercise could induce changes in the DNA methylation of various genes such as *L3MBTL1* in peripheral blood leukocytes, suggesting a link between DNA methylation and patient's survival.

The effects of physical activity on histone modifications have been explored previously in cancer patients, but have largely focused on blood-based measurements, and tumor-specific responses have never been evaluated. The team of Zimmer conducted two studies evaluating the impact of exercise on histone alterations in immune cells (51,52). They observed an increase in histone acetylation and *NKG2D* expression in circulating natural killer (NK) cells isolated from blood drawn from cancer patients (and healthy individuals) following an intense endurance run (51,52). These results suggest an activation of NK cells due to epigenetic modifications after a vigorous exercise. Interestingly, physical activity has been shown to reduce tumor growth through a direct regulation of NK cell trafficking in preclinical models (53). In non-Hodgkin lymphoma patients, moderate exercise induced changes in cytokine levels (i.e. IL6 and MIF), thereby influencing histone modifications and activity of circulating lymphocytes (52). Our data contrasts with these findings, since no modifications of histone acetylation were detected in prostate tumor tissues in response to exercise training and highlights potential site- or cancer-specific differences in the impact of physical activity on epigenetic alterations that is an important area of future investigation.

miRNA expression is highly dysregulated in prostate cancer, but their functional role remains incompletely understood. Nevertheless, several miRNAs have been shown to be involved in driving cell growth, inhibiting cell death and promoting metastasis in prostate cancer, thereby representing attractive therapeutic targets (54). For instance, microRNA let-7 is commonly downregulated in prostate cancer where it acts as a tumor suppressor (55). Overexpressing let-7a was shown to suppress prostate cancer cell proliferation both *in vitro* and *in vivo*, and may act as a radiosensitizer in prostate cancer cells, supporting its use for therapy (56,57). Surprisingly, data linking cancer growth and exercise-induced changes in miRNAs expression are scarce. Here, we demonstrated an impact of exercise training on intra-tumor miRNA expression in prostate cancer, with 75 significantly differentially regulated miRNAs. Pre-clinical studies have previously reported similar results. Exercise training has indeed been shown to down-regulate miR-21 and up-regulate miR-206 and let-7 in breast cancer, which was associated with decreased tumor growth (43,58). Our results therefore complement these previous findings, by suggesting a different miRNA expression pattern between trained and sedentary rats and by indicating an overexpression of miR-27a-5p in tumor tissue in response to ET, concomitantly with caspase-3 cleavage. Interestingly, it was shown that PC3 cells transfected with anti-miR-27a-5p had a significant increased cell viability and decreased apoptosis, suggesting a growth-suppressive function of this miRNA in prostate cancer cells (59).

However, further researches are needed to elucidate the impact of exercise on epigenetic modifications and its link with cancer progression.

CONCLUSION AND FUTURE DIRECTIONS

Our study demonstrates that exercise training can affect global epigenetic modifications in the prostate tumor tissue. More specifically, while exercise training did not seem to impact histone acetylation, it may be able to normalize genome-wide methylation and DNMT expression in the tumor tissue, as well as reverse some of the miRNA alterations associated with prostate cancer. Epigenetic modifications in the tumor tissue could therefore represent a mechanism mediating some of the anti-cancer benefits observed with physical activity and exercise. Future studies should establish the relationship between exercise-induced epigenetic alterations and cancer prognosis.

AUTHORS CONTRIBUTION

A. Rebillard and E. Ho designed research; S. Dufresne, J Guéritat, CP.Wong, A. Isanejad, E. Serna, MC. Gomez-Cabrera and A. Rébillard performed experiments; S. Dufresne, CP.Wong, E. Serna and MC. Gomez-Cabrera analyzed data; S. Dufresne and A. Rébillard wrote the manuscript.

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DISCUSSION

DISCUSSION

In this section, we will discuss several of the major points raised in the manuscript and notably: (1) the potential of epigenetic mechanisms in mediating physical activity anti-cancer effects and possible implications for cancer treatment sensitivity; (2) the possible interactions between physical activity and radiotherapy efficiency through the modulation of the tumor microenvironment (notably the immune system and tumor vascularization) ; and (3) the plausible barriers to physical activity benefits on tumor growth and treatment response particularly in regards to physical activity modalities and cancer types/subtypes.
Finally, we will discuss the development of personalized physical activity in the oncology setting, from a preclinical and clinical point of view.

I – Epigenetic mechanisms and physical activity: a link to cancer and cancer treatments?

The adherence to physical activity guidelines in cancer survivors remains low (Coletta et al. 2019) despite increasing evidence supporting its benefit on treatment side effects and survival. Unraveling the biological mechanisms mediating the anti-cancer effects of physical activity may not only increase the awareness of health care practitioners on the value of this strategy but also help evaluate its safety in patients undergoing treatment.

In our work we showed that exercise training led to several epigenetic modifications in the tumor tissue of rats bearing prostate tumors which may reflect a less aggressive tumor type. Notably, global DNA methylation was higher in trained *versus* inactive rats while the expression of *Dnmt*s, which catalyzes the transfer of methyl groups to DNA, was reduced. Tumor miRNA profile was also modified in response to treadmill running, while HDAC activity did not appear to be altered. Importantly, both DNA methylation and miRNA expression may be associated with treatment resistance and represents an interesting therapeutic target

(Azad et al. 2013; Easwaran, Tsai, and Baylin 2014). The innate or acquired treatment resistance in prostate cancer (PCa) results in treatment failures and eventually death when no more therapeutic options are available. Interestingly, epigenetic regulation has emerged as a promising strategy to counteract treatment resistance. For instance, it was found that treating different cancer cell lines (including the PCa cell line DU145) with zebularine – a DNMT inhibitor – resulted in enhanced radiation-induced cell death (Dote et al. 2005). It notably prevented the hypermethylation of the Ras associated domain family 1A (*RASSF1A*) promoter region, a tumor suppressor gene involved in cell cycle regulation and apoptosis signaling. Similarly, the knockdown of DNMT3B has been shown to sensitize PC-3 PCa cells to radiation *in vitro* (Xue et al. 2015). In our model, *Dnmt1*, *Dnmt3a* and *Dnmt3b* expression was lower in trained rats compared to inactive controls. Therefore, even though the rats did not receive any treatment, these results suggest that physical activity prior to receiving radiotherapy (RT) might enhance treatment efficiency.

Global DNA hypomethylation is a sign of genome instability commonly observed in cancer, and is associated with a poor prognosis (Miousse and Koturbash 2015), even though its exact role remains to be understood. In PCa, DNA hypomethylation typically arises in the more advanced stages of the disease and participates in metastatic tumor heterogeneity, which may contribute to treatment resistance (Yegnasubramanian et al. 2008). By counteracting global DNA hypomethylation in the tumor tissue, exercise training may favor a tumor profile more responsive to treatment.

miRNA expression in the tumor tissue is another epigenetic signature associated with cancer progression and treatment resistance (Ma, Dong, and Ji 2010; Zheng et al. 2010; Iorio and Croce 2012). In PCa, current efforts are aiming to identify miRNA involved in radioresistance in order to develop novel drugs sensitizing cancer cells to RT (Malla et al. 2017; McDermott et al. 2017). Physical activity on the other hand has been shown to modulate miRNA expression in the muscle and blood of humans (Zacharewicz, Lamon, and Russell 2013; Sapp et al. 2017). Additionally, miR-206, let-7a and miR-21 expression measured in the tumor tissue were modulated by treadmill running in a preclinical model of breast cancer (Isanejad et al. 2016). Furthermore, in our work, an array showed that 75 miRNAs measured in the prostate tumor tissue were differently expressed between trained rats and inactive controls. The mechanisms mediating the modulation of miRNA expression by exercise in the tumor tissue remains unknown, but it could potentially be attributed to, at least partly, miRNA trafficking between

the muscles and the tumor through microvesicle transportation, as hypothesized in our review (Dufresne et al. 2018). Importantly, whether the miRNA profile induced by treadmill running is more favorable for treatment efficiency remains to be evaluated.

Finally, HDAC activity is associated with treatment resistance in a wide variety of cancer types including PCa (Frame et al. 2013), but exercise was not able to modulate this epigenetic mechanism in our study.

Overall, the observation that DNA methylation and miRNA expression are modulated in the tumor tissue by exercise training in a murine model of PCa provides additional elements to support the benefits of physical activity in PCa patients. This epigenetic remodeling of the tumor tissue in response to exercise may have consequences for treatment efficiency, but additional studies are warranted to investigate this hypothesis (Figure D-1).

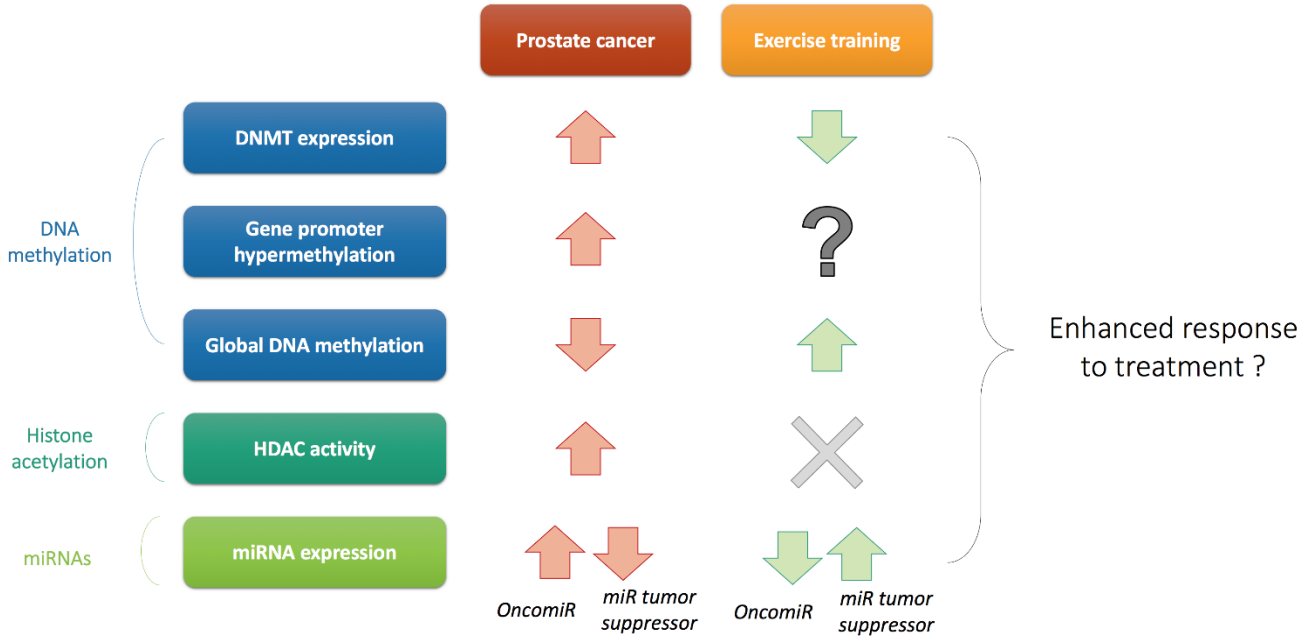

Figure D-1: Epigenetic modifications modified by exercise training observed in our work. Prostate cancer is typically characterized by increased DNMT expression, promoter hypermethylation of specific genes, genome-wide DNA hypomethylation, increased HDAC activity, and altered miRNA expression. Exercise training lowered DNMT expression, exhibit higher levels of global DNA methylation, did not alter HDAC activity and modified the expression of several miRNA expression compared to the cancer control group. The impact of these modifications on treatment efficiency remain unknown.

II – Interactive effects of physical activity and radiotherapy in prostate cancer: a new perspective

The core of this PhD was directed towards the investigation of how physical activity could impact RT efficiency. The first experiments conducted showed that treadmill running (TR) could (1) slow down tumor growth and (2) enhance the response to RT in mice bearing PPC-1 PCa tumors.

At the molecular level, the proliferation marker phospho-ERK1/2:ERK1/2 ratio was decreased in response to TR but not modified by RT nor by the combination of RT and TR. This effect may explain the reduced cancer cell proliferation (assessed by Ki67 staining) and the slower tumor growth observed with TR alone. Nevertheless, these findings suggest that interactions at the molecular level between cancer treatments and exercise can occur (Figure D-2).

The vast majority of preclinical studies investigating the effects of physical activity on cancer do not use additional treatments in their experiments. Hence, while numerous biological processes mediating the anti-cancer effects of physical activity have been identified from these studies, several of these mechanisms may be counteracted when in the presence of a treatment. Caution is thus warranted when prescribing exercise to cancer patients undergoing treatments.

Our work in PPC-1 xenografts also showed that apoptotic marker cCASP3 was not modified by TR but enhanced with RT, this increase being the highest with both strategies combined. Furthermore, RT increased gene expression for NK cell receptor Nkg2d and Il-2rβ, this effect being more important when combined with TR. NK1.1 immunostaining followed a similar pattern. These findings suggest that while TR alone had no effect on NK cells present in the tumor tissue, it led to an enhanced RT response through increased NK cell infiltration and activation (Figure D-2). RT induces DNA damage to the cancer cells, facilitating their killing by the immune system (Hodge et al. 2008; Wang et al. 2018). Priming the immune system through physical activity may result in similar benefits (though to a lower extent) than the combination of immunotherapy and RT. These findings are interesting as they show that exercise training can sensitize the prostate tumor to anti-neoplastic therapies and produce interactive effects at the molecular level.

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

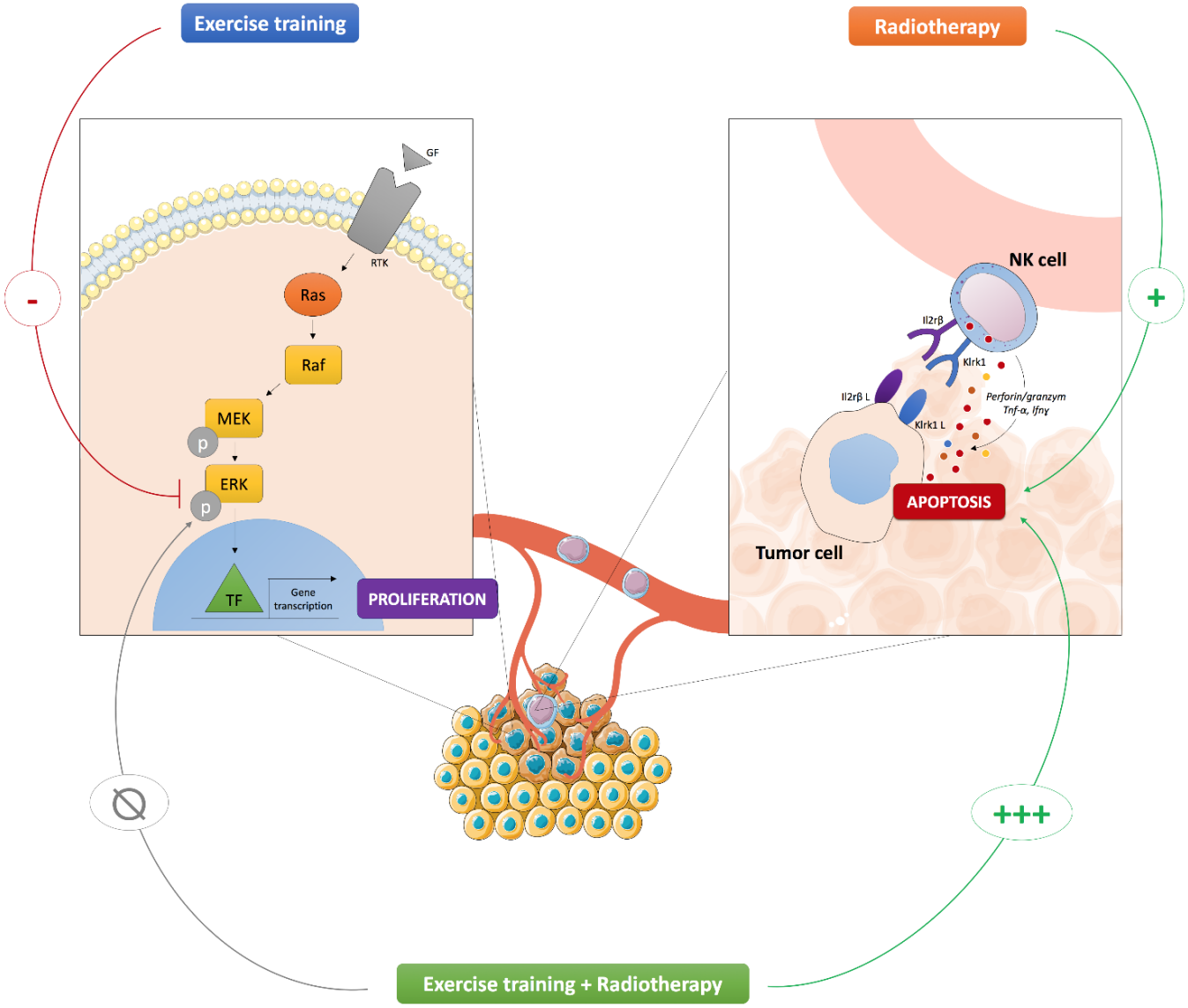

Figure D-2 : Graphical representation of molecular interactions observed between treadmill running and radiotherapy in PPC-1 tumors. Exercise training inhibits ERK phosphorylation, contributing to reduced cancer proliferation, and not impact NK cell activity. Radiotherapy increases the infiltration and activation of NK cell, resulting in enhanced apoptosis. The combination of radiotherapy and exercise training does not influence the phosphorylation of ERK but enhances NK cell trafficking to the tumor tissue, resulting in higher levels of apoptosis.

In view of these findings at the molecular level, the results obtained from preclinical studies evaluating the impact of physical activity alone on cancer growth might not apply when combined with treatments, as interactive effects may occur. Especially because numerous preclinical studies (21%) have reported that physical activity had no impact on tumor growth when used as an isolated strategy (Ashcraft et al. 2016), but its effect when combined with treatments could hypothetically be different. Supporting this idea, several studies evaluating

the combination of physical activity and chemotherapy in preclinical cancer models have found that physical activity alone did not result in slower tumor growth but improved chemotherapy efficiency (Sturgeon et al. 2014; Schadler et al. 2016; Lemke et al. 2016; Florez Bedoya et al. 2019), suggesting potential interactive effects at the molecular level. In addition to the potential implication for the immune system described above, another plausible mechanism that has been discussed in other studies is improved tumor vascularization. Changes in tumor vascularization in response to physical activity is not always associated with slower tumor growth, including in PCa (Jones et al. 2012), but may result in enhanced treatment delivery. Indeed, in preclinical models of breast cancer, pancreatic cancer and Ewing sarcoma, physical activity (voluntary wheel running (VWR) or TR) was shown to increase tumor perfusion and was associated with improved tumor sensitivity to chemotherapy (cyclophosphamide or gemcitabine) (Betof et al. 2015; Schadler et al. 2016; Florez Bedoya et al. 2019; Morrell et al. 2019). Interestingly, it was hypothesized that similar effects could be observed for the combination of RT and physical activity (Schumacher et al. 2020) which we evaluated in another study (unpublished).

III – Physical activity in prostate cancer: one size does not fit all?

The last study presented in this thesis showed that VWR enhanced tumor perfusion in mice bearing PC-3 tumors but did not impact tumor growth. This led us to test whether VWR had the ability to improve RT efficiency. No effect of VWR on the RT response was observed however, and no difference in major signaling pathways were reported. These results were surprising in view of our previous findings and led us to question whether different physical activity modalities could result in different effects on tumor growth in PCa. For instance, two preclinical studies using *APC*$^{Min/+}$ mice (a transgenic model of colon cancer) found that TR but not VWR may reduce the tumor burden (Mehl et al. 2005; Basterfield and Mathers 2010). In our study however, we found that TR, similar to VWR, did not slow down tumor growth. Molecular analyses are ongoing to determine if, despite a lack of effect on tumor growth, TR has the ability to modulate major signaling pathways. Furthermore, the evaluation of TR on RT efficiency remains to be investigated. Indeed, TR and VWR are two distinct physical activities with different physiological effects (Kim et al. 2020) and their impact on the tumor tissue may differ. Notably, immune cell mobilization in response to physical activity is

DUFRESNE, Suzanne. Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models - 2020

proportional to its duration and intensity. TR (depending on the setting used) may represent a more intense physical activity than VWR and thus mobilize the immune system to a greater extent. The immune system may be a key player in the interactive effects of physical activity and RT (as previously reported) and therefore the choice of physical activity modality may condition its effect on the RT response. However, this remains highly hypothetical and remains to be investigated.

The type and subtype of cancer may also represent key determinants in the anti-cancer effects of physical activity. When treating PC-3 and LNCaP cancer cells with exercise-conditioned media *in vitro*, we observed that LNCaP, but not PC-3, showed reduced proliferation. Interestingly, *in vitro* experiments suggest that p53 status of PCa cells might play a pivotal role in the anti-cancer effects of exercise. Leung *et al*. indeed reported that LNCaP (p53 functional) but LN-56 prostate cancer cells (p53 non-functional) were sensitive to exercise-conditioned serum (Leung et al. 2004). PC-3 is p53 null which could explain in parts our findings. Even though these results need to be confirmed *in vivo*, they raise the question of whether some PCa patients may benefit from physical activity interventions more than others in terms of survival.

IV – Implications for the clinical setting and future perspectives

In view of the above-mentioned findings, it can be hypothesized that PCa patients could benefit from personalized physical activity interventions in regard to (1) their cancer characteristics and (2) their treatment plan. Furthermore, a better knowledge of how different physical activity modalities may influence PCa biology and its treatment could improve PCa patient health care.

Firstly, as suggested by *in vitro* data, physical activity might affect tumor cell growth differently depending on the PCa subtype. Determining which cancers are the most sensitive to physical activity might help identify the individuals which are the most likely to benefit from this lifestyle factor in terms of survival. Facilitating access to physical activity in these patients appears essential. However, even though physical activity may affect disease outcome in some men with PCa, the promotion of this lifestyle remains important as it could still improve

treatment side effects, quality of life, and physiological outcomes (i.e., cardiorespiratory fitness and muscle strength).

Secondly, the choice of treatment may determine whether physical activity is encouraged or not but also when it should be performed. For instance, men undergoing active surveillance do not have risk of interference between physical activity and treatment. The preclinical studies evaluating the effect of physical activity on PCa growth support the integration of exercise interventions in the health care plan of PCa patients. Indeed about half of the preclinical studies show reduced tumor growth (Zheng et al. 2008; Esser et al. 2009; X. Zheng et al. 2011; 2012; Gueritat et al. 2014; Saedmocheshi et al. 2019; Vahabzadeh et al. 2020; Dufresne et al. 2020) and the other half show no effect (Jones et al. 2012; McCullough et al. 2013; Baumfalk et al. 2018; Taylor et al. 2020; D. I. Patel, Wallace, et al. 2019; D. I. Patel, Abuchowski, et al. 2019; Opoku-Acheampong et al. 2019), with only limited evidence supporting a possible negative impact of physical activity on PCa outcomes (McCullough et al. 2013). Interestingly, our work showed that exercise could alter DNA methylation and miRNA expression in the tumor tissue, which could mediate some of the beneficial effects observed. Whether this translates to the clinical setting remains to be investigated. The question of whether PCa patients engaging in regular physical activity while undergoing active surveillance could delay the transition to active therapy has raised scientific interest (Galvão et al. 2016). Even though findings from a retrospective study suggest no association between physical activity and active surveillance discontinuation (Papadopoulos et al. 2019), additional results from an ongoing RCT should provide valuable insights on this question (Galvão et al. 2018).

For PCa patients undergoing prostatectomy, obesity is a risk factor of poor outcomes and the combination of low-calorie diet and exercise may counteract these adverse effects (Wilson et al. 2020). However, the evidence remains scarce and RCTs evaluating the effect of physical activity on prostatectomy outcomes are warranted. Furthermore, such studies could provide valuable information from a molecular point of view as the resected prostate could be used for biological analyses.

The findings from our work supports the safety of physical activity for PCa patients undergoing RT. Nevertheless, caution should be warranted as molecular interferences may occur. Additionally, the timing of physical activity in regard to RT treatment is unknown. Engaging in physical activity before a RT session may improve vascularization, decrease hypoxia and

increase immune cell infiltration in the tumor tissue, which could result in an enhanced response to RT. However, receiving RT several hours after exercise cessation may lead to drastically different results as the physiological adaptations are likely to differ. Nevertheless, this is hypothetical and remains to be investigated.

Finally, the effect of physical activity on other anti-cancer treatments such as chemotherapy and immunotherapy in PCa patients has not yet been investigated, and therefore physical activity during these treatments should be carefully monitored.

Interestingly, a large-scale RCT, the Intense Exercise for Survival Among Men with Metastatic Castrate-Resistant Prostate Cancer (INTERVAL) trial, is currently ongoing and should provide valuable information on the effect of physical activity following a PCa diagnosis on disease outcomes according to treatment type (Newton et al. 2018).

Thirdly, it would seem logical that the effect of physical activity on cancer outcomes depends on the type of physical activity. However, very little is known to date on how the different physical activity modalities impact tumor growth and treatment efficiency. The data from preclinical studies are highly divergent (likely because of differences in study design) which makes it difficult to draw any solid conclusions. Furthermore, how to extrapolate physical activity doses from preclinical models to the clinical setting remains unknown. In addition, each PCa patient possesses unique physiological characteristics which will impact the adaptive response to training. Nevertheless, efforts should be made to characterize the effects of different physical activity modalities and intensities on tumor growth. These experiments should be coupled to molecular analyses to help determine the specificity of each modality on tumor growth.

The current recommendations from the American College of Sports Medicine (ACSM) for physical activity in cancer patients is to engage in regular aerobic activities or in a combination of aerobic and resistance activities. These should represent 150-300 min of moderate intensity or 75-150 min of vigorous intensity activities per week (Campbell et al. 2019). These guidelines follow a one-size-fits-all approach which may not maximize the anti-cancer effects associated with physical activity. Personalizing physical activity in this population by taking into account the specificities of their cancer and their treatment plan, as well as adjusting the physical activity modalities accordingly, may provide additional benefits, as suggested by Jones *et al*.

(Jones, Eves, and Scott 2017). To date however, there is however too little knowledge to incorporate such practice, but ongoing RCTs and preclinical studies should help to personalize the approach to physical activity in cancer patients in the upcoming years.

BIBLIOGRAPHY

BIBLIOGRAPHY

A 209
B 211
C 214
D 217
E 218
F 219
G 221
H 223
I 226
J 226
K 227
L 230
M 232
N 236
O 237
P 237
Q 239
R 239
S 241
T 246
U 248
V 248
W 249
X 251
Y 251
Z 251

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APPENDIX

Suzanne DUFRESNE



Ph.D student
Movement, Sport and Health Lab
Rennes 2 University
France
www.M2Slab.com

Contact :
1 rue porçon de la barbinais
35000 RENNES
France
(+33)6 42 72 92 45
suzanne.dufresne@ens-rennes.fr

EDUCATION

2016 – Present
Ph.D – Sports Science and Molecular Biology
Movement, Sport and Health Lab (Rennes 2 University, Rennes, France)
Ph.D supervisor : Amélie REBILLARD, Sport and Health Lab (Rennes 2 University, Rennes, France)

2011 – 2016
MSc – Physical Education & Sports Science
École Normale Supérieure (ENS) de Rennes (Bruz, France) - National competitive examination

2013 – 2015
MSc – Movement, Health and Sport
Rennes 2 University (Rennes, France) and University of Limerick (Limerick, Ireland)
Ranked 3rd/33 in first year and 3rd/25 in second year

2012 – 2013
BSc – Sports Science – Sports coaching
Rennes 2 University (Rennes, France)

2009 – 2012
BSc – Sports Science - Physical Education
Rennes 2 University (Rennes, France) and Paris XI University (Orsay, France)

WORK EXPERIENCE

2016 – Present
Ph.D student
Movement, Sport and Health Lab (Rennes 2 University, Rennes, France)

2015 – 2016
Research lab intern – ENS program
"School of Biological & Population Sciences", Oregon State University (USA)
Internship (7 months) on the topic "impact of exercise training on epigenetic markers in prostate cancer ", supervised by Emily Ho.

Summer 2015
Research lab intern – optional internship
"Southern Alberta Cancer Research Institute", University of Calgary (Canada)
Internship (2 months) supervised by Christine Friedenreich. Involved in the fitness assessment of women diagnosed with breast cancer before and after a training program (ALPHA Ancillary and AMBER studies)

Summer 2014
Research lab intern – ENS program
"Bond University of Health and Sport", Bond University (Australia)
Internship (2 months) supervised by Justin Keogh. Involved in the physical testing of elderly individuals diagnosed with sarcopenia

2007 – 2016
Gymnastics Coach
Club Sportif de Betton (France)
Olympic Club Cessonais Gymnastique (France)
Club Athlétique d'Orsay (France)

2011 – 2012
Sports Instructor
Françoise Dolto Secondary School (Pacé, France)
Saclay Primary School (Saclay, France)

2009 – 2011
Sports Instructor in Adapted Physical Activity
Fresnes penitentiary centre (France)
Medical and Educational Institute Le Val d'Essonnes (France)

SKILLS

Animal experimentation
Level 1 animal experimentation accreditation obtained in 2017 (ONIRIS, Nantes, France)

Informatics
Certificate in informatics and internet (C2i French diploma)
Basic computer skills (Word, Excel, PowerPoint, Graphpad Prism, Image J, etc.)

English language
Fluent (numerous experiences abroad)
TOEIC diploma obtained in 2012 (score of 975/990)

Sport
Gymnast for 18 years (participation in French National Championships)
Gymnastics judge (French national judge)

First aid
Certificate of first aid (Level 1)

CONFERENCE ARTICLES

« Could Exercise Training Alter Radiotherapy Efficiency in a Murine Model of Prostate Cancer ? »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Rannou-Bekono F., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
Metabolism in Action – Copenhagen (Denmark), October 27-31st, 2019
Poster communication

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« 3ème Congrès de Physiologie et Biologie Intégrative » (National conference) - Montpellier (France), June 12-14, 2019
Oral communication

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Oral communication

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« Journées Scientifiques de l'École Doctorale Biologie Santé » (National conference) - Rennes (France), December 12 and 13, 2018
Oral communication

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Dufresne S., Guéritat J, Chiavassa S., Noblet C., Rannou-Bekono F., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
1st conference « Exercise, Locomotion and Musculoskeletal System » - Lyon (France), May 28, 2018
Poster communication

« Could Exercise Training Alter Radiotherapy Efficiency in a Murine Model of Prostate Cancer ? »
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Journées NACRe 2018 (National conference) - Paris (France), March 13, 2018
Oral communication

« Could Exercise Training Alter Radiotherapy Efficiency in a Murine Model of Prostate Cancer ? »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Rannou-Bekono F., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
Metabolism in action – Copenhaguen (Danemark), October 1st-5, 2017
Poster communication

« Combination of Exercise Training and Radiotherapy in Prostate Cancer »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
« 11ème Journées du Cancéropole Grand Ouest » - Vannes (France), June 29-30 2017
Poster communication

« Combination of Exercise Training and Radiotherapy in Prostate Cancer »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
Cancer Cell Death & Therapy - Saint-Malo (France), May 10-12, 2017
Poster communication

« Combination of Exercise Training and Radiotherapy in Prostate Cancer »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
« Chimie, Biologie, Mathématiques et Physique, la Recherche au Service du Médicament et de la Santé » (National conference) – Rennes (France), January 18, 2017
Oral communication

« Combination of Exercise Training and Radiotherapy in Prostate Cancer »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
Troisième Journée Signalisation et Cancer (SCAN) (National conference) – Rennes (France), December 2nd, 2016
Poster communication

SCIENTIFIC PUBLICATIONS

Dufresne S., Guéritat J., Wong C.P., Isanejad A., Ho E., Serna E., Gomez-Cabrera MC., Rebillard A. *Exercise training as a modulator of epigenetic events in prostate tumors.* Article in review

Dufresne S., Guéritat J., Chiavassa S., Noblet C., Assi M., Rioux-Leclercq N., Rannou-Bekono F., Lefeuvre-Orfila L., Paris F., Rebillard A. *Exercise training improves radiotherapy efficiency in a murine model of prostate cancer*. FASEB J. 2020 Feb 11 - https://doi.org/10.1096/fj.201901728R

Assi M., **Dufresne S.**, Rebillard A. *Exercise shapes redox signaling in cancer*. Redox Biol. 2020 Jan 10 - https://doi.org/10.1016/j.redox.2020.101439

Dufresne S., Rebillard A., Muti P., Friedenreich C.M., Brenner D.R. *A review of physical activity and circulating-mirna expression: implications in cancer risk and progression*. Cancer Epidemiol Biomarkers Prev. 2018;27:11–24 - https://doi.org/10.1158/1055-9965.EPI-16-0969

GRANTS & AWARDS

2019
Grant for a 4th year of Ph.D awarded by the Association for Cancer Research (ARC) foundation - 21 000 €

2018
Best talk award
« Could Exercise Training Alter Radiotherapy Efficiency in a Murine Model of Prostate Cancer ? »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Rannou-Bekono F., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
« Journées Scientifiques de l'École Doctorale Biologie Santé » (National conference) - Rennes (France), December 12 and 13, 2018

Poster flash talk award
« Could Exercise Training Alter Radiotherapy Efficiency in a Murine Model of Prostate Cancer ? »
Dufresne S., Guéritat J, Chiavassa S., Noblet C., Rannou-Bekono F., Assi M., Lefeuvre-Orfila L., Paris F., Rebillard A.
1st conference « Exercise, Locomotion and Musculoskeletal System » - Lyon (France), May 28, 2018

2016
Grant for a 4th year of Ph.D attributed by the École Normale Supérieure (ENS) – 64 000 €

TEACHING, SUPERVISION & OTHER SCIENTIFIC ACTIVITIES

TEACHING EXPERIENCE

2020-2019
Biomechanics and physiology of strength training
Level: 1st year BSc Sports Science

2017-2018
Physical activity and environmental constraints: thermoregulation
Level: 3rd year BSc Sports Science

Since 2017
Theory and practice of gymnastics
Level: 1st year BSc Sports Science

2016-2017
Biomechanics in exercise and physical activity (introduction)
Level: 1st year BSc Sports Science

Assessment of physical functioning in the elderly population
Level: 3rd year BSc Sports Science

SUPERVISION

Master students
Mrs Cindy Richard (Msc, grad 2018); co-supervision with Amélie Rebillard – Integrating physical activity in the healthcare of prostate cancer patients: from reducing side effects to improving survival?
Univ Rennes 2, France

Prune Mesyngier (Msc, grad 2017); co-supervision with Claudia Lefeuvre and Vincent Daniel – The impact of Dragon Boat racing on joint pain in breast cancer patients undergoing hormonotherapy
ENS Rennes, France

Undergraduate students
Caroline Bour and Amélie Broudissou
ENS Rennes, France

PEER REVIEW

Reviewer for the *FASEB* journal and the *Experimental Biology* journal.

L'activité physique, une stratégie permettant d'améliorer l'efficacité de la radiothérapie ? Nouvelles perspectives issues de données précliniques

Par Suzanne DUFRESNE

Sous la direction d'**Amélie Rebillard** et de **Christine M. Friedenreich**

Thèse présentée et soutenue à Rennes le 13 novembre 2020

Unité de recherche : ***Laboratoire Mouvement, Sport, Santé – EA7470***

Rapporteurs avant soutenance :

Alice Carrier, Directrice de Recherche – Centre de Recherche en Cancérologie de Marseille, équipe stress cellulaire, Marseille, France

Adrien Rossary, Maitre de Conférences des Universités – Unité de Nutrition Humaine, UFR Pharmacie, Université Clermont Auvergne, Clermont-Ferrand, France

Examinateurs :

Yann S. Gallot, Maitre de Conférences des Universités – Laboratoire de Biologie de l'Exercice pour la Performance et la Santé, Université d'Evry-Val-d'Essone, Evry, France

Romain Mathieu, Professeur des Universités, Praticien Hospitalier – CHU Pontchaillou Rennes, Service Urologie, Rennes, France

Directrice de thèse :

Amélie Rébillard, Professeur des Universités, laboratoire Mouvement Sport Santé, Bruz, France

Co-directrice de thèse :

Christine M. Friedenreich, Directrice scientifique, Department of Cancer Epidemiology and Prevention Research, Alberta Health Services, Holly Cross Centre, Calgary, Alberta, Canada

SOMMAIRE

INTRODUCTION **4**

REVUE DE LITTÉRATURE **5**

CHAPITRE I : LE CANCER DE LA PROSTATE 5

I. Épidémiologie du cancer de la prostate *5*

II. Les étapes de la carcinogenèse prostatique, caractéristiques du cancer et signatures épigénétiques *6*

III. Les traitements contre le cancer de la prostate *9*

CHAPITRE II : ACTIVITE PHYSIQUE ET CANCER DE LA PROSTATE 11

I. Impact de l'activité physique sur la psychologie et la physiologie des patients atteints d'un cancer de la prostate *11*

II. Impact de l'activité physique sur la survie des patients atteints d'un cancer de la prostate et sur la croissance tumorale prostatique *14*

III. Impact de l'activité physique sur l'efficacité des traitements *17*

OBJECTIFS DE LA THÈSE **21**

RÉSULTATS **22**

Étude 1 : *L'entrainement de course sur tapis roulant améliore l'efficacité de la radiothérapie dans un modèle murin de cancer de la prostate* *22*

Étude 2 : *La roue d'activité n'améliore pas l'efficacité de la radiothérapie chez des souris porteuses de tumeurs PC-3* *25*

Étude 3 : *Revue de questions sur activité physique et miARN circulants : implications pour le risque et la progression du cancer* *27*

Étude 4 : *L'entrainement comme modulateur d'évènements épigénétiques dans le cancer de la prostate* *30*

DISCUSSION **33**

Mécanismes épigénétiques : un mécanisme impliqué dans les effets anti-cancer de l'activité physique ? *33*

Interactions entre activité physique et radiothérapie dans le cancer de la prostate : nouvelles perspectives *35*

Activité physique et cancer de la prostate : la nécessité d'une approche personnalisée. *36*

CONCLUSION ET PERSPECTIVES **37**

BIBLIOGRAPHIE **38**

INTRODUCTION

La modernisation de nos sociétés et de nos habitudes de vie a entrainé une limitation de nos mouvements et une réduction de notre activité musculaire. Par exemple, le temps passé devant un écran a considérablement augmenté au cours de ces dernières années, participant à accroitre le temps passé assis (Owen et al. 2010). Ainsi, la sédentarité et l'inactivité sont devenus de plus en plus fréquentes, ce qui n'est pas sans conséquence sur la santé. En effet, l'inactivité physique est considérée comme contribuant fortement aux maladies non-communicables (dont le cancer) et a été estimée comme entrainant le décès de 5.3 millions de personnes en 2008, un fardeau comparable au tabac (Lee et al. 2012).

Alors que l'inactivité physique est considérée comme un facteur de risque pour de nombreux cancers, l'activité physique à l'inverse est de plus en plus reconnue comme une stratégie efficace pour limiter les effets secondaires des traitements, améliorer la qualité de vie, et même augmenter la survie des patients atteints d'un cancer (Rock et al. 2012; Patel et al. 2019; Campbell et al. 2019; Friedenreich et al. 2020). La pratique d'une activité physique perturbe l'homéostasie corporelle et entraine de nombreuses adaptations dans les cellules, tissus et organes (Hawley et al. 2014). Certaines de ces adaptations seraient responsables des effets anti-cancer de l'activité physique, mais pourraient également potentiellement interagir avec les traitements utilisés pour lutter contre cette maladie (Ashcraft et al. 2019).

Dans ce contexte, les travaux de thèse présentés ici cherchent à étudier l'effet de l'activité physique sur la croissance tumorale et sur l'efficacité de la radiothérapie en utilisant différents modèles précliniques de cancer de la prostate.

REVUE DE LITTÉRATURE

Chapitre I : Le cancer de la prostate

I. Épidémiologie du cancer de la prostate

Le cancer de la prostate représente un enjeu de santé publique majeur. En 2018, cette maladie était le second cancer le plus fréquent chez les hommes avec 1 276 106 nouveaux diagnostiqués dans le monde, représentant 7.1% des nouveaux cas de cancer au niveau mondial (Bray et al. 2018). Il existe toutefois de grandes disparités géographiques, avec une forte incidence en Océanie et une beaucoup plus faible en Asie (Bray et al. 2018; Taitt 2018). Le cancer de la prostate est considéré comme la cinquième cause de décès dans le monde et a été responsable du décès d'environ 358 989 personnes en 2018 (Bray et al. 2018), avec un nombre de décès important dans les régions en développement. Du fait de son incidence élevée et de son taux de survie relativement important, la prévalence du cancer de la prostate est très forte : en effet, on estime qu'il y a 10 millions d'hommes vivant avec un cancer de la prostate dans le monde, le second cancer le plus prévalent au niveau mondial, derrière le cancer du sein (Institute for Health Metrics and Evaluation (IHME) 2018). Ces données montrent l'importance de ce type de cancer et soulignent la nécessité de développer des stratégies visant à améliorer non seulement l'efficacité des traitements mais aussi la qualité de vie des patients.

Le diagnostic d'un cancer de la prostate est réalisé lorsque le niveau du taux d'antigène spécifique de la prostate (PSA) est élevé (>4ng/mL) et que la présence de cellules cancéreuses est confirmée par biopsie. Depuis les années 1980, l'utilisation à grande échelle des tests PSA

a permis de détecter le cancer de la prostate de manière précoce, permettant de traiter un cancer localisé plutôt qu' avancé et métastatique (Thompson and Ankerst 2007; Etzioni et al. 2008). Cependant, ces méthodes de dépistage ont également entrainé un sur-diagnostic de cancers de la prostate, c'est à dire un dépistage de cancers qui n'auraient pas eu de conséquences cliniques si non détectés (Loeb et al. 2014).

Afin d'améliorer la prévention du cancer de la prostate, plusieurs études épidémiologiques ont identifiés certains facteurs de risques associés à cette maladie qui peuvent être regroupés en deux catégories : (1) facteurs de risque non modifiables et (2) facteurs de risque modifiables. Les facteurs de risques non modifiables sont irréversibles et incluent l'âge, l'ethnicité, la taille, et les antécédents familiaux. Les facteurs de risques modifiables, quant à eux, comprennent l'obésité, l'exposition aux pesticides, certains régimes alimentaires, le manque d'activité physique et la consommation de tabac (Gann 2002; Bostwick et al. 2004). Connaitre ces différents facteurs permet de mieux cibler les personnes à risques et aide à la mise en place de stratégies visant à modifier certains comportements afin d'améliorer la prévention du cancer de la prostate.

II. Les étapes de la carcinogenèse prostatique, caractéristiques du cancer et signatures épigénétiques

La prostate est une glande de l'appareil génital masculin dont la fonction principale est de produire une partie du liquide séminal qui s'intègre au sperme et permet son expulsion lors de l'éjaculation (Verze, Cai, and Lorenzetti 2016).

La structure de la prostate peut être divisée en quatre zones qui se distinguent par leurs caractéristiques histologiques et anatomiques, leurs fonctions, ainsi que par leur potentiel à

développer des pathologies (McNeal 1981). On distingue une zone fibromusculaire et trois zones glandulaires représentant respectivement 33% et 66% du volume total de la prostate (McNeal 1981). La zone glandulaire de la prostate est constituée de différents types de cellules épihtéliales et est responsable de la sécrétion de fluides qui composent en partie le sperme. La zone fibromusculaire contient majoritairement des fibroblastes et des cellules musculaires lisses qui participent à l'expulsion des sécrétions prostatiques.

Un cancer résulte de la perte d'homéostasie entre prolifération cellulaire et mort cellulaire. Cela survient à la suite de modifications génétiques et épigénétiques qui vont transformer des cellules saines en cellules malignes (Harris 1991; Sharma, Kelly, and Jones 2010). Le cancer de la prostate a la particularité d'avoir une croissance lente qui peut se diviser en plusieurs étapes : (1) néoplasie intraépithéliale prostatique (PIN) caractérisée par une dysplasie des cellules épithéliales et une perte progressive des cellules basales ; (2) adénocarcinome androgéno-dépendant où la couche basale est perdue, ce qui entraine l'invasion des cellules cancéreuses et la formation d'un carcinome prostatique sensible aux androgènes ; (3) cancer de la prostate hormono-résistant caractérisé par une croissance qui n'est plus dépendante des androgènes et qui devient donc résistant à l'hormonothérapie ; (4) cancer de la prostate métastatique, qui correspond à l'invasion des cellules cancéreuses dans différentes zones du corps via la circulation sanguine ou le système lymphatique. Le développement de métastases peut toutefois avoir lieu avant ou après l'apparition de résistances aux hormones.

Les cellules cancéreuses acquièrent de nombreuses caractéristiques au cours des différentes étapes de la carcinogénèse prostatique qui les différencient des cellules saines. En 2000, Hanahan et Weinberg ont proposé de regrouper ces caractéristiques en six grandes catégories

(Hanahan and Weinberg 2000) avant d'en rajouter quatre supplémentaires quelques années plus tard (Hanahan and Weinberg 2011). Ces caractéristiques comprennent (1) l'autosuffisance en signaux de prolifération ; (2) l'insensibilité aux signaux inhibiteurs de croissance ; (3) la capacité d'invasion et de formation de métastases ; (4) l'aptitude à se répliquer indéfiniment ; (5) l'induction de l'angiogenèse ; (6) l'échappement à l'apoptose ; (7) la résistance à la destruction immunitaire ; (8) l'inflammation pro-tumorale ; (9) l'instabilité du génome et les mutations ; et (10) la dérégulation du métabolisme énergétique cellulaire (Hanahan and Weinberg 2000; 2011).

De plus, alors que le cancer est généralement perçu comme une maladie génétique, les altérations épigénétiques sont de plus en plus reconnues comme ayant un rôle majeur dans l'initiation et la progression du cancer (Sharma, Kelly, and Jones 2010). En effet, celles-ci peuvent participer à la mise en place des caractéristiques précédemment évoquées (Flavahan, Gaskell, and Bernstein 2017). L'épigénétique correspond à la modification de l'expression de gènes mais sans changement de la séquence d'ADN. Ce sont plutôt des modifications structurelles de l'ADN ou de la chromatine qui vont favoriser ou réprimer l'expression de certains gènes. Les mécanismes épigénétiques les plus connus sont : (1) la méthylation de l'ADN ; (2) les modifications d'histone ; et (3) l'expression de microARNs (miARNs).

L'ensemble des efforts réalisés pour mieux comprendre les caractéristiques des cellules cancéreuses et les mécanismes sous-jacents ont permis le développement de certains traitements contre le cancer.

III. Les traitements contre le cancer de la prostate

Aujourd'hui, il existe plusieurs options thérapeutiques pour les hommes diagnostiqués avec un cancer de la prostate et notamment la prostatectomie, l'hormonothérapie par privation androgénique, la radiothérapie, la chimiothérapie, et plus récemment l'immunothérapie. Ces traitements peuvent être réalisés seuls ou combinés entre eux, selon le contexte clinique. Le choix de la thérapie dépend notamment de l'agressivité du cancer de la prostate mais également de la longévité estimée et du risque de décéder d'une autre cause.

Les patients ayant un cancer de la prostate localisé évoluant lentement peuvent être en surveillance active, qui consiste seulement à monitorer l'évolution de la maladie (Filson, Marks, and Litwin 2015). En effet, chez ces patients, l'utilisation de traitements conventionnels pourraient induire plus de risques que de bénéfices.

Chez les patients diagnostiqués avec un cancer de la prostate localisé, la maladie va être traitée soit par prostatectomie, soit par radiothérapie (Mohler et al. 2010).

La prostatectomie est une procédure chirurgicale qui consiste à retirer la glande prostatique, les vésicules séminales et les ganglions lymphatiques environnants. Cette technique a été utilisée dans le traitement du cancer de la prostate depuis plus de 100 ans (Young 2002) et a évolué avec les avancées technologiques. Cependant, environ 30% des patients traités par prostatectomie auront des récidives biochimiques (Stephenson et al. 2006).

La radiothérapie a pour but de délivrer une dose de radiation létale à la tumeur tout en épargnant les tissus environnants. La radiothérapie exerce ses effets anti-cancer en créant des dommages à l'ADN de manière directe et indirecte par la radiolyse de l'eau et par la génération

d'espèces réactives à l'oxygène (ROS). Cela va activer des voies de signalisation qui vont entrainer soit la réparation des dommages, soit la mort cellulaire. La radiothérapie a également la capacité de modifier le micro-environnement tumoral et de moduler les réponses immunitaires (Demaria, Golden, and Formenti 2015; Dar, Henson, and Shiao 2018). En effet, les signaux de danger (tels que la libération de cytokines pro-inflammatoires, de chimiokines ou d'antigènes tumoraux) émis par les cellules irradiées ou mortes peuvent initier des réponses immunitaires (de Andrade Carvalho and Villar 2018).

Malgré une première phase de rémission à la suite de traitements chirurgicaux ou de radiothérapie, environ un tiers des patients atteints d'un cancer de la prostate aura une récidive biochimique caractérisée par une augmentation du taux de PSA (Roehl et al. 2004). Pour les cancers de la prostate agressifs et métastatiques, les traitements utilisés sont souvent l'hormonothérapie combinée ou non à la radiothérapie (Warde et al. 2011; Heidenreich et al. 2014).

Le potentiel de l'hormonothérapie pour lutter contre le cancer de la prostate a été mis en évidence par Huggins et Hodges en 1941 (Huggins and Hodges 1941) et a entrainé le développement de nouveaux médicaments qui visent à bloquer les androgènes au maximum tout en minimisant les effets secondaires associés. Aujourd'hui, les traitements utilisés agissent soit en empêchant la synthèse de testostérone, soit en bloquant le récepteur à l'androgène (Sharifi, Gulley, and Dahut 2005). Alors que les traitements anti-androgéniques sont efficaces dans un premier temps chez les patients, ceux-ci développent presque tous par la suite un cancer qui devient insensible à l'action des androgènes. Lorsque le cancer progresse en cancer hormono-indépendant, il n'y a pas de traitement curatif. Cependant, la

chimiothérapie et l'immunothérapie peuvent être recommandées afin de ralentir la progression de la maladie et améliorer la qualité de vie.

Chapitre II : Activité physique et cancer de la prostate

Alors qu'il était auparavant conseillé aux patients atteints d'un cancer de se reposer et d'éviter de réaliser de l'activité physique, de nombreuses études montrent aujourd'hui que pratiquer une activité physique régulière à la suite d'un diagnostic de cancer apporte de nombreux bénéfices. Ces bénéfices peuvent être psychologiques, physiologiques, et même anti-cancer.

I. Impact de l'activité physique sur la psychologie et la physiologie des patients atteints d'un cancer de la prostate

Être diagnostiqué d'un cancer peut entrainer de nombreux évènements psychologiques stressants et des troubles de l'humeur, d'où une diminution de la qualité de vie et un certain mal-être. De plus, le cancer de la prostate et les traitements utilisés pour lutter contre cette maladie peuvent impacter certaines fonctions de l'organisme (comme par exemple induire des dérégulations hormonales) ce qui peut entrainer une altération de la force musculaire et une détérioration des capacités cardiorespiratoires. La pratique régulière d'une activité physique a été proposée comme stratégie permettant de limiter ces effets néfastes.

L'effet de l'activité physique sur la qualité de vie des patients atteints d'un cancer a été largement étudié, y compris chez les patients atteints d'un cancer de la prostate. Plusieurs revues systématiques et méta-analyses ont mis en évidence que des entrainements de type

aérobie ou de type renforcement musculaire avaient des effets bénéfiques sur la qualité de vie des patients atteints d'un cancer de la prostate, même si ces effets ont été jugés relativement faibles (Keogh and MacLeod 2012; Hasenoehrl et al. 2015; Bourke et al. 2016; Baguley et al. 2017; Cormie and Zopf 2020).

De plus, l'activité physique pourrait également limiter la dépression des patients atteints d'un cancer de la prostate. La dépression est un effet secondaire du cancer fréquemment observé chez les patients atteints d'un cancer de la prostate, avec une prévalence de 15-20% (Watts et al. 2014). Plusieurs études ont montré que des programmes d'activité physique diminuaient les symptômes dépressifs dans cette population (Culos-Reed et al. 2010; Livingston et al. 2015; Gaskin et al. 2017).

La fatigue liée au cancer est également un symptôme très souvent retrouvé chez les patients atteints d'un cancer de la prostate, présent chez plus de 74% d'entre eux (Langston et al. 2013). Cet effet indésirable peut les décourager de pratiquer une activité physique, ce qui peut diminuer leur force musculaire et ainsi augmenter la sensation de fatigue (Schulz et al. 2017). Pratiquer une activité physique pourrait ainsi briser ce cercle vicieux. En effet, il a été montré que la combinaison d'entrainement aérobie et de renforcement musculaire diminuait la fatigue chez les patients atteints d'un cancer de la prostate, notamment lorsque les exercices étaient supervisés (Baguley et al. 2017). Dans le cancer de manière générale, l'activité physique est même considérée comme la stratégie la plus efficace pour réduire la fatigue, même lorsqu'elle est comparée à des interventions médicamenteuses ou psychologiques (Mustian et al. 2017).

Au-delà de ces bénéfices psychologiques, l'activité physique pratiquée par des patients atteints d'un cancer de la prostate apporte également des bénéfices physiologiques. On

observe souvent chez les patients atteints d'un cancer une détérioration importante de la capacité cardiorespiratoire. En effet, ils ont une consommation pic d'oxygène (VO_{2peak}) inférieure de 30% en moyenne à celle de personnes saines de même âge et de même sexe (Jones et al. 2007; Haykowsky et al. 2009). Chez les patients atteints d'un cancer de la prostate, la $VO2_{peak}$ diminue au cours de la période de traitement (Segal et al. 2009) ce qui pourrait être une conséquence du cancer lui-même ou de ses traitements (Jones et al. 2009). Ces altérations de la capacité cardiorespiratoire peuvent avoir des conséquences majeures. En effet, ce paramètre est considéré comme un indicateur de bon état de santé et est un prédicateur de mortalité (Sandvik et al. 1993; Blair et al. 1995; Myers et al. 2002; Arena et al. 2007; D. Lee et al. 2010). De plus, il est estimé qu'une VO_{2peak} d'au moins 18mL/kg/min chez les personnes âgées est nécessaire pour vivre une vie de manière indépendante (Paterson et al. 1999). Dans le cancer de la prostate, plusieurs études ont montré qu'un programme d'entrainement permettait d'améliorer les capacités cardiorespiratoires ou au moins de limiter leur déclin (Baumann, Zopf, and Bloch 2012; Keogh and MacLeod 2012; Gardner, Livingston, and Fraser 2014; Bourke et al. 2016; Bigaran et al. 2020).

Les patients atteints d'un cancer peuvent également développer une cachexie cancéreuse caractérisée par une perte de masse musculaire importante. La pratique d'activité physique pourrait limiter ces effets et améliorer la force musculaire chez les patients atteints d'un cancer de la prostate (Baumann, Zopf, and Bloch 2012; Keogh and MacLeod 2012; Gardner, Livingston, and Fraser 2014; Bourke et al. 2016). En particulier, le suivi d'un programme d'entrainement permettrait de limiter la perte de masse musculaire chez les patients atteints d'un cancer de la prostate traités par hormonothérapie (Gardner, Livingston, and Fraser 2014; Winters-Stone et al. 2015) et de préserver, voire améliorer, la force musculaire chez ceux

traités par radiothérapie et/ou traitements anti-androgéniques (Segal et al. 2009; Gardner, Livingston, and Fraser 2014; Chen et al. 2019).

Ainsi, l'activité physique peut induire de nombreux bénéfices – psychologiques et physiologiques – chez les patients atteints d'un cancer, ce qui pourrait potentiellement avoir des répercussions sur leur survie.

II. Impact de l'activité physique sur la survie des patients atteints d'un cancer de la prostate et sur la croissance tumorale prostatique

L'inactivité physique est considérée comme responsable du décès de plus de 5.3 millions de personnes au niveau mondial (Lee et al. 2012). De plus, il est aujourd'hui largement reconnu que la pratique régulière d'une activité physique entraine une grande variété de bénéfices dans de nombreuses pathologies et augmente la survie de la population générale (Erikssen 2001; Manini et al. 2006; Schoenborn and Stommel 2011; Pedersen and Saltin 2015). Chez les patients atteints d'un cancer de la prostate, plusieurs études suggèrent que des effets similaires pourraient être observés. Il est toutefois important de distinguer l'activité physique réalisée avant le diagnostic, appelée activité physique pré-diagnostic, et celle pratiquée à la suite d'un diagnostic, dénommée activité physique post-diagnostic. En effet, ces deux paramètres représentent des facteurs pronostics indépendants (Friedenreich et al. 2020).

Chez les patients atteints d'un cancer de la prostate, il a été montré que ceux ayant une activité physique pré-diagnostic élevée avaient une diminution de leur taux brut de mortalité par rapport à ceux en pratiquant peu (Patel et al. 2019; Friedenreich et al. 2020). Cependant, l'activité physique pré-diagnostic n'apparaît pas avoir d'effets sur la mortalité spécifique du

cancer de la prostate, ce qui contraste avec ce qui est observé pour d'autres cancers tels que le cancer du côlon et le cancer du sein (Patel et al. 2019; Friedenreich et al. 2020).

En ce qui concerne l'activité physique post-diagnostic, celle-ci a été associée à une amélioration de la survie chez les patients atteints d'un cancer de la prostate mais également à une diminution de 30% de la mortalité spécifique du cancer (Patel et al. 2019; Friedenreich et al. 2020). Ainsi, ces résultats soulignent l'importance de pratiquer une activité physique régulière à la suite d'un diagnostic de cancer de la prostate.

Pour étayer ces résultats et mieux comprendre les mécanismes moléculaires impliqués, de nombreuses études précliniques ont également été réalisées. Cependant, leurs protocoles expérimentaux sont très hétérogènes. Une grande variété de modèles de cancer ont été utilisés, y compris les modèles transgéniques, les modèles de tumeurs syngéniques transplantées, les xenogreffes humaines, et les modèles de tumeur induite par carcinogènes. De plus, différentes modalités de pratique d'activité physique et d'entrainement peuvent être choisies. Les plus couramment utilisées avec les animaux de laboratoire sont la roue d'activité volontaire, qui correspond à une activité peu stressante où une roue est placée dans la cage des animaux qui y accèdent quand ils le souhaitent, et l'entrainement sur tapis roulant, où les animaux sont forcés de courir à une certaine vitesse et pendant une durée déterminée et contrôlée. La pratique d'activité physique chez les animaux peut être réalisée avant et/ou après formation de la tumeur.

Parmi le grand nombre d'études précliniques qui ont traité de l'effet de l'activité physique sur le cancer, la majorité (64%) a trouvé qu'elle était associée à une diminution de la croissance tumorale tandis que seulement 9% ont trouvé qu'elle entrainait son accélération (Ashcraft et al. 2016). Pour le cancer de la prostate plus précisément, il existe à notre connaissance quinze

études publiées dont les résultats sont très contrastés. En effet, huit d'entre-elles ont montré un ralentissement de la croissance tumorale en réponse à l'activité physique (Zheng et al. 2008; Esser et al. 2009; Zheng et al. 2011; 2012; Gueritat et al. 2014; Saedmocheshi et al. 2019; Vahabzadeh et al. 2020; Dufresne et al. 2020), tandis que les sept autres études n'ont pas montré d'effet de l'activité physique sur ce paramètre (Jones et al. 2012; McCullough et al. 2013; Baumfalk et al. 2018; Taylor et al. 2020; Patel, Wallace, et al. 2019; Patel, Abuchowski, et al. 2019; Opoku-Acheampong et al. 2019). De plus, certains résultats ont même indiqué que des rats nudes avec une tumeur prostatique de Dunning R-3327 AT-1 entrainés sur tapis roulant pendant sept semaines avaient une croissance tumorale accélérée par rapport au groupe contrôle. Cet effet n'était toutefois pas observé lorsque l'expérience était répétée chez des rats immunocompétents (McCullough et al. 2013). D'un point de vue moléculaire, quatre études ont montré une diminution de la prolifération des cellules cancéreuses chez les animaux actifs (Zheng et al. 2008; 2012; Gueritat et al. 2014; Dufresne et al. 2020) tandis qu'une autre n'a pas indiqué de différence (McCullough et al. 2013). De plus, deux études ont montré qu'un entrainement sur tapis était capable de diminuer l'activation de ERK1/2 (Gueritat et al. 2014; Dufresne et al. 2020), une protéine qui participe à la voie de signalisation MAPK et PI3K qui favorise la prolifération des cellules cancéreuses (De Luca et al. 2012). L'activité physique pourrait également augmenter l'apoptose des cellules cancéreuses prostatiques, comme cela a été rapporté dans deux études (Zheng et al. 2008; 2012), même si cela ne semble pas toujours être le cas (Gueritat et al. 2014; Dufresne et al. 2020). Les apparentes contradictions de l'ensemble de ces résultats sont surement liées en grande partie aux différences des protocoles expérimentaux utilisés. En effet, plusieurs études suggèrent que la période à laquelle est réalisée l'activité physique (avant *vs.* après implantation de la tumeur), le modèle de cancer utilisé et la modalité d'activité physique

pratiquée, peuvent drastiquement modifier l'impact de l'activité physique sur la croissance tumorale (Ashcraft et al. 2016).

Néanmoins, plusieurs mécanismes moléculaires sous-jacents aux effets anti-cancer potentiels de l'activité physique ont été identifiés. Toutefois ces études ne concernent pas seulement le cancer de la prostate, qui ne représente qu'une petite partie des études précliniques réalisées. Parmi les mécanismes identifiés, on retrouve l'amélioration de la perfusion tumorale, l'amélioration du système immunitaire, la modulation du métabolisme tumoral, et le dialogue entre le muscle et la tumeur (Pedersen, Christensen, and Hojman 2015).

III. Impact de l'activité physique sur l'efficacité des traitements

Au vu de l'impact de l'activité physique sur de nombreux mécanismes biologiques et la diminution de la mortalité associée à cette stratégie observée en clinique, il est probable que l'activité physique puisse interagir avec les traitements anti-cancer conventionnels (Ashcraft et al. 2019). Plusieurs études ont en effet montré que l'activité physique réalisée de manière pré-chirurgicale chez les personnes opérées d'une tumeur du poumon permettait de diminuer les complications post-opératoires et de réduire la durée d'hospitalisation (Sebio Garcia et al. 2016; Cavalheri and Granger 2017; Steffens et al. 2018; Rosero et al. 2019). L'amélioration des capacités cardiopulmonaires induite par l'activité physique pourrait limiter les dommages et augmenter la survie de ces patients. En ce qui concerne les autres types de cancer, les données actuelles ne sont pas assez nombreuses pour établir de conclusions (Steffens et al. 2018).

La combinaison de l'activité physique et de l'hormonothérapie pourrait également avoir des bénéfices et améliorer la survie des patients, notamment en limitant le risque de développer des pathologies cardiovasculaires et métaboliques, maladies qui peuvent être induites par

l'hormonothérapie (Kiwata et al. 2016). En ce qui concerne l'efficacité du traitement en lui-même, des études précliniques suggèrent que la combinaison de l'activité physique avec des traitements antiandrogéniques pourrait être plus efficace que les traitements seuls. Dans ces études, les souris ne recevaient pas de traitement mais étaient castrées afin de mimer l'effet d'un traitement anti-androgénique. Les auteurs ont observé que les souris qui avaient accès à une roue d'activité avaient une croissance tumorale plus lente que les souris non actives (Zheng et al. 2011; 2012). De plus, l'activité physique était associée à une moindre prolifération cellulaire et à une plus grande apoptose des cellules cancéreuses (Zheng et al. 2012). Des études précliniques réalisées dans le cancer du sein suggèrent également que l'activité physique pourrait améliorer l'efficacité du tamoxifen et du letrozol, deux hormonothérapies couramment utilisées dans ce type de cancer (Khori et al. 2015; Isanejad et al. 2016). Ces effets s'expliqueraient notamment par une diminution importante de l'expression de miR-21 et de BCL-2 en réponse à l'activité physique (Khori et al. 2015).

En ce qui concerne la chimiothérapie, plusieurs études suggèrent que la combinaison de ce traitement avec l'activité physique serait une stratégie efficace. Une étude clinique publiée en 2007 a notamment montré que des patients qui suivaient un programme de renforcement musculaire avaient un taux d'achèvement de leur chimiothérapie plus important que ceux qui ne pratiquaient pas d'activité physique (Courneya et al. 2007). Pour venir renforcer ces résultats, plusieurs études précliniques ont montré que combiner chimiothérapie et activité physique entrainait une réduction de la croissance tumorale plus importante que celle observée avec la chimiothérapie seule (Sturgeon et al. 2014; Betof et al. 2015; Schadler et al. 2016; Florez Bedoya et al. 2019; Lemke et al. 2016; Morrell et al. 2019; Ballarò et al. 2019). Ces effets seraient notamment attribués à une amélioration de la perfusion tumorale qui

entrainerait une meilleure distribution de l'agent chimiothérapeutique à l'ensemble de la tumeur (Betof et al. 2015; Schadler et al. 2016; Florez Bedoya et al. 2019; Morrell et al. 2019). Plus récemment, l'immunothérapie a également été testée en combinaison avec l'activité physique dans des études précliniques. Une première étude utilisant des xenogreffes dérivées de patients atteints d'un cancer du côlon a montré que l'association de l'activité physique et de l'immunothérapie ne permettait pas d'obtenir d'effets supplémentaires sur la croissance tumorale par rapport à l'immunothérapie seule (Martín-Ruiz et al. 2020). Cependant, une autre étude combinant immunothérapie et radiothérapie chez des souris porteuses de tumeurs mammaires a montré que la pratique d'activité physique permettait une réduction de la croissance tumorale encore plus importante par rapport aux traitements conventionnels réalisés seuls (Wennerberg et al. 2020). Les auteurs ont notamment observé que l'entrainement était associé à une plus grande activation des lymphocytes ainsi qu'à une diminution des cellules myéloïdes suppressives (myeloid-derived suppressor cells, MDSC) à la fois dans le foie et dans la tumeur, ce qui expliquerait la meilleure efficacité des traitements (Wennerberg et al. 2020). Avoir étudié comment l'activité physique pouvait impacter la combinaison de l'immunothérapie et de la radiothérapie est intéressant car cela mime ce qui se fait en clinique, mais ne permet pas de distinguer l'effet de l'activité physique sur l'immunothérapie *vs* de ceux sur la radiothérapie.

À l'exception de la dernière étude mentionnée, aucune étude ne s'est intéressée à l'effet de l'activité physique sur l'efficacité de la radiothérapie. Pourtant, plusieurs mécanismes biologiques induits par l'activité physique pourraient interagir avec ce traitement (Ashcraft et al. 2019). En effet, activité physique et radiothérapie sont des stratégies qui toutes deux modulent les niveaux de stress oxydatifs. La radiothérapie induit une partie de sa toxicité en produisant une grande quantité de ROS (Riley 1994; Azzam, Jay-Gerin, and Pain 2012).

L'activité physique de son côté peut, soit participer à la production de ROS dans le muscle si elle est réalisée de manière aigue (isolée), soit, à l'inverse, augmenter les défenses anti-oxydantes lorsqu'elle est pratiquée de manière régulière (He et al. 2016). Dans la tumeur, il a été observé qu'un entrainement de course sur tapis roulant apportait une diminution des dommages oxydatifs à l'ADN (Gueritat et al. 2014). Ainsi, si l'on considère les effets chroniques de l'activité physique, on peut penser que cette stratégie limitera les dommages à l'ADN créé par la radiothérapie et donc réduira son efficacité, tandis que si on prend en compte les effets aigus de l'activité physique, il est possible, à l'inverse, que cela augmente ces dommages et améliore la réponse à la radiothérapie. La modulation de la vascularisation tumorale par l'activité physique pourrait également moduler l'efficacité de la radiothérapie en diminuant l'hypoxie de la tumeur, reconnue comme un mécanisme de résistance aux irradiations (Rockwell et al. 2009). Enfin, l'amélioration de la fonction immunitaire qui est observée en réponse à la pratique d'activité physique pourrait améliorer la réponse à la radiothérapie, comme ce qui est observé avec l'immunothérapie.

Ainsi, il existe plusieurs mécanismes biologiques qui rendent plausible l'idée que l'activité physique puisse impacter l'efficacité de la radiothérapie, et ces effets sont probablement dépendants du type de tumeurs et de la modalité d'activité physique choisie.

OBJECTIFS DE LA THÈSE

L'objectif premier de la thèse est d'étudier l'impact de l'activité physique sur la croissance tumorale et l'efficacité de la radiothérapie dans différents modèles précliniques de cancer de la prostate et selon différentes modalités de pratique. Nous chercherons également à identifier les mécanismes moléculaires impliqués dans les effets observés. Ainsi, les deux premières études présentées dans cette thèse ont été conduites dans le but :

1) D'évaluer l'impact d'un entrainement de course sur tapis roulant sur l'efficacité de la radiothérapie dans un modèle murin de cancer de la prostate (Étude n°1)
2) D'étudier l'effet de la roue d'activité volontaire sur la réponse à la radiothérapie dans un modèle préclinique de cancer de la prostate (Étude n°2)

Un second objectif de cette thèse est d'évaluer la possibilité de l'activité physique à moduler des mécanismes épigénétiques dans le cancer de la prostate. Pour répondre à cet objectif, deux études incluses dans cette étude ont été réalisées dans le but :

1) De réaliser une revue de question sur la modulation de l'expression des miARNs circulant en réponse à l'activité physique, et de discuter comment ces changements peuvent potentiellement impacter la croissance tumorale (Étude n°3).
2) D'étudier l'effet d'un entrainement de course sur tapis sur des évènements épigénétiques au sein du tissu tumoral prostatique (Étude n°4).

RÉSULTATS

Étude 1 : L'entrainement de course sur tapis roulant améliore l'efficacité de la radiothérapie dans un modèle murin de cancer de la prostate

Le cancer de la prostate est le deuxième cancer le plus fréquemment diagnostiqué chez les hommes (Bray et al. 2018). Parmi les différentes options thérapeutiques disponibles, la radiothérapie est couramment utilisée chez les patients atteints de cette maladie. Cependant, malgré les avancées médicales majeures, des radiorésistances peuvent se développer, entrainant l'échec du traitement. Aussi, de nombreux efforts sont faits afin de développer des stratégies permettant de contrer ces résistances. En parallèle, de nombreuses données épidémiologiques et précliniques montrent que la pratique régulière d'une activité physique est associée à une diminution de la mortalité dans de nombreux types de cancer (Ashcraft et al. 2016; Patel et al. 2019; Friedenreich et al. 2020). Plusieurs mécanismes biologiques sous-jacents à ces effets ont été identifiés et peuvent être regroupés en quatre grandes catégories : (1) vascularisation et perfusion tumorale, (2) fonction immunitaire, (3) métabolisme tumoral et (4) dialogue muscles-tumeur (Pedersen, Christensen, and Hojman 2015). Au vu de ces éléments, il est possible que l'activité physique puisse améliorer l'efficacité des traitements anti-cancer. Dans la présente étude, nous avons cherché à évaluer l'impact de l'exercice sur la réponse à la radiothérapie dans un modèle murin de cancer de la prostate.

Pour cela, des souris nudes athymiques inoculées avec des cellules cancéreuses prostatiques PPC-1 étaient randomisées en quatre groupes : (1) cancer contrôle (sans intervention), (2) cancer entrainement (entrainement sur tapis roulant cinq fois par semaine pendant deux semaines), (3) cancer radiothérapie (quatre séances de radiothérapie de cinq Grays chacune),

et (4) cancer radiothérapie-entrainement (combine les interventions des groupes radiothérapie et entrainement).

Comme attendu, la radiothérapie a permis de diminuer la croissance tumorale. L'entrainement a également eu un effet puisqu'il a ralenti la croissance tumorale, qu'il soit réalisé seul ou en combinaison avec la radiothérapie. L'entrainement a ainsi permis d'améliorer l'efficacité de la radiothérapie, les tumeurs les plus petites et les plus légères ayant été observées chez les souris combinant radiothérapie et entrainement.

Comparé au groupe contrôle, les trois groupes interventionnels ont montré une diminution de la prolifération des cellules cancéreuses. Cependant, en analysant le ratio des protéines pERK1/2:ERK1/2 dans le tissu tumoral, on a constaté qu'uniquement l'entrainement seul a permis de diminuer ce paramètre.

Nous avons également analysé l'apoptose dans le tissu tumoral et nous avons trouvé que l'expression de la caspase-3 clivée, effecteur d'apoptose, n'était pas modifiée avec l'entrainement seul mais était augmentée avec la radiothérapie. Surtout, cette augmentation était encore plus importante lorsque la radiothérapie était combinée avec l'entrainement. Ainsi, l'entrainement a permis de potentialiser la réponse à la radiothérapie en majorant l'activation de la caspase-3.

Nous avons ensuite évalué si le système immunitaire pouvait être impliqué dans ces effets. L'activité physique est capable de moduler le système immunitaire dans de nombreux tissus et fluides corporels (Pedersen and Hoffman-Goetz 2000). Dans notre étude, nous avons trouvé que le marquage NK1.1 (marquage de cellules NK) était augmenté avec la radiothérapie et que cette augmentation était encore plus importante lorsque celle-ci était combinée à

l'entrainement. De même, les gènes codant pour le killer cell lectin-like receptor K1 (Klrk1) et l'interleukin 2 receptor β (Il2rβ), deux récepteurs activateurs de cellules NK, ainsi que les cytokines pro-inflammatoires tumor necrosis factor α (Tfn-α) et interferon ɣ (Ifn-ɣ) étaient augmentés chez les souris ayant reçu de la radiothérapie et ceci était majoré avec la combinaison radiothérapie et entrainement. Ainsi, l'entrainement semble améliorer l'efficacité de la radiothérapie en potentialisant l'activation des cellules NK au sein du tissu tumoral, entrainant davantage de processus apoptotiques et une diminution plus importante de la croissance tumorale.

Ainsi, cette étude montre que (1) l'entrainement peut améliorer la réponse à la radiothérapie dans un modèle préclinique de cancer de la prostate en augmentant l'infiltration de cellules NK et l'apoptose et (2) même si l'entrainement seul ou combiné à la radiothérapie limite dans les deux cas la croissance tumorale, les mécanismes moléculaires sous-jacents étant différents. En effet, l'entrainement seul diminue la prolifération des cellules cancéreuses en limitant l'activation de ERK1/2 mais n'a pas d'effet sur l'apoptose des cellules tumorales, tandis que l'entrainement combiné à la radiothérapie n'a pas joué sur l'activation de ERK1/2 mais a majoré l'apoptose des cellules tumorales induite par la radiothérapie. Ainsi, l'exercice semble pouvoir interagir avec les traitements anti-cancer conventionnels : dans cette étude, cette interaction a été bénéfique et a permis d'améliorer l'efficacité de la radiothérapie, mais il est plausible que des interactions négatives aient lieu dans d'autres modèles. Néanmoins, nos résultats sont en faveur de la promotion de l'activité physique chez les patients atteints d'un cancer, y compris ceux suivant un traitement par radiothérapie.

Étude 2 : La roue d'activité n'améliore pas l'efficacité de la radiothérapie chez des souris porteuses de tumeurs PC-3

Afin de mieux comprendre l'effet de l'activité physique sur la radiothérapie dans le cancer de la prostate, nous avons réalisé une deuxième étude visant à évaluer l'impact d'une autre modalité d'activité physique, la roue d'activité volontaire, sur l'efficacité de la radiothérapie dans un modèle murin PC-3. Il a notamment été montré que cette modalité de pratique était capable d'améliorer la perfusion tumorale et de limiter l'hypoxie de la tumeur (Betof et al. 2015) ce qui pourrait améliorer la réponse à la radiothérapie (Schumacher et al. 2020).

Dans notre étude, des souris nudes auxquelles on avait injecté des cellules cancéreuses prostatiques PC-3 ont été randomisées soit dans le groupe de souris non-actives, soit dans le groupe de souris actives qui avaient à disposition une roue d'activité dans leur cage. La roue d'activité n'a pas impacté la croissance tumorale et semble améliorer la perfusion tumorale dans notre étude. En effet, en utilisant une échographie de la tumeur combinée avec un agent de contraste, nous avons pu observer que la divergence du flux était plus importante chez les souris actives que chez les souris inactives. Une deuxième expérience a alors été réalisée dans un même modèle de cancer murin où la roue d'activité était cette fois combinée à la radiothérapie. Les résultats obtenus ont montré que la combinaison des deux stratégies n'était pas plus efficace sur la croissance tumorale que l'effet de la radiothérapie seule. Au niveau moléculaire, aucun effet de la roue d'activité n'a été mesuré. Ces résultats contrastent avec notre étude précédente (Dufresne et al. 2020) et nous ont amené à nous demander si ces différences d'effets pouvaient s'expliquer par l'utilisation de roue d'activité *vs* de tapis roulant. Nous avons alors évalué l'effet de ces deux modalités chez des souris avec des xenogreffes de PC-3, et nous avons observé que seul l'entrainement sur tapis roulant

permettait de ralentir la croissance tumorale. Ainsi, la modalité de pratique apparait comme un facteur essentiel à prendre en compte dans les effets anti-cancer de l'activité physique. De plus, nous avons également cherché à évaluer in vitro si différentes lignées de cellules cancéreuses prostatiques pouvaient réagir différemment à l'effet de l'activité physique. Pour cela, du sérum d'hommes actifs et inactifs a été prélevé et utilisé pour traiter des cellules PC-3 et LNCaP. Le sérum d'hommes actifs a permis de diminuer la prolifération des cellules LNCaP mais n'a pas eu d'effets sur la prolifération des cellules PC-3 par rapport au sérum d'hommes inactifs, suggérant que selon la lignée de cancer, les effets de l'activité physique peuvent être différents. Ces résultats nécessitent toutefois d'être confirmés *in vivo*.

Pris dans leur ensemble, les résultats de cette étude suggèrent que la roue d'activité initiée après formation de la tumeur n'a pas d'effet sur la croissance tumorale prostatique (même lorsque combinée avec la radiothérapie) malgré une modification de la perfusion tumorale. En utilisant un modèle similaire, nous avons pu observer qu'un entrainement sur tapis roulant était cependant capable de ralentir la croissance tumorale, soulignant l'importance de mieux comprendre l'impact de différentes modalités de pratique sur le cancer. De plus, des expériences *in vitro* où deux lignées cancéreuses prostatiques (LNCaP et PC-3) ont été traitées avec du sérum d'hommes actifs *vs* inactifs suggèrent que la lignée cancéreuse ou le type de cancer peuvent également influencer les effets anti-cancer de l'activité physique. Il paraît dès lors essentiel de poursuivre les efforts visant à déterminer les modalités de pratique les plus à même d'améliorer la survie des patients atteints de cancer, et de déterminer si certains patients pourraient bénéficier plus que d'autres de cette stratégie.

Étude 3 : Revue de questions sur activité physique et miARN circulants : implications pour le risque et la progression du cancer

Bien que plusieurs processus biologiques sous-jacents aux effets anti-cancer de l'activité physique aient été identifiés, il reste encore de grandes zones d'ombre concernant ces mécanismes. Lors d'un exercice physique, les muscles sont largement sollicités et leur contraction pourrait être impliquée dans les effets anti-cancer de l'activité physique. Le muscle squelettique est capable d'agir sur de nombreux organes grâce à des signaux autocrines, paracrines, ou endocrines (Pedersen and Febbraio 2012; Severinsen and Pedersen 2020). Plusieurs études ont démontré que des cytokines sécrétées par les muscles (appelées myokines) pouvaient impacter la croissance tumorale. Aoi et collaborateur ont notamment identifié la myokine SPARC, capable d'augmenter l'apoptose des cellules cancéreuses et limiter le développement du cancer du côlon (Aoi et al. 2013).

Au-delà des myokines, le tissu musculaire squelettique est également capable de sécréter des miARNs. Les miARNs sont des ARNs courts non codants impliqués dans la régulation post-transcriptionelle de l'expression de gènes. Certains d'entre eux se trouvent en grande quantité dans certains organes spécifiques, et ceux principalement présents dans le muscles sont connus sous le nom de myomiRs. À la suite de stimulations ou de dommages, les miARNs peuvent passer d'un organe à un autre par le biais de mécanismes actifs ou passifs (Chen et al. 2012). Il y a aujourd'hui en oncologie un grand engouement autour des miARNs circulants (c-miARNs) présents dans la circulation sanguine car leur expression est modifiée dans de nombreux cancers (Bryant et al. 2012). Ces c-miARNs apparaissent non seulement comme des biomarqueurs prometteurs mais également comme des outils thérapeutiques (Kosaka, Iguchi, and Ochiya 2010). En effet, les miARNs extracellulaires peuvent avoir des effets biologiques

sur d'autres cellules (Mittelbrunn et al. 2011; Fabbri et al. 2012). Des vésicules extracellulaires, comme les exosomes, peuvent transporter les miARNs aux cellules avoisinantes mais aussi à des organes distants. Ainsi, les miARNs pourraient être impliqués dans la communication inter-cellulaire et inter-organes. Cependant, le rôle des miARNs dans les effets anti-cancers de l'activité physique reste largement inexploré.

Dans cette revue de littérature, nous avons rassemblé les données disponibles concernant l'expression de c-miARNs en réponse à de l'activité physique aigue ou chronique chez des individus sains, et discuté leurs possibles effets anti-cancers. Parmi les études inclues, quatorze ont analysé l'expression de c-miARNs en réponse à une unique séance d'exercice (exercice aigu) et six ont évalué l'effet d'un entrainement de plusieurs semaines (exercice chronique). Exercice aigu et exercice chronique ont tous deux entrainés des différences significatives concernant l'expression de c-miARNs, et ces différences semblent être dépendantes des modalités de pratique (Banzet et al. 2013) et/ou des caractéristiques des sujets. De manière importante, plusieurs c-miARNs dont l'expression a été modifiée par l'activité physique sont connus comme étant altérés dans de nombreux cancers. Cela nous a amené à formuler l'hypothèse selon laquelle la modulation de l'expression de miARNs par l'activité physique pourrait jouer un rôle important dans la prévention et le développement de cancers, notamment en jouant sur la communication inter-cellulaire des miARNs. Par exemple, le miR-133 est un myomiR également connu comme ayant un rôle de suppresseur de tumeur. En réponse à l'activité physique, il est modifié à la fois dans le muscle (Nielsen et al. 2010; Russell et al. 2013) et dans la circulation sanguine (Banzet et al. 2013; Uhlemann et al. 2014; Mooren et al. 2014; Nielsen et al. 2014; Baggish et al. 2014; Gomes et al. 2014; Clauss et al. 2016; Min et al. 2016). Nous avons alors fait l'hypothèse que les miARNs peuvent se

transmettre depuis les muscles *à* la circulation sanguine (par exemple via la libération d'exosomes), puis éventuellement jusqu'à la tumeur où ils peuvent être exprimés.

En parallèle, une étude utilisant un modèle préclinique de cancer du sein a récemment montré qu'un entrainement sur tapis roulant apportait une diminution de l'expression du mir-21 au sein du tissu tumoral, associé à un ralentissement de la croissance tumorale (Isanejad et al. 2016). Les auteurs ont également observé que l'entrainement augmentait l'expression de miR-206 et let-7 dans la tumeur. Cette étude est la première à montrer que l'activité physique est capable de moduler l'expression de miARN au sein du tissu tumoral. Comme ces miARNs sont connus pour être impliqués dans la carcinogenèse, l'altération de leur expression par l'activité physique pourrait limiter l'initiation et le développement de cancer. De plus, il est intéressant de noter que ces trois miARNs (mir-21, miR-206 et let-7) sont modulés dans la circulation sanguine en réponse à l'exercice chez des personnes saines (comme relevé dans cette revue de littérature), mais également dans le muscle suite à un effort physique (Drummond et al. 2008; Nielsen et al. 2010; Fernandes et al. 2012). Ainsi, l'ensemble de ces résultats amène à penser qu'il existe un dialogue entre le muscle et la tumeur en partie médié par le transport de miARNs. Malgré le manque de données disponibles, la modulation des miARNs pourrait représenter un mécanisme biologique sous-jacent aux bénéfices de l'activité physique dans le risque et la progression de cancer. Des études supplémentaires sont toutefois nécessaires pour évaluer cette hypothèse.

Étude 4 : L'entrainement comme modulateur d'évènements épigénétiques dans le cancer de la prostate

Le cancer de la prostate représente une maladie complexe résultant de mutations génétiques, mais également de modifications épigénétiques (Jeronimo et al., 2011 ; Kgatle et al., 2016 ; Ngollo et al., 2014). L'épigénétique correspond à des modifications de l'expression génique qui ne sont pas liées à des altérations dans la séquence de l'ADN. Il existe trois principaux mécanismes épigénétiques : (1) l'expression d'ARN non codant (comme les miARNs), (2) la méthylation de l'ADN, et (3) les modifications d'histones. Au-delà des traitements thérapeutiques, des stratégies de changements de comportement apparaissent prometteuses pour moduler ces différents processus. L'activité physique en particulier est de plus en plus intégrée dans la prise en charge des patients atteints de cancer et des données récentes suggèrent que cette pratique est capable, dans cette population, de modifier des marqueurs épigénétiques au sein de la circulation sanguine (Isanejad et al., 2016). Cependant, l'effet de l'activité physique sur les processus épigénétiques au sein de la tumeur reste largement inexploré. Ainsi, le but de cette étude est d'évaluer, au sein d'un modèle préclinique de cancer de la prostate, l'impact de l'entrainement sur la signature épigénétique du tissu tumoral.

Pour cela, des échantillons de tumeur issus d'une étude précédemment publiée par notre équipe (Guéritat et al., 2014) ont été analysés. Des rats auxquels on avait implanté une tumeur prostatique AT1 étaient randomisés en deux groupes : (1) cancer contrôle (sans intervention) et (2) cancer entrainement (entrainement sur tapis roulant cinq fois par semaine pendant quatre semaines). Il avait notamment été montré que l'entrainement était capable de ralentir la croissance tumorale (Guéritat et al., 2014). Dans la présente étude, nous avons utilisé ces échantillons de tumeur afin d'évaluer si les effets anti-cancer de l'activité physique étaient

associés à des modifications dans l'expression de miARN, méthylation de l'ADN ou à des modifications d'histones.

L'expression de miARN est fortement dérégulée dans le cancer de la prostate. À l'aide d'un microarray, nous avons évalué l'expression de plus de 1000 miARNs au sein de la tumeur, et les résultats obtenus ont montré que l'entrainement avait altéré l'expression de 75 d'entre eux. Parmi ces miARNs, le miR-27a-5p, connu comme ayant des propriétés pro-apoptotiques dans le cancer de la prostate (Barros-Silva et al., 2018), était augmenté avec l'entrainement. De plus, l'expression de la caspase-3 clivée, marqueur d'apoptose, était également plus importante dans le groupe entrainement que dans le groupe contrôle. Ainsi, l'activité physique est capable de modifier l'expression de miARNs au sein du tissu tumoral prostatique, ce qui pourrait représenter un mécanisme anti-tumoral associé à cette pratique.

Le cancer de la prostate est également caractérisé par une hypométhylation globale de l'ADN, qui est associée à une plus grande instabilité des chromosomes et favorise la progression de la maladie Dans notre étude, nous avons montré que l'entrainement limitait l'hypométhylation globale de l'ADN, ce qui pourrait être un signe de meilleur pronostic. De manière paradoxale, le cancer de la prostate est également caractérisé par une hyperméthylation spécifique de la région promotrice de certains gènes, et notamment les gènes suppresseurs de tumeur, ce qui entraine leur inhibition. Dans notre étude, nous avons pu observer que l'entrainement diminuait l'expression des DNA methyl-transferases (DNMTs) qui catalysent cette réaction.

Enfin, de nombreuses modifications d'histones participent à l'initiation et à la progression du cancer de la prostate. En particulier, les tumeurs prostatiques sont caractérisées par une augmentation de l'expression d'histones deacetylases (HDACs), qui favorisent la prolifération

des cellules cancéreuses. Dans notre étude cependant, l'entrainement n'a pas provoqué de modifications dans l'activité ou l'expression des HDACs.

En conclusion, notre étude a montré que l'activité physique était capable de modifier des processus épigénétiques majeurs au sein du tissu tumoral prostatique. Plus précisément, l'entrainement a permis de moduler l'expression de miARNs, de normaliser la méthylation globale de l'ADN, et de diminuer l'expression des DNMTs. L'ensemble de ces résultats suggère que les modifications épigénétiques pourraient représenter un mécanisme biologique sous-jacent aux effets anti-cancer de l'activité physique.

DISCUSSION

L'adhésion des patients atteints de cancer aux recommandations en matière d'activité physique est relativement faible (Coletta et al. 2019) malgré l'accumulation d'éléments montrant un bénéfice de l'activité physique sur les effets secondaires du cancer et de ses traitements, ainsi que sur la survie. Mieux comprendre les mécanismes impliqués dans ces effets est essentiel pour sensibiliser davantage le personnel soignant sur l'intérêt de cette stratégie ainsi que pour aider à confirmer la sûreté de cette pratique chez les patients sous traitement.

Mécanismes épigénétiques : un mécanisme impliqué dans les effets anti-cancer de l'activité physique ?

Dans nos études, nous avons montré dans un modèle préclinique qu'un entrainement de course sur tapis roulant était associé à plusieurs modifications épigénétiques au sein du tissu tumoral, ce qui pourrait être le reflet d'une tumeur moins agressive. Plus particulièrement, nous avons observé que chez les rats actifs, la méthylation globale de l'ADN était plus importante, l'expression *des Dnmt*s était diminuée, et l'expression du miR-27a-5p augmentée. Il est important de noter que la méthylation de l'ADN et l'expression de miARNs sont deux facteurs qui peuvent être impliqués dans les résistances aux traitements et représentent des cibles thérapeutiques prometteuses (Azad et al. 2013; Easwaran, Tsai, and Baylin 2014). Par exemple, il a été montré *in vitro* que traiter des cellules cancéreuses prostatiques avec du zebularine, un inhibiteur de DNMT, entrainait une plus grande efficacité de la radiothérapie (Dote et al. 2005). De même, une autre étude a montré que l'inhibition de DNMT3B permettait

de sensibiliser les cellules cancéreuses PC-3 à la radiothérapie *in vitro* (Xue et al. 2015). Dans notre étude, nous avons observé que l'expression des gènes *Dnmt1*, *Dnmt3a* et *Dnmt3b* était diminuée chez les rats actifs par rapport aux rats non actifs. Ainsi, ces résultats suggèrent que pratiquer une activité physique avant traitement par radiothérapie pourrait permettre d'améliorer l'efficacité de la radiothérapie en diminuant l'expression de DNMTs, mais cette hypothèse reste à être évaluée.

De plus, nous avons observé que l'entrainement sur tapis limitait l'hypométhylation globale de l'ADN, qui est un signe d'instabilité du génome fréquemment observé dans les tumeurs et qui pourrait être impliqué dans les résistances aux traitements (Yegnasubramanian et al. 2008). La modulation de la méthylation globale du génome pourrait ainsi potentiellement représenter un mécanisme par lequel l'entrainement améliorerait la réponse aux traitements.

L'expression aberrante de miARNs dans le tissu tumoral est un autre événement épigénétique impliqué dans avec la progression du cancer et la résistance aux traitements (Ma, Dong, and Ji 2010; Zheng et al. 2010; Iorio and Croce 2012). Dans le cancer de la prostate, de nombreux efforts sont réalisés pour identifier les miARNs impliqués dans la radioresistance afin de développer de nouvelles thérapies permettant de sensibiliser les cellules cancéreuses à la radiothérapie (Malla et al. 2017; McDermott et al. 2017). L'activité physique, comme observé dans notre étude, est capable de modifier l'expression de miARNs au sein du tissu tumoral, et pourrait ainsi potentiellement moduler l'efficacité de la radiothérapie.

La modification par l'activité physique de certains évènements épigénétiques dans la tumeur pourrait donc avoir des conséquences sur les réponses aux traitements, mais des études supplémentaires doivent être réalisées afin de tester cette hypothèse.

Interactions entre activité physique et radiothérapie dans le cancer de la prostate : nouvelles perspectives

Le cœur de cette thèse s'attache à comprendre comment l'activité physique impacte l'efficacité de la radiothérapie dans différents modèles précliniques de cancer de la prostate. La première étude réalisée chez des souris nudes auxquelles on a injecté des cellules PPC-1 a permis de montrer que l'entrainement de course sur tapis roulant (1) ralentissait la croissance tumorale et (2) améliorait la réponse à la radiothérapie.

Au niveau moléculaire, l'activation de ERK1/2 était diminuée avec l'entrainement (ce qui était associé à une moindre prolifération des cellules cancéreuses), mais cet effet n'était pas observé lorsque l'entrainement était combiné à la radiothérapie. Ainsi, ces résultats suggèrent que des interactions au niveau moléculaire peuvent avoir lieu entre traitement et activité physique. De plus, nous avons également constaté que le marqueur d'apoptose caspase 3 clivée ainsi que plusieurs marqueurs de cellules NK n'étaient pas modifiés par l'entrainement et qu'ils étaient augmentés avec la radiothérapie, cette augmentation étant encore plus importante lorsque l'entrainement était combiné à la radiothérapie. Ces résultats sont intéressants car ils montrent que l'entrainement peut sensibiliser les cellules cancéreuses à la radiothérapie *via* le système immunitaire, et souligne également la présence d'interactions potentielles au niveau moléculaire. Ainsi, il est important de garder à l'esprit que les résultats obtenus par les études précliniques qui se sont intéressées à l'effet de l'activité physique seule sur la croissance tumorale ne seront pas forcément les mêmes que si l'activité physique est combinée avec un traitement (car des interactions entre les stratégies peuvent avoir lieu). Par exemple, certaines études précliniques ont montré que l'activité physique seule n'avait pas d'effet sur la croissance tumorale, tandis qu'elle permettait d'améliorer l'efficacité de la

chimiothérapie lorsque combinée à celle-ci (Sturgeon et al. 2014; Schadler et al. 2016; Lemke et al. 2016; Florez Bedoya et al. 2019). L'ensemble de ces éléments permet donc de supposer la possibilité d'interactions au niveau moléculaires entre activité physique et traitement.

Activité physique et cancer de la prostate : la nécessité d'une approche personnalisée

Pour mieux comprendre les effets de l'activité physique sur la radiothérapie nous avons réalisé une seconde étude dans un modèle de xenogreffe PC-3. Dans un premier temps, nous avons observé que la roue d'activité était capable d'améliorer la perfusion tumorale, ce qui nous a amené à nous demander si cela pouvait améliorer la réponse à la radiothérapie. Cependant, nos résultats montrent que dans notre modèle, la roue d'activité n'a pas eu d'effet sur la croissance tumorale, que ce soit seule ou combinée à la radiothérapie. Cela peut surprendre au vu de notre précédente étude, et nous a amené à nous demander si différentes modalités d'activité physique pouvaient induire des effets différents sur la croissance tumorale prostatique. Lorsque l'effet de la roue d'activité *vs* l'effet d'un entrainement sur tapis roulant a été étudié au sein d'un même modèle de xenogreffe PC-3, nous avons observé que le tapis ralentissait la croissance tumorale, alors que la roue n'avait aucun effet (comme observé précédemment). L'entrainement sur tapis et l'utilisation régulière d'une roue d'activité engendrent des adaptations physiologiques différentes (Kim et al. 2020), ce qui peut expliquer pourquoi leurs impacts sur la croissance tumorale diffèrent. Par exemple, la mobilisation du système immunitaire en réponse à l'activité physique est proportionnelle à la durée et à l'intensité de l'activité pratiquée. L'entrainement de course sur tapis roulant peut (selon les paramètres fixés) représenter une activité plus intense que la roue d'activité et pourrait donc

potentiellement être capable mobiliser le système immunitaire de manière accrue. Le système immunitaire joue un rôle clé dans les effets anti-cancer de l'activité physique (Pedersen et al. 2016) et dans la réponse à la radiothérapie, et le choix des modalités de pratique ne doit donc pas être négligé.

De plus, le type et le sous-type de cancer semblent également représenter des facteurs déterminants dans les effets anti-cancer de l'activité physique. En effet, nous avons observé *in vitro* que lorsque les cellules PC-3 et LNCaP étaient traitées avec du sérum d'hommes actifs *vs* inactifs, seuls les LNCaP étaient sensibles à l'effet anti-prolifératif de l'activité physique. Ainsi, cela pose la question de savoir si certains cancers sont plus sensibles à l'effet de l'activité physique que d'autres, et si les identifier permettrait de mieux cibler les patients à prendre en charge avec des programmes d'entrainement.

CONCLUSION ET PERSPECTIVES

Au vu des résultats exposés dans cette thèse, il parait important de personnaliser l'approche de l'activité physique chez les patients atteints d'un cancer de la prostate en fonction (1) des caractéristiques de leur cancer et (2) de leur traitement. De plus, une meilleure compréhension de l'impact de différentes modalités de pratiques sur la croissance tumorale apparait essentielle pour améliorer la prise en charge de ces patients. Ainsi, des études supplémentaires sont nécessaires afin de mieux comprendre comment ces différents paramètres influencent le cancer de la prostate et de permettre une approche personnalisée et efficace de l'activité physique dans le milieu de l'oncologie.

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Titre : **L'activité physique : une stratégie permettant d'améliorer l'efficacité de la radiothérapie ? Nouvelles perspectives issues de données précliniques**

Mots clés : Activité physique, exercice, cancer de la prostate, radiothérapie

Résumé : Le cancer de la prostate est un fléau mondial et l'échec des traitements est un défi majeur pour combattre cette maladie. La pratique d'une activité physique chez des patients atteints d'un cancer est associée à une amélioration du pronostic et certains mécanismes biologiques sous-jacents à ces bénéfices ont été identifiés grâce à des modèles précliniques. Il est dès lors plausible que l'activité physique améliore l'efficacité des traitements utilisés contre le cancer. Toutefois, l'effet de l'activité physique sur l'efficacité de la radiothérapie reste méconnu.
L'objectif a cette thèse est d'étudier l'impact de l'activité physique sur la croissance tumorale et la réponse à la radiothérapie dans différents modèles précliniques de cancer de la prostate.
Notre travail montre (1) qu'un entrainement sur tapis roulant permet d'améliorer l'efficacité de la radiothérapie dans un modèle préclinique de cancer de la prostate PPC-1 en améliorant l'infiltration des cellules tueuses naturelles dans le tissu tumoral ; (2) qu'un modèle de roue volontaire ne permet pas d'obtenir ces effets chez des souris porteuses de tumeurs PC-3, ce qui semble être attribuable au sous-type de cancer plutôt qu'à la modalité d'activité physique ; et (3) que les processus épigénétiques au sein de la tumeur sont régulés par l'activité physique chez des rats porteurs de tumeurs AT1, le rôle de cet effet sur l'efficacité des traitements restant à explorer. Nos travaux suggèrent donc que l'activité physique pourrait sensibiliser les cellules cancéreuses prostatiques à la radiothérapie et souligne également la nécessité de programmes d'entrainement personnalisés pour les patients atteints de cancer pourrait améliorer l'efficacité de la radiothérapie

Title : **Physical activity : a strategy to improve radiotherapy efficiency in prostate cancer? Novel insights from preclinical models**

Keywords : Physical activity, exercise, prostate cancer, radiotherapy, radiation therapy

Abstract : Prostate cancer is a major health issue worldwide and treatment failure represents a major challenge in this disease. Physical activity in cancer patients is associated with improved outcomes and several biological mechanisms mediating these benefits have been identified in preclinical models. It thus appears plausible that physical activity could improve anti-cancer therapy efficiency. The effect of physical activity on radiotherapy efficiency in prostate cancer remains however largely unknown.
The objective of this thesis was to investigate the impact of physical activity on tumor growth and radiotherapy response using different physical activity modalities and preclinical prostate cancer models.
Our work showed that (1) treadmill running enhanced the radiotherapy response in a preclinical model of PPC-1 prostate cancer through increased natural killer cell infiltration to the tumor tissue; (2) voluntary wheel running did not provide the same benefits in mice bearing PC-3 tumors, which might be attributable to differences in prostate cancer subtype rather than physical activity modalities; and (3) tumor epigenetic processes in rats with AT1 tumors were regulated by exercise, but their influence on cancer treatment remains unknown. Hence, our work suggests possible improvements in radiotherapy efficiency in prostate cancer with physical activity but also shows the need for tailored exercise programs in the oncology setting.