Using bioinformatic analyses to understand Prostate Cancer Cell Biology

Mémoire

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Résumé

Le cancer de la prostate (CaP) affecte 1 homme sur 7 au cours de sa vie. C'est le cancer numéro un diagnostiqué chez l'homme. Il s'agit du quatrième cancer le plus fréquent au Canada. Le CaP est une maladie hormonodépendante diagnostiquée chez l'homme. Les androgènes jouent un rôle vital dans la progression de la maladie. La première ligne de traitement, suivant une intervention chirurgicale ou un traitement de radiothérapie, est la thérapie de déprivation aux androgènes. Malgré une réponse initiale positive à l'inhibition des androgènes, la progression de la maladie vers un cancer de la prostate résistant à la castration (CRPC) est presque inévitable. Aux différentes étapes du CaP, le récepteur des androgènes joue un rôle majeur. Ainsi, cette thèse décrit les méthodes développées et utilisées pour mieux comprendre la biologie du CaP et le rôle joué par les androgènes dans cette maladie. Le travail démontré dans cette thèse se compose principalement d'analyses bioinformatiques effectuées sur des ensembles de données accessibles au public et d'un « pipeline » construit pour analyser des données RNA-Seq. Un pipeline RNA-Seq a été développé pour comprendre l'impact des androgènes et des gènes régulés lors du traitement aux androgènes dans les modèles de cellules de CaP. Ce pipeline bioinformatique se compose de divers outils qui ont été décrits ci-dessous dans le chapitre 1. L'objectif principal de ce projet était de développer un pipeline pour analyser les données RNA-Seq qui aide à comprendre et à définir les voies et les gènes métaboliques qui sont régulés par les androgènes, et qui jouent un rôle important dans la progression du CaP. Le flux de travail expérimental consistait en deux lignées cellulaires positives aux récepteurs aux androgènes LNCaP et LAPC4. Toutes les données utilisées dans ce projet ont été rendues publiques pour que la communauté de recherche puisse effectuer diverses autres études et analyses comparatives pour comprendre les fonctions des androgènes dans un sens beaucoup plus profond afin de développer de nouvelles thérapies pour traiter le CaP.

Dans un autre projet décrit au chapitre 2, des analyses bioinformatiques ont été réalisées sur des données accessibles au public pour comprendre la fréquence de la perte et de l'altération génomique du gène *PTEN* localisé à 10q23. Ces analyses ont mis en évidence la fréquence d'altération génomique de PTEN qui est beaucoup plus élevée dans le CRPC que dans le CaP localisé. Ces analyses ont également aidé à identifier d'autres gènes altérés dans le CaP. Ces gènes n'ont pas été beaucoup étudiés dans la littérature, mais il semble que certains d'entre eux possèdent des caractéristiques de suppresseurs de tumeurs. Ces résultats pourraient être un bon début pour des analyses plus approfondies concernant la perte de gènes.

La compréhension des fonctions de AR et de la suppression de *PTEN* aidera à développer de nouvelles stratégies et approches pour diagnostiquer et traiter le CaP. L'intégration des analyses bioinformatiques à la recherche clinique ouvre une nouvelle perspective dans le domaine de la recherche du CaP.

Abstract

Prostate Cancer (PCa) affects 1 in 7 men in their lifetime and is the number one diagnosed cancer in men. It is the 4th most common cancer in Canada. PCa is a hormone-dependent disease diagnosed in men. Androgens play a vital role in the disease progression. The standard of care to treat PCa, following surgery or radiation therapy, is the androgen deprivation therapy (ADT). In spite of initial positive response to androgen inhibition, the progression of the disease to castration-resistant prostate cancer (CRPC) is almost inevitable. Across the various stages of PCa, the androgen receptor (AR) plays a major role. This thesis portrays the methods developed and used to understand PCa biology. The work demonstrated in this thesis majorly consists of bioinformatic analyses performed on publicly available data sets and a pipeline built to analyse RNA-Seq data. An RNA-Seq pipeline has been developed to understand the impact of androgens and the genes regulated upon androgen treatment in PCa cell models. This bioinformatic pipeline consists of various tools which have been described below in chapter 1. The major goal of this project was to develop a pipeline to analyse the RNA-Seq data which helps to understand and define the metabolic pathways and genes regulated by androgens which play an important role in PCa disease progression. The experimental workflow consisted of two androgen receptor positive cell lines LNCaP and LAPC4. All the data used in this project has been made publicly available for the research community to perform various other comparative studies and analyses to understand the functions of androgens in a much deeper sense to develop novel therapies to treat PCa.

In another project described in chapter 2, bioinformatic analyses have been performed on publicly available data to understand the loss and genomic alteration frequency of the gene *PTEN* occurring at *10q23*. These analyses highlighted that the genomic alteration frequency of *PTEN* is much higher in CRPC than in localised PCa, and also helped in identifying other genes which are lost along with *PTEN*. The lost genes have not been studied much in literature, but few studies demonstrated that they might possess tumor suppressor characteristics. These results might be a good start for further deeper analyses regarding the lost of genes.

Understanding the functions of AR and the deletion of *PTEN* will help for the development of novel strategies and approaches to diagnose and treat PCa. Integration of bioinformatic analyses with clinical research open up a new perspective in the PCa research domain.

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ADT	Androgen Deprivation Therapy
AR	Androgen Receptor
CNA	Copy Number Alterations
CGP	Cancer Genome Project
CRPC	Castration-Resistant Prostate Cancer
DHT	Dihydrotestosterone
ER	Estrogen Receptors
FDR	False Discovery Rate
GEO	Gene Expression Omnibus
GnRH	Gonadotropin-releasing hormone
GR	Glucocorticoid Receptors
GSEA	Gene Set Enrichment Analyses
ISUP	International Society of Urologic Pathology
LBD	Ligand Binding Domain
LH	Luteinizing Hormone
MR	Mineralocorticoid Receptors
NES	Normalised Enrichment Score
NGS	Next generation sequencing
NTD	N Terminal Domain
PCa	Prostate Cancer
PHTS	PTEN hamartoma tumor syndrome
PR	Progesterone Receptors
PSA	Prostate Specific Antigen
QC	Quality Control
RCT	Randomised controlled clinical trials

RP	Radical Prostatectomy
TCA	Tricarboxylic Acid
TCGA	The Cancer Genome Atlas
TPM	Transcripts Per Million
TSG	Tumor Suppressor Genes

Dedicated to my granny Mrs.Vijaya Lakshmi Sathyavolu

गतस्य शोचनं नास्ति
Gone is gone. You shouldn't worry about that.

Remerciements

I don't understand the concept of acknowledgements and thanking every person for inspiration and non-existence assistance or some casual reference. Going with the flow and formality, I would like to "THANK" people who have provided me with impeccable "existing and real" support during my stay in #LABEAW housed at CHU-Quebec.

First things first, a super big Thank you (genuinely) to "The Boss" Dr. Étienne Audet-Walsh for accepting me as your first grad student. I'm pretty sure I will never be erased out of your memory but Mr. Kevin Gonthier can be erased, removed and deleted (as he is the second grad student :P). I might have let you down and I might have given you nightmares on various occasions, but all this was on purpose so that you can never forget me ☺. Leaving the fun aside, thank you for letting me grow and for guiding me onto the right path. Prostate cancer wouldn't have never become a huge part of my career if it wasn't for you. I still remember (I'm sure even you do too) my first lab meeting where I presented my slides with yellow borders and grey background consisting of all computational information and no biology. My career graph after joining your lab is almost similar (let's say identical) to the prostate cancer disease progression graph. After the initial identification and deployment in your lab, the first standard of care is poster and oral presentations followed by ChIP protocols and TC protocols. In spite of initial positive response to TC protocols, the progression of fear in the hood of TC was inevitable. Across all these stages, EAW played a major role in progression of my career. In more than 80% of the cases (instances), EAW is always reactivated when my brain starts working on PCa. We (#LABEAW) all by now know that AR (EAW) indirectly controls various pathways in disease progression through mTOR and SREBF1. Here, mTOR and SREBF1 are Mr. Poluri and Mr. Gonthier. Terrific and troubleshooting Thursday lab meetings were always exhausting (at least when I was presenting) but I learnt the art of destroying every slide (inclusive of title slide). Tuesday individual meetings at the temple of enlightenment (EAW's cave) consisted of flashes of pure awesomeness and epiphany jiffs. Drive to Château Bromont in 2018, Bombay Sapphire at Estérel in 2019, playing cards for one whole day at airports (YQB, YYZ) along with Dr.JLP will be the ultimatum. I believe I have been absolutely lucky and blessed to have you as my PI/Boss for my master's. It is sad that I'm leaving the team as things didn't go as well as we planned, but still I'm taking tonnes of positives on my way out. I also thank you for putting out a super huge Avengers' team around me who became my tight knit circle much more than just lab mates or colleagues. Thank you to the members of #LABEAW (especially KG and CL) for supporting me emotionally and mentally during KitKat, Oreo, Hazelnut Hot Choc and Butter phases.

**theme music plays* Bad boys … bad boys … Whatcha gonna do …. Whatcha gonna do ... When they come for you!!!*

Mr. Gonthier, I have no words to describe the moments we had and the time we spent together. You have told my mother that you are my Canadian twin and you stayed on what you have said. I believe we have equally discussed bullcrap and PCa over the past 2 years (I think the lab will not agree to this but it's a fact). One day we might find a cure to PCa but nothing can cure your absence around me when I move to Kingston. The experiment performed with you at 6:00 AM on a beautiful Thursday changed me into a better student/researcher. CRCQ and CCRC 2019 are the elite highlights for various reasons, but Luigi's mansion and sharing the same blanket with you will be on the zenith of my moments. The 4/5 hours preparation for our first journal club during the C'mas season of 2018 was as good as Oreo shake and Domino's pizza. C'mas 2018 reminds me of fun filled dinner party at EAW's domicile and the drive back in your beautiful blue "FROZEN" car. I have honed my skills in bullcrapping from you. One example of this is the spontaneously knit beautiful story of elephants and tigers. Thank you for introducing me to the rousse and noire boréale. Along with you these two are the best things that have happened to me. The conversation with you after orally consuming the amalgamation of the left-over liquids at La Cage/3 Brasseurs felt like a budding Buddha preaching under a bodhi tree. Every cup of mushroom soup at the cafeteria with you was a pure nirvana. You have promised me to teach WB but never did. Will never ever forgive you for this. Thank you for letting me be me. Thank you for not making me wear any masks or put on any fronts. Thank you for being part in my happiest moments, and for genuinely feeling the same; for listening to my weird exotic stories and radiating compassion and empathy from wherever you are. Thank you for being the only person I ever want to confide in.

You will always be my BLUE DRAGON. « **I love you** ».

Ms. Lafront, I'm privileged to share my workstation next to yours. I have learnt so much just by staring at your color annotated lab experiment book and watching you work. It was a pure bliss. The wonderful first lab meeting of yours was legendary. The presentation along with you for the scientific communication course was a heroic saga. I know I have irritated and disturbed you on various ocassions by bullcrapping but thank you for taking them on your chin and being a sport. You have destroyed me and Kevin on every opportunity given to you. Class given by you on economy of Zimbabwe at CRCQ 2019 was historic. Thank you for being the ***Wizard***. And super huge shout out for critical reading of this thesis (with out which this would have been a pure mess with spelling and grammatical errors :P)

Cindy, you have been an absolute blessing and it was wonderful working with you. You have always been super positive and had the patience to hear out my frustrations. Stealing cookies with Mr.KG from your office felt like a heist. Thank you for the unlimited cookies, snacks and chocolates, thank you for helping me out on various experiments and thank you for sharing your office space for couple of months.

I don't even know the names of the summer interns (being mean here :P). Thank you, Mr. Luke the Nuke, for all the donkey jokes and elephant stories. You are the only person who could understand the bioinfo errors and frustrations. Working with you for last minute analyses for EAW's grant was epic … Felt like sitting on a grenade without pin. Loved working for the article you have authored (sarcasm). Thanks mate for everything. Thank you Lilliane for teaching me how to be social, thank you Jolyane for showing 8 full qPCR plates can be done in one single day. Thank you Virginie for showing me how to be productive, punctual and focussed.

Thank you, Mariam Dessay, for being the shoulder I can lean on whenever I needed one. Thank you for keeping my desi taste buds active. Thank you for torturing me to death to attend the 6:00 AM swimming sessions even during the chilly winters. In spite of so many ups and downs in the past year and a half, your love, affection, generosity and concern towards me remained the same. You are an unbelievable pocket-sized beast.

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Special 'Thank you' to Baba, Amma, Chellai and Ammama (Thanking you people doesn't make sense … Your acts have been selfless so that I could take a step forward). This would have never been possible without you (Technically I would have never been possible without you). Thank you for being super supportive, encouraging and trusting my choices and career decisions. Thank you for giving me the strength and belief to reach for the stars and chase my dreams.

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have been pizza sauce to my pizza base (I mean pizza without sauce is horrible … At least super bad). The gaming sessions (from Assassins Creed, NFS Most Wanted, FIFA to LUDO King and COD Mobile) we had/ we are having/we will be having are best I had/I'm having/I will be having. Thank you for being with me during my hardest times. As our family is growing bigger year by year, I believe and hope the fun and bonding will be doubled and the journey in the next coming decades will be a terrific ride.

"*Not all races are won on the track*." - Darius, NFS Carbon, 2006

Thank you to my dearest Gauri Matta. I don't say it too often but thank you. Thank you for being that person that I know I can rely on even if I feel like the world has turned against me. Most of all, thank you for showing me that love still exists and for always sticking around when things get hard. Thank you for being the light of my life and for showing me that I am yours too. Thank you for supporting all my stupid ideas and my ambitious choices. Thank you for introducing me to Grey's Anatomy, due to which this thesis writing has been put on hold :P for straight couple of days.

"*If there's a crisis, you don't freeze, you move forward. You get the rest of us to move forward because you've seen worse, you've survived worse, and you know we'll survive too. You say you're all dark and twisty. That's not a flaw; that's a strength. It makes you who you are. I love you and I want to spend the rest of my life with you.*" - Derek Shepherd, season 5, episode 19.

Thank you, Patrick Russo and Mario Russo at Patrizio & co., and at Birra & Basta. It has been absolute fun and pleasure to work along with you guys and grow into an average « Pizzaolo ». Thank you for being patient and teaching me the art of cooking Italian dishes. #ciaobella

Merci à tous ☺ <3 #desperatefrenchattempt

Finally, I would like to leave the remaining space (including the next page with a star) in the memory of people who lost their lives in the process of fighting with/against COVID-19.

*

Avant-propos

This thesis titled “Using bioinformatic analyses to understand the Prostate Cancer Cell Biology” is written and adapted to article insertion format and is presented to the Graduate Faculty of Université Laval for a M.Sc. Degree in Molecular Medicine. This section explains and defines contribution of other authors in my articles. I have also contributed as a co-author in other projects from the lab which are not discussed in this thesis.

“Genomic Deletion at *10q23* in Prostate Cancer: More Than *PTEN* Loss?” is the title of the first article I contributed and authored. This article was published in Frontiers of Oncology journal on June 29th, 2018. The impact factor of this journal is 4.137. This study was conceptualized and established by Dr. Étienne Audet-Walsh. The analyses were performed by Dr. Étienne Audet-Walsh. For my part, my contribution was involved in the interpretation of the results obtained from the analyses, and also involved in manuscript writing along with Dr. Étienne Audet-Walsh. All the data to perform the analyses has been obtained from The Cancer Genome Atlas (TCGA) portal. I estimate the percentage of my contribution to this article would be around 20%.

The article titled “RNA Sequencing Data of Human Prostate Cancer Cells Treated With Androgens” was published in the Data in Brief journal in August 2019 with an impact factor of 0.970. This study was designed and conceptualized by Dr. Étienne Audet-Walsh and me. I have contributed to this article by performing the wet lab experiments and bioinformatic analyses. I have performed wet lab experiments like cell treatments and RNA extraction. I have also majorly participated in the writing of the manuscript and graphical representations. Dr. Étienne Audet-Walsh also contributed by fine tuning the manuscript and the graphical representation. The RNA-Seq bioinformatic was established in collaboration with Dr. Charles Joly-Beauparlant and Dr. Arnaud Droit. They have contributed in building and debugging the pipeline. I believe my contribution in this article would be around 75%.

Introduction

1. Cancer Genomics

1.1 Role and Impact

In the recent decade, the rapid surge in awareness that somatic mutations and other aberrations drive human malignancies has led us within reach of personalized cancer medicine. The cumulative phenotypic consequence of somatically acquried genetic, genomic and epigenetic alterations in cancer cells are influenced by both heterotypic interactions with a unique tissue microenvironment and the host germline. The greatest advantage that cancer genomics holds is the potential to inform the prognosis and guide evidence-based management of early stage diseases, which comprise an increasing proportion of cancer diagnoses. Cancer genome drives the transition from a morphology-based to a genetics-based taxonomy of cancer, which means point of care decisions will become increasingly customized to the unique genomic and proteomic features of a patient's tumor. These molecular features may be assessed in tumor biopsies, in circulating tumor cells or in body fluids using companion diagnostics that can predict the likelihood of clinical benefit for all treatment options[1].

The information which is being gained from studying cancer genome has changed the dimensions of cancer research. Defining the biological relevance of genomic data is a key step in realizing the full clinical potential of cancer genetics and genomics.

A point mutation in *HRAS* gene was the first cancer related mutation discovered nearly thirty years ago, which causes a glycine to valine mutation at codon 12[2]. The subsequent identification of similar mutations in the family members KRAS3–5 and NRAS6 ushered in a new field of cancer research activity[3]. The high cost and time taking efforts like using a PCR-based exon-by-exon direct sequencing approach concentrated in a highly draggable class of genes. Two cancer genes V600 and BRAF, which are now the subject of intense therapeutic development, were identified during the early phase of the work[4]. The extension of these PCR-based resequencing efforts to larger and larger segments of the coding genome has substantially increased the number of newly identified cancer genes and broadened the range of biology that has been shown to be subverted by mutations in human cancer.

The sudden hike in clinical opportunities has been because of the analysis of ever-increasing genomic datasets. The identification of mutations in the JAK2 kinase in myelo-proliferative neoplasms has resulted in new drug development opportunities for diseases for which there are currently no approved therapies[5]. Targeted PCR-based sequencing of candidate genes has yielded an expanding list of genomic discoveries that are being translated into personalized cancer management. Some notable ones include kinase activating mutations in the oncogene KIT. Integrative cancer genome characterization can generate the genomic and statistical evidence

for new candidate cancer targets for therapeutic and diagnostic development. Converting these into therapeutic agents and biomarkers will require deep biological insights to inform drug discovery and to define a clinical path for development.

1.1.1 Accession of Cancer Genomics Data

Most of the large-scale cancer genomics data has been made publicly available but accessing and using that data remains a major obstacle. Depending on the degree of computational modification and integration applied to the data, it is divided into four categories: (1) raw, (2) processed or normalized, (3) interpreted, and (4) summarized categories. These are also even known or referred as levels I to IV data. Projects like The Cancer Genome Atlas (TCGA), Cancer Genome Project (CGP) at Wellcome Trust Sanger Institute are huge large-scale projects, whose genomics data is generally available for the public or can be accessed upon request[6]. The data available does not have to be a high-quality data. Various quality control analyses have to be performed to check for experimental noise, analytical accuracy and batch effects, as these have an impact on validation steps. Multiple factors have to be considered before performing or progressing ahead with publicly accessed data.

1.2 Computational considerations in Cancer genome analysis

For the analysis of cancer genome data, several general considerations are looked upon apart from the challenges thrown by the process. These include Quality Control (QC) of data, the accurate estimation of the signal and noise in large data sets, reproducible approaches to complex genomic analysis and achieving sufficient power in the face of multiple hypotheses testing. The quality of the genomic data should first be verified whether it is sufficient for the analysis and experimental purposes. The QC can be performed using multiple computational QC tools, but the most commonly used is the FastQC[7]. This tool generally checks for Per Base Sequence Quality, Adaptor Content, Kmer Content and other parameters. The sample mismatching is also one of the biggest problems in the generation of the data. One should ensure that the data obtained or downloaded is from the intended sample. To address the aforementioned challenge, TCGA road map developed by Robbins et al. can be implemented, which provides a data model and system for capturing, indexing and annotating metadata for a subset of TCGA data[8]. One of the key objectives of the TCGA roadmap project was to develop a programmatic interface that enables the end-user to navigate and discover subsets of files based on file-level provenance annotations and rich metadata. This enables, for example, discovery of the exact set of files that led to a particular research result, even if each patient sample, experiment, and platform have several raw data files[9].

There are several limitations to using next-generation sequencing. Next-generation sequencing provides information on a number of molecular aberrations. For many of the identified abnormalities, the clinical significance is currently unknown. Building of validated bioinformatic pipelines is another major step in the

analyses. The pipelines must be updated and maintained in order to be working on par with latest and validated tools as well as data. The performance of the pipelines should be very much optimized without compromising quality and results, in order to give more room for the people with low configuration units to implement the pipelines in their workflow.

Reproducibility is another key element which should be considered in the genome analysis. For example, if 25 genes are differentially expressed between two tumor subtypes, one would expect to obtain the approximate result even when the process is done for the second time using the exact parameters, tools used, and process involved.

Building of validated bioinformatic pipelines is another major step in the analyses. The pipelines must be updated and maintained in order to be working on par with latest and validated tools as well as data. The performance of the pipelines should be very much optimized without compromising quality and results, in order to give more room for the people with low configuration units to implement the pipelines in their workflow.

In the next coming years, the considerations or the challenges mentioned above will be drastically reduced as the current era is largely progressing forward. Further developments in "omic" analysis of data must aim to ease interoperability of multiple data sets and to develop a framework that can help in seamless analysis of data.

1.3 Transcriptomics

The health science research sector is currently undergoing a huge transformation in order to integrate latest technologies and produce new data to unravel the mystery of cancer. The importance of identification of proper predictive biomarker has been show by the success and failures of integration of transcriptomics in the new age of oncology care and research[10, 11]. Concentrations of RNA molecules in biological samples and mRNAs for protein coding genes can be understood and analysed precisely by transcriptomics[12]. Transcriptomics is the go-to approach in this era to generate top quality high throughput gene expression data[13]. Transcriptomics can be defined as the study of all RNA molecules consisting in a cell. This approach focuses more messenger (m)RNA molecules only, which provides with expression levels or state of the genes. Transcriptomics uses RNA sequencing (RNA-Seq) data, which uses high-throughput sequencing to capture all sequences to determine the quantity of a transcript[14]. Many labs across the globe are working towards personalized medication through transcriptomics, which has increased the deposition of transcriptomic data on public repositories like Gene Expression Omnibus (GEO)[15] or TCGA, Array-Express[16]. The use of transcriptomics for functional interrogation of gene expression has been justified by drawing a correlation between ribosomal profiling and transcriptomic data for mRNA molecules[17-19].

Over the period, the potential to understand gene expression between genotypes, drug treatments or tissues has been transformed by evaluating the content and abundance of RNA. This technique helps to improve the depth of investigation to the entire transcriptome of known and novel RNAs[20]. This is because RNA-Seq is not only limited to genomic sequences but can also identify transcripts. Low background signal and RNA-Seq data can be more quantitative than the data obtained from microarrays[21]. Pathway analyses, differentially expressed genes, identification of novel transcripts and splicing events can be accessed by various bioinformatic tools like Gene Set Enrichment Analysis, DESeq2, g:Profiler and Whippet.

RNA-Seq is a powerful tool which can help the researchers to better understand genes. For example, a gene with an unknown function expressed in various tissues can be emphasized through transcriptome. Novel alternative splicing events/regions can be discovered (Fig.1). These events cannot be identified by DNA sequencing[20].This approach will enable the identification of the genes that may be susceptible to prostate cancer (PCa)[22]. On the basis of RNA-seq data, the transcriptome profiles of primary PCa are identified, including gene fusions, long non-coding RNAs, alternative splicing and somatic mutations[23, 24].

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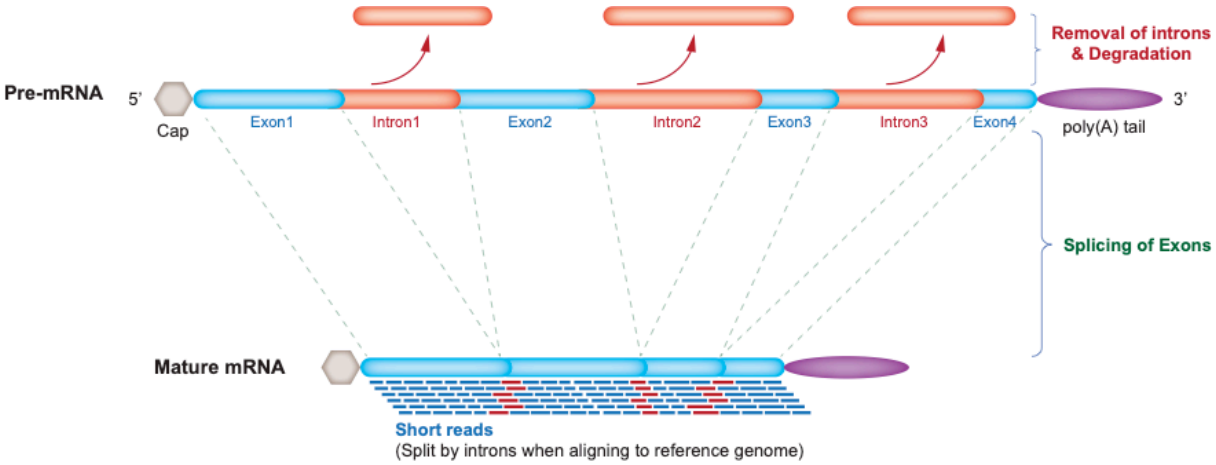

Figure 1: RNA-Seq data uses short reads of mRNA which is free of intronic non-coding DNA. The reads must be aligned to the reference genome. Aligning the reads to the reference genome will help for the identification of novel gene expressions and splicing events/regions.

Next generation sequencing (NGS) played an important role in the recent cancer biological insights and breakthroughs; however, the main challenge in translating the large amounts of data into information which can be interpretable and accessible for cancer care still lies ahead. A fruitful relationship has to be established between bioinformaticians, statistical geneticists and molecular biologists to increase the translating rate of

omics data into clinical practice. The access to the databases and the availability of proper workflows and pipelines for NGS will greatly increase omics-based cancer diagnosis and personalized treatment strategies[20, 21].

The induction of omics approaches in clinical practice will allow analysis of changes in patients at a global level by improving diagnosis and choice of therapeutic plans which are so far based on a few markers only. This approach also helps in developing models that can accurately predict patient survival using prognostic and predictive biomarkers in the era of precision oncology. In the near future, omics technologies will lead to a significantly improved biomarkers identification, compared to for example the top genes obtained by conventional differential gene expression analysis. It will also help in guided biomarker identification, which will play an important role in early tumor prognostic so that treatments can start earlier. Moreover, the identification of novel targets will decrease reliance on morbid therapies, thus improving the quality of life for PCa patients. A revolutional step forward in the field of precision medicine could derive from a pan-cancer analysis of multiple omics profiles on a genome-wide scale, in order to understand the shared patterns across multiple cancer types and identify shared actionable targets at a multilayered level. It therefore appears evident that the integration of omics data represents a powerful tool to allow clinical translation of this integrated dissection of cancer biology.

2. What is a Prostate ?

Behind the man's penis is the localization of a walnut-sized gland called prostate. In the male reproductive system, the prostate gland is the largest accessory gland. It is important for the proper functioning of the male reproductive system. The vital function of the prostate is the production of thin, slightly alkaline fluid that forms a portion of the seminal fluid which is responsible for the protection and transportation of the sperms. The collagen forms an outer layer of the prostate capsule and the inner layer consists of a smooth muscle[25].

Another important function of prostate gland is the accumulation and secretion of high levels of citrate. Healthy prostate epithelial cells exhibit a highly specialized behaviour regarding their metabolic pathways. Typically, cells rely on citrate oxidation as a key step in the Krebs cycle for the progression of aerobic respiration[26, 27]. Benign prostatic cells accumulate zinc in high concentrations, and this excess zinc inhibits the oxidation and metabolism of citrate within the citric acid cycle, resulting in the production of citrate (Fig.2). The citrate is subsequently secreted as a component of semen.

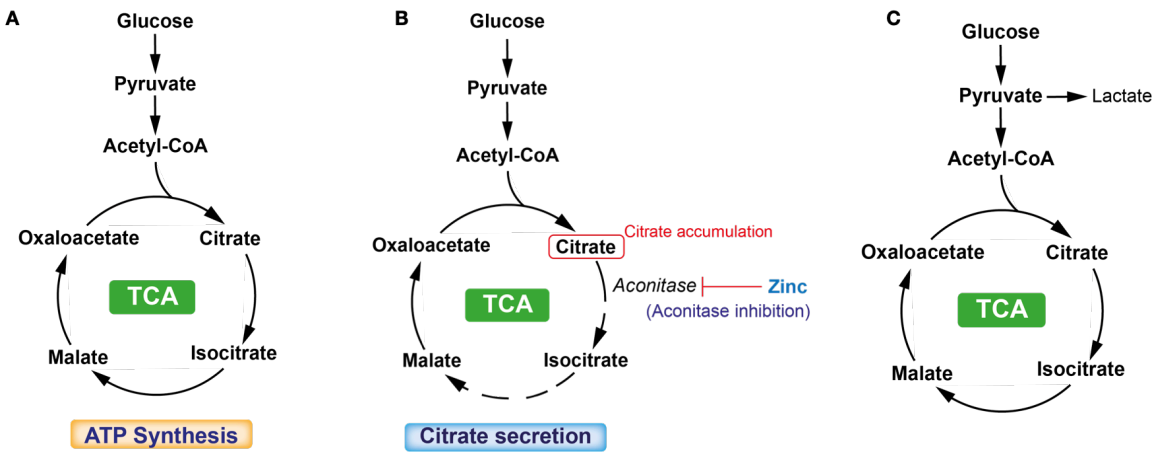

Figure 2**:** TCA cycle is a central route for oxidative phosphorylation in cells, and fulfills their bioenergetic, biosynthetic, and redox balance requirements. (A) TCA cycle in the classic cells. (B) TCA cycle in normal prostate cells. These healthy cells exhibit a highly specialized behavior with respect to their metabolic pathways. Prostate cells, especially epithelial cells in the peripheral zone of the prostate, are programmed to produce and not oxidize citrate. (C) TCA cycle in PCa cells. PCa cells lose their ability to accumulate zinc, thus leading to a continuation of the TCA cycle.

The prostate gland is located posterior to the lower portion of the symphysis pubis, anterior to the rectum, and inferior to the urinary bladder in the sub peritoneal compartment between the pelvic diaphragm and the peritoneal cavity. The prostate gland surrounds the proximal urethra as it exits from the bladder[28].

The prostate consists of 4 regions, the Central Zone, Transition Zone, Peripheral Zone, and Anterior fibromuscular stroma, and also abides an apex, base and anterior, posterior, and inferior-lateral surfaces (Fig.3)[28]. One third of the lower prostate is taken by the apex, the middle one third of the prostate is midprostate which comprises of the verumontanum in the midprostatic urethra, and the upper one third of the prostate is formed by the base situated right below the urinary bladder[28].

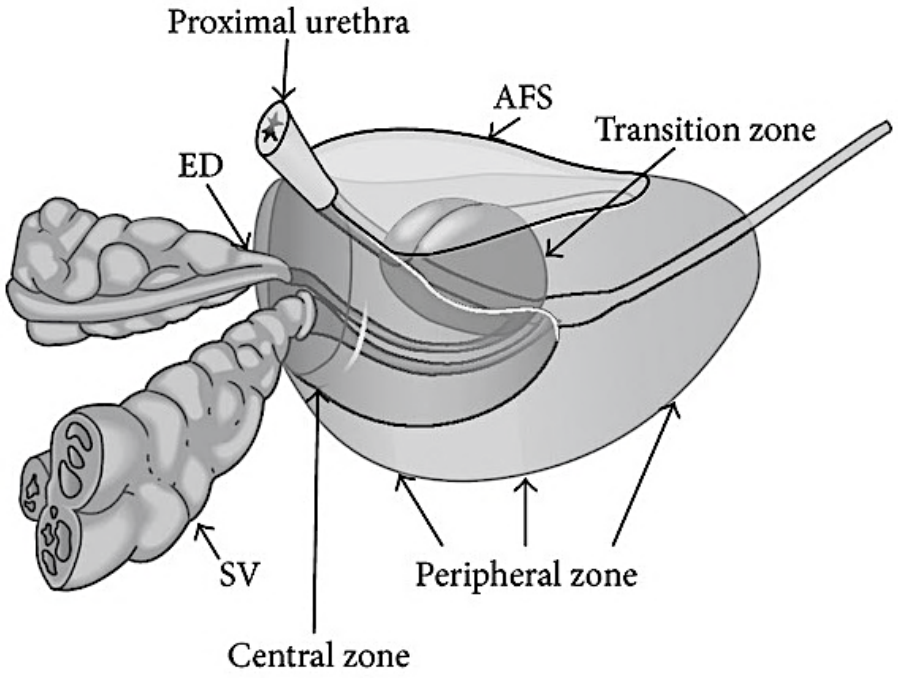

Figure 3: Zonal anatomy of the prostate gland. Ejaculatory Ducts (ED), Seminal Vesicles (SV), Anterior Fibromuscular Stroma (AFS). [28]

Out of all the 4 zones, peripheral zone is the largest of all the zones, comprising around 70% of the glandular tissue. It surrounds the distal urethra and it extends from the base to the apex along the posterior surface. Approximately around 70% of all PCa arise from peripheral zone, which is primarily derived from the urogenital sinus[29]. By contrast, a very low incidence of prostate cancer is found in the central zone which is derived from the Wolffian duct[30]. The transition zone shares a similar embryologic origin as the peripheral zone; however, the percentage of prostate cancer arising from the transition zone is lower, in the order of 25%[28].

The location of the central zone is at the base of the prostate between peripheral and transition zones, and is responsible for approximately 25% of the glandular tissue[28]. It is in the form of a cone and narrows down to an apex at verumontanum, and adjoining are the ejaculatory ducts. The transition zone comprises of two small lobules of glandular tissue that surrounds the proximal prostatic urethra just superior to the verumontanum and this zone makes up 5% of glandular tissue. This is the portion of the glandular tissue that enlarges due to benign prostate hyperplasia[28]. Finally, the anterior fibromuscular stroma forms the convexity of the anterior external surface and is devoid of glandular tissue; it is instead composed of fibrous and smooth muscular elements[28].

2.1 The Etiology of Prostate Cancer

PCa is the most commonly diagnosed cancer in men in Canada[31]. PCa is often diagnosed in North American men when compared to rest of the world. The majority of PCa cases are diagnosed in men aged 50 and above[31]. A tumor is formed when the healthy epithelial cells of prostate change and grow out of control. The malignancy occurs by a major alternation in the mechanism of epithelial cells[32]. The tumor formed can be either cancerous or benign. The major difference between the former two is cancerous tumor can grow and spread to other parts of the body whereas benign cancer can grow but will not spread to other parts of the body. At the time of initial diagnosis, more than 90% of patients have PCa confined in the organ or only locally advanced[33]. PCa can be a slow-progression disease, such that many men die with PCa rather than from PCa. Many men have tumors that grow very slowly, whereas others develop a very aggressive disease, which metastasizes rapidly spreading elsewhere in the body.

PCa clinical follow-up has been revolutionized by the discovery of prostate-specific antigen (PSA), and to this day it is the only widely used biomarker for prognosis and follow-up of the disease. However, the survival benefit of PSA screening for PCa diagnostic is still being studied[34, 35]. This human protease is a protein encoded by the gene *KLK3*, which is located on chromosome 19q13.4 and is a member of the tissue kallikrein family of proteases[36]. PSA is responsible for dissecting semenogelin I and II in the seminal coagulum; it is produced in prostate ductal and acinar epithelium and is secreted into the lumen where the dissecting happens[37, 38]. Research studies conducted during the early 1990s suggested the use of PSA for detecting the presence of PCa. Serum PSA was clearly a much more sensitive screening tool, but it lacked specificity when compared digital rectal examination[39, 40]. On the other hand, when compared to prostatic acid phosphatase, serum PSA was demonstrated to be a more sensitive marker for PCa detection. The introduction of these tools for screening of PCa led to the increase of organ-confined PCa cases[38].

Another method to evaluate the prognosis of men with prostate cancer is the Gleason grading system with prostate biopsy samples[41]. The classical Gleason system defines five histological growth patterns (grades). It scales from favourable prognosis which is Gleason 1 to poor prognosis which is Gleason 5. The scoring has been significantly modified after two major consensus meetings conducted by the International Society of Urologic Pathology (ISUP) in 2005 and 2014, respectively. A new simplified grading system is integrated into a strategy of prostate cancer staging that predicts prognosis and helps to guide treatment[42]. By using these known baseline clinical parameters, we can predict the prognosis and efficacy of novel treatments for prostate cancer. However, they are not perfect for selecting the best treatment sequence. Achieving precision medicine will require more precise tissue- or liquid-based biomarkers with prognostic and predictive value beyond these clinical parameters.

The treatment approaches for localized PCa involve radical prostatectomy (RP) and radiotherapy. These approaches are considered to be curative approaches for localized PCa[43, 44]. Observation studies have been conducted to understand the outcomes of these above-mentioned approaches, with surgery being beneficial especially in young men with intermediate and high risk of localized PCa[44]. The majority of patients with localized PCa will be cured after local therapy with a five-year survival near 100%; but once the tumor progresses by developing distant metastasis, the disease often become incurable[45]. Various comparative studies have been done by teams across the globe to understand the outcomes of radiotherapy and surgery, but no clear and strong evidence has been found[46].

3. Androgen Receptors

3.1 What are Androgens and Androgen receptors?

Testosterone and Dihydrotestosterone (DHT) together are known as androgens. Androgens are the male sex hormones which play a vital role in the development of the male reproductive system. Testosterone can be converted into DHT by the enzyme 5α-reductase, which is the most potent form of androgens, and to estradiol by the enzyme aromatase (Fig.4). Actions of DHT and testosterone are mediated through the androgen receptor AR, a ligand-dependent transcription factor. The androgen receptor is part of the hormonal nuclear receptor family which consists of estrogen receptors (ER), glucocorticoid receptors (GR), progesterone receptors (PR) and mineralocorticoid receptors (MR)[47]. The localization of the gene *AR* is on the X chromosome and is also expressed in a variety of tissues. Androgens are studied and subjected to various biological functions in bone, muscle, prostate, and immune and neural systems. The initiation of male sexual development is done when AR binds to its native ligands testosterone and DHT. The AR, when bound by a ligand, complexes with DNA at androgen response elements in the promoter region of target genes. The transcriptional regulatory effects of the AR include pathways involved in cell growth and proliferation, cell cycle progression, protein synthesis, and cell death.

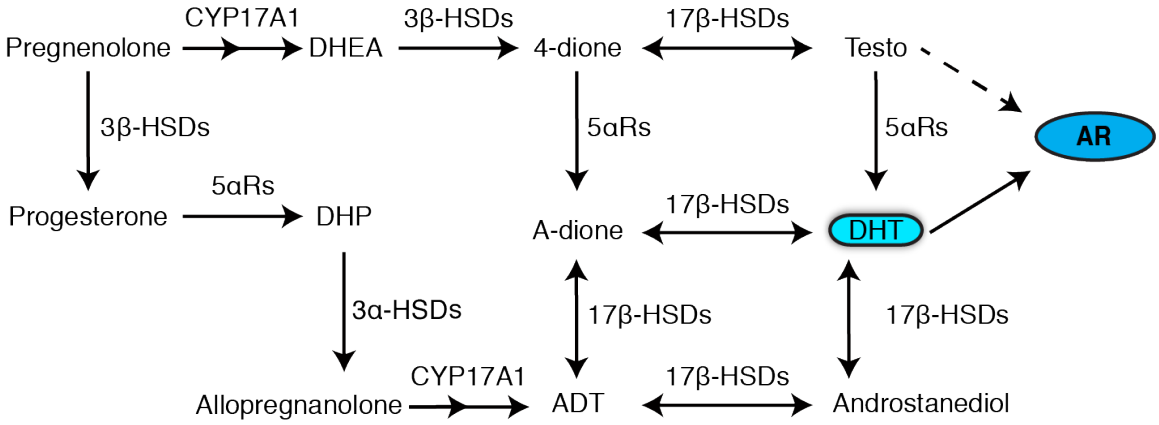

Figure 4: Schematic chart depicting the synthesizing of DHT, the most potent form of androgen. [48]

Prostate cancer is driven by the androgen receptor signalling[27, 49]. AR signalling axis is considered to be an important driver of prostate carcinogenesis and subsequent phases of the disease because of the vital and critical role of AR in the normal prostate. Androgen receptor indirectly/directly promotes tumor growth and disease progression through reprogramming specific cellular metabolic pathways such as aerobic glycolysis, mitochondrial respiration, fatty acid ß-oxidation, and de novo lipid synthesis. For that reason, androgen inhibition or deprivation therapy is the standard of care to treat PCa following disease recurrence after surgery or radiotherapy. In most of the cases, in spite of initial positive responses to the AR inhibition/deprivation therapy, the progression of the disease to CRPC is almost inevitable, which generally occurs within the period of 18-24 months[50]. This is the most lethal form of PCa[51, 52].

Recent research work has shown that CRPC remains dependant on AR signalling pathways, and the most important therapeutic target has been the AR signalling pathway even in low androgen environment. The incremented expression, gene duplication and gene copy number of *AR* is believed to be the cause of dependence on AR in CRPC. Increased levels of *AR* gene copy number and mRNA amplification has been recorded in patients with CRPC[53]. The promotion of resistance to variety of AR targeting agents[54] and conversion of AR antagonists to agonists have been linked to the elevated levels of AR.

3.2 Androgen Receptor Structure

The localization of the AR gene is on the X chromosome at the locus Xq11-Xq12 [47, 55, 56]. The protein coding region comprises of 8 exons, and the introns varies in sizes from 0.7 to 2.6kb. A 110kDa protein consisting of 919 amino acids is encoded by *AR*[57, 58]. AR is the only receptor in the nuclear receptor family which consists of 3 functions domains, namely (i) the N-Terminal Domain (NTD), (ii) the DNA binding domain (DBD) and (iii) the C-Terminal ligand Binding Domain (LBD), which is connected to the DBD by a flexible hinge region (Fig.5)[47]. For

the receptor to function, all the three domains are important. Currently, there is no complete structural information of the full-length AR receptor, but both DBD and LBD have been studied separately[57], revealing critical details of the ligand dependant androgen receptor mechanism action of this receptor.

Figure 5: The three functional domains of AR: (i) the N-Terminal Domain (NTD), (ii) the DNA binding domain (DBD) and (iii) the C-Terminal ligand Binding Domain (LBD). (H – Hinge Region, AF-1 – transcriptional activating function 1, AF-2 – Transcriptional activating function 2, NLS – Nuclear localisation Signal, NES – Nuclear Export Signal)[47].

3.3 Androgen Deprivation Therapy

It was demonstrated by Huggins and Hodges that PCa is dependent on AR activation for its growth and survival[59]. The main therapy to treat PCa is Androgen Deprivation Therapy (ADT) or Androgen Inhibition Therapy[60]. ADT is the process of testosterone deprivation produced by testicles, which is done either by chemical castration or by surgery (orchidectomy).

By using ADT, AR ligand availability and subsequent AR mediated proliferative effects on prostate have been decreased by the reduction of circulating androgen levels and the rates of testicular androgen synthesis since androgens play a major role in PCa disease progression. The common approaches of ADT include orchiectomy or chemical castration through the chronic administration of gonadotropin-releasing hormone (GnRH) agonists and analogs of the LHRH[61]; other medical approaches include estrogen therapy, which also results in impaired androgen production and castration levels of circulating testosterone[62]. The use of GnRH agonists is the preferred means of achieving anorchid testosterone levels in clinical practice. Cyclic GnRH stimulation of the anterior pituitary stimulates the release of luteinizing hormone (LH), which in turn stimulates testicular androgen synthesis in the normal host. Depot GnRH agonists, such as leuprolide, goserelin, buserelin and others, induce an initial transient increase in LH release, followed by tachyphylaxis resulting from nonphysiologic and nonpulsatile stimulation of GnRH receptors. The potential therapeutic benefit of inhibiting both AR ligand production and binding led to the development of AR antagonists, such as bicalutamide, enzalutamide, flutamide and nilutamide, but this remains controversial[62].

3.4 Progression of Prostate Cancer to Castration-Resistant disease

PCa cannot be completely cured with ADT; indeed, after the median of 1-2 years of ADT, the clinical progression occurs[63]. This stage is termed as CRPC. AR signaling is maintained by various mechanisms like mutations or truncations, amplifications and extragonadal androgen production even in the drastically low circulating levels of serum testosterone[64]. Randomized controlled clinical trials (RCT) are the clinical proof that showed CRPC are androgen dependent and also showed the survival benefits with more innovative ways of androgen inhibition or blockade. Enzalutamide, an androgen inhibitor and a second-generation AR antagonist with higher affinity for AR than first-generation antagonists, blocks and degrades AR, resulting in survival benefits in CRPC stage[65]. The reactivation of AR in CRPC stage should be studied in much deeper sense to understand the functions of AR in order to develop novel therapies.

If castrate levels of serum testosterone are less than 20ng/dL during ADT, along with satisfying at least one of the below mentioned three criteria, then PCa is considered as being at the castration-resistant stage. The three criteria are: (1) a rise of prostate-specific antigen (PSA) serum levels (biochemical progression), (2) development of symptoms in the presence of pre-existing cancer (clinical progression), or (3) detection of new metastatic lesions on imaging (radiographic progression)[66, 67]. CRPC was also termed as “androgen-independent” or “hormone-refractory” PCa[68], but as the research led to new discoveries, especially the discovery of reactivation of AR in CRPC, it is now referred as “castration-resistant”[69].

The concept of disease progression to CRPC is still unclear and is being studied to understand and discover novel therapies. Few researchers pointed out that the continued AR signaling despite of ADT might be the central point for the disease progression to CRPC[70, 71]. In recent times, majority of the research is done to understand how AR signaling is reactivated in CRPC and to understand the functions of AR in CRPC[69].

The mammalian target of rapamycin (mTOR), which is a serine/threonine kinase that plays central roles in various biological processes, is a major component of the protein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2)[72]. mTOR phosphorylates distinct sets of substrates in response to growth factors (GFs), stress, nutrient availability, and other stimuli[73, 74]. Nuclear mTOR has been shown as an important transcriptional effector of PCa cellular metabolism and a key integrator of the androgen signaling pathway. It has also been shown that activation of mTOR-dependent metabolic gene networks is essential for the androgen-mediated metabolic reprogramming of cancer cells[75].

In metastatic CRPC, mTOR is often hyperactivated through one of the most frequent genomic alternations which is loss of *PTEN*[76, 77].

4. *PTEN*: Tumor Suppressor

The impact on Tumor Suppressor Genes (TSG) from the alterations that lead to the development of cancer are higher than compared to oncogenes in the cancer genomes. The major set of cancer driving genes consisted of TSGs which was justified by the results from exome sequencing analyses performed on various human cancers[78, 79]. In the recent times, the analyses on data obtained from The Cancer Genome Atlas (TCGA) supported the initial findings, with the majority of copy number alterations (CNA) in this genome analyses comprising deletions of putative TSGs. According to the findings provided by Zack et al., approximately 60% of peak regions of copy number alteration in cancer are deletions, and the majority of genes within these peaks are either known TSGs or appear to be novel TSGs[80].

A novel gene *PTEN* was identified on the chromosome 10q23 in the year 1997 as a tumor suppressor redefining the regulation of cell behaviour[81, 82]. *PTEN* is abbreviated for phosphate and tensin homolog. *PTEN* is also known to be playing vital roles in the processes of apoptosis, embryonic development and cell migration[83, 84]. The mutation or deletion of this gene on chromosome 10 has serious impacts on the progression of human brain, breast and prostate cancer.

The dephosphorylation of phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P3) to phosphatidylinositol 4,5-biphosphate (PtdIns(4,5)P2) is the major biological function of *PTEN*. This biological process inhibits the phosphatidylinositide 3-kinase (PI3K) signaling pathway (Fig.6)[85]. The PI3K signaling pathway is known to play major roles in cell metabolism, survival, proliferation, apoptosis, growth and migration. The suppression of PI3K pathway allows *PTEN* to bring its tumor suppressive functions to play[86]. *PTEN* loss also hyperactivates mTOR and its upstream regulator (PI3K/Akt) (Fig.4), and has been observed in metastatic castration-resistant prostate cancer (CRPC)[87, 88]. Activated AR enhances and reprograms mTOR chromatin-binding profiles and that nuclear mTOR activity is essential for androgen-mediated transcriptional reprogramming of metabolism in PCa cells[75](Fig.7).

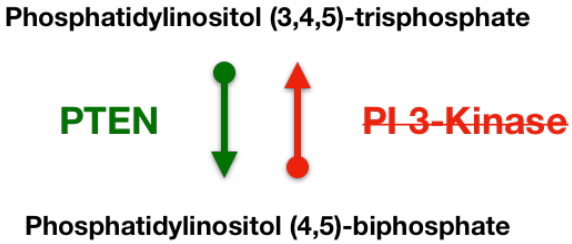

Figure 6**:** The major biological function of *PTEN*: the inhibition of PI3K pathway, which plays an important role in cell metabolism, survival, proliferation, apoptosis, growth and migration.[85]

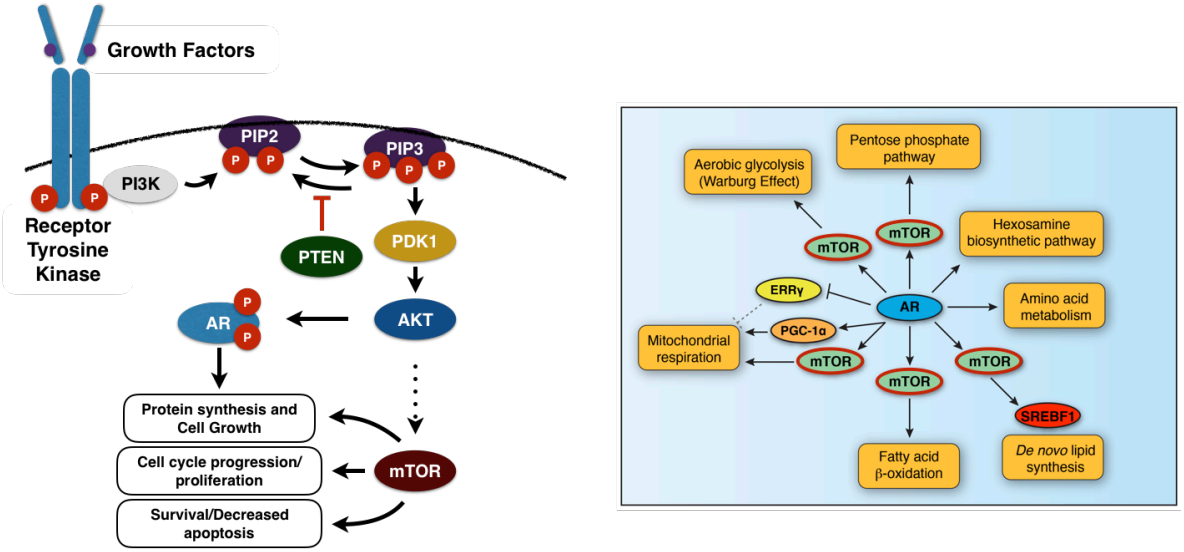

Figure 7**:** mTOR pathway (left). mTOR directly/indirectly controls various cellular metabolic pathways linked with disease progression (right).

From the database of catalogue of somatic mutations in cancer (COSMIC) by sanger institute, 1478 unique mutations of *PTEN* have been recorded. The major enzymatic activity of *PTEN* can be impaired by these unique mutations[89]. The most typical pathology induced by *PTEN* mutation is *PTEN* hamartoma tumor syndrome (PHTS). PHTS confers an increased risk for specific malignancies, mostly breast, thyroid, renal and endometrial cancers[90].

Various studies have demonstrated the tumor suppressor characteristics of *PTEN* gene[86, 91]. In the recent times, numerous studies have shown that *PTEN* regulates many proteins involved in immune cell development and immune signaling pathways, as well as other immunological activities[89].

5. Hypotheses, Objectives and Methodologies

AR is known as the master regulator of cellular energy metabolism as it controls metabolic pathways like Glycolysis, lipid metabolism and protein synthesis along with Electron Transport Chain and TCA cycle in the mitochondria. AR also indirectly controls various pathways related to disease progression, majorly through mTOR. For example, AR controls pathways like Fatty acid β oxidation and mitochondrial respiration through mTOR. Here mTOR pathway is an important intracellular signaling pathway in regulating cell cycle. There it is directly related to proliferation. Phosphorylation and activation of AKT can have many downstream effects out of which one is the activation of mTOR (Fig.7). This pathway is antagonized by various factors along with *PTEN*, which will be further detailed in this thesis[92].

The major hypotheses for my master's degree are: (i) AR controls mRNA expression of various genes associated with various metabolic pathways, and (ii) the loss of *PTEN* is hyperactivating the mTOR pathway and is much more than just a *PTEN* loss at 10q23. The objectives developed and designed to understand and assess the above mentioned hypothesis are: (i) to develop and establish a RNA-Seq bioinformatic pipeline which identifies differentially expressed genes and pathways regulated upon treatment with androgens (R1881) from the sequencing data, then define metabolic genes regulated by androgen receptor, and (ii) to perform bioinformatic analyses on the publicly available data to study *PTEN* in details. The results of the above-mentioned work are described in chapters 1 and 2 respectively.

For the wet lab experiments, two AR positive cell lines LNCaP and LAPC4 have been selected, maintained and treated with androgens. RNA extraction was performed, and samples were sent for sequencing. The sequencing of these samples was performed by CHU-Québec sequencing platform.

The knowledge of methodologies obtained and used in this work are majorly bioinformatic tools required to analyze the data. The bioinformatic pipeline for RNA-Seq analyses has been built from scratch in collaboration with Dr. Arnaud Droit team. The pipeline consists of tools for quality control, pseudo alignment and quantification, and is used to identify differentially expressed genes and pathways regulated. The majority of the analyses were performed on compute canada servers. Statistical analyses were perfomed on local system using R-Studio.

Chapitre 1. RNA Sequencing Data of Human Prostate Cancer Cells Treated With Androgens

Résumé

Le cancer de la prostate (CaP) est le cancer le plus fréquent chez les hommes en Amérique du Nord et les cellules de CaP dépendent du récepteur aux androgènes (AR) pour leur croissance et leur survie. Pour comprendre l'effet du AR dans les cellules cancéreuses, nous avons traité des cellules LNCaP et LAPC4, deux lignées cellulaires humaines immortalisées de CaP *in vitro*, avec l'androgène synthétique R1881, puis nous avons effectué des analyses par RNA-Seq. Les données de séquençage de haute qualité obtenues ont été analysées en utilisant notre pipeline bioinformatique qui comprend le *FastQC* pour le contrôle de la qualité, *Trimmomatic* pour l'épuration des données et *Kallisto* pour le pseudo-alignement sur le transcriptome. Les gènes présentant un différentiel d'expression ont été identifiés en utilisant *DESeq2* suivant un ajustement pour le False Discovery Rate (FDR) (valeurs *q* FDR < 0,05) et une normalisation de l'expression logarithmique relative (RLE). Une analyse de l'enrichissement des ensembles de gènes (GSEA) a également été effectuée pour identifier les voies biologiques significativement modulées par les androgènes. Les analyses GSEA ont permis d'identifier la voie de signalisation des androgènes, ainsi que plusieurs sentiers métaboliques, comme étant significativement enrichis après une stimulation aux androgènes. Ces analyses ont mis en évidence les sentiers métaboliques les plus significativement régulées à la hausse suite à l'activation du AR. Les données brutes et traitées de RNA-Seq ont été déposées et rendues disponibles publiquement sur le *Gene Expression Omnibus* (GEO ; GSE128749). Ces données ont été intégrées dans un récent article décrivant les fonctions du AR en tant que régulateur clé du métabolisme des cellules CaP. Pour plus de détails sur l'interprétation de ces résultats, veuillez-vous référer à l'article "*Functional genomics studies reveal the androgen receptor as a master regulator of cellular energy metabolism in prostate cancer*" par Gonthier K et al. (doi : 10.1016/j.jsbmb.2019.04.016).

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Abstract

Prostate cancer (PCa) is the most frequent cancer in North American men and PCa cells rely on the androgen receptor (AR) for growth and survival. To understand the effect of AR in cancer cells, we have treated LNCaP and LAPC4 cells, two immortalized human PCa cells *in vitro*, with the synthetic androgen R1881 and then performed RNA-seq analyses. High quality sequencing data have been analyzed using our bioinformatic pipeline which consists of FastQC for quality controls, Trimmomatic for trimming, and Kallisto for pseudoalignment to the transcriptome. Differentially expressed genes were identified using DESeq2 after adjustment for false-discovery rate (FDR *q* values < 0.05) and Relative Log Expression (RLE) normalization. Gene Set Enrichment Analysis (GSEA) was also performed to identify biological pathways significantly modulated by androgens. GSEA analyses identified the androgen signaling pathway, as well as several metabolic pathways, as significantly enriched following androgen stimulation. These analyses highlight the most significant metabolic pathways up-regulated following AR activation. Raw and processed RNA-seq data were deposited and made publicly available on the Gene Expression Omnibus (GEO; GSE128749). These data have been incorporated in a recent article describing the functions of AR as a master regulator of PCa cell metabolism. For more details about interpretation of these results, please refer to "Functional genomics studies reveal the androgen receptor as a master regulator of cellular energy metabolism in prostate cancer" by Gonthier et al. (doi: 10.1016/j.jsbmb.2019.04.016).

This article is published in Data in Brief 2019; 25:104372

Keywords: Castration-resistance; Fatty acid metabolism; Glycolysis; Hormone receptor; Metabolic reprogramming; Metabolism; Mitochondria; Nuclear receptor; Steroid.

Value of the Data

- Bioinformatic analyses of differentially expressed genes and biological pathways regulated by androgens can be studied for a better understanding of the effect of AR in PCa.
- Validation in two distinct PCa cell lines allow for the identification of more reproducible results.
- These data highlight a new function of AR in PCa as a master regulator of cellular energy metabolism.
- These data may allow the discovery of new therapies targeting the unique PCa cell metabolic program.

Data

The raw data (.fastq files) generated from Illumina sequencing were deposted on the Gene Expression Omnibus (GEO) with the reference number GSE128749. The comma separated value files (.csv) which have been produced after the quantification and pseudoalignment with the transcriptome hg38 using Kallisto were also uploaded on GEO. These files contain the raw counts, the transcripts per million (TPM) values, and the fragments per kilobase million (FPKM) values for every sample. Differentially expressed genes on normalized data were identified using a FDR *q* value < 0.05.

Experimental Design, Materials, and Methods

Cells

LNCaP and LAPC4, two androgen receptor (AR) positive human PCa cell lines, were initially obtained from the ATCC and re-authentified in 2016 [93]. After resuscitation, the cells were not kept in culture for more than 3 months. Cells were grown in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), streptomycin, penicillin, and sodium pyruvate in 37°C incubators with 5% CO_2. Before androgen stimulation, cells were trypsinized and seeded at a 70% confluence in RPMI-1640 media with no phenol-red and supplemental with 5% charcoal-stripped serum (CSS), streptomycin, penicillin, and sodium pyruvate, as described previously [75]. After hormonal deprivation (48h), media was changed and fresh media containing 10nM of the synthetic androgen R1881 or vehicle (EtOH 96%). 24h later, cells were harvested for RNA purification using the RNA purification kit RNeasy plus mini kit from QIAGEN.

Sequencing

Excellent RNA integrity was confirmed using a TapeStation 2200 (Agilent); all samples had an RNA integrity number equivalent (RIN^e) > 8.5. mRNA enrichment and library preparation were performed using the NEBNext Ultra II Directional RNA library prep kit following the manufacturer's protocol. RNA was then sent to the Genomic

Centre of the Centre de recherche du CHU de Québec - Université Laval for sequencing using a HiSeq 2500 (125bp paired-end sequencing).

RNA-seq analysis

After sequencing, raw data were obtained in the fastq format. FastQC[7] was used for validating the quality of the data. Trimming of the adaptor content and over-represented sequences was performed using Trimmomatic[94]. Also note that trimming was performed with the minimal length (MINLEN) set at 36. Quality check using FastQC was performed again on the trimmed sequences (Table 1). For the pseudoalignment of the trimmed sequences to the hg38 transcriptome, the Kallisto tool was used[95]. Final normalization was performed using the Relative Log Expression (RLE) method[96]. We have used the R-package called Tximport to convert the transcript quantifications to gene quantifications [97].

Differential gene expression and GSEA analysis

To study genes regulated by AR in PCa cells, differential expressed genes were identified using a FDR *q* value < 0.05 with DESeq2[98]. Overall, 1868 and 716 genes were up-regulated in LNCaP and LAPC4 cells and 2294 and 847 genes were significantly down-regulated in LNCaP and LAPC4 cells, respectively (Fig. 1A). Of these, 321 common genes were up-regulated while 314 common genes were down-regulated in both cell lines (Fig. 1B). GSEA analyses [99] were also performed using TPM values to identify the most significantly up-regulated pathways following activation of AR in these human PCa cells. In both cell lines, the androgen signaling pathway was highly enriched following R1881 treatment (Fig. 1C). In addition, several metabolic pathways were also enriched in both LNCaP and LAPC4 cells following AR activation (Fig. 1D).

Acknowledgments

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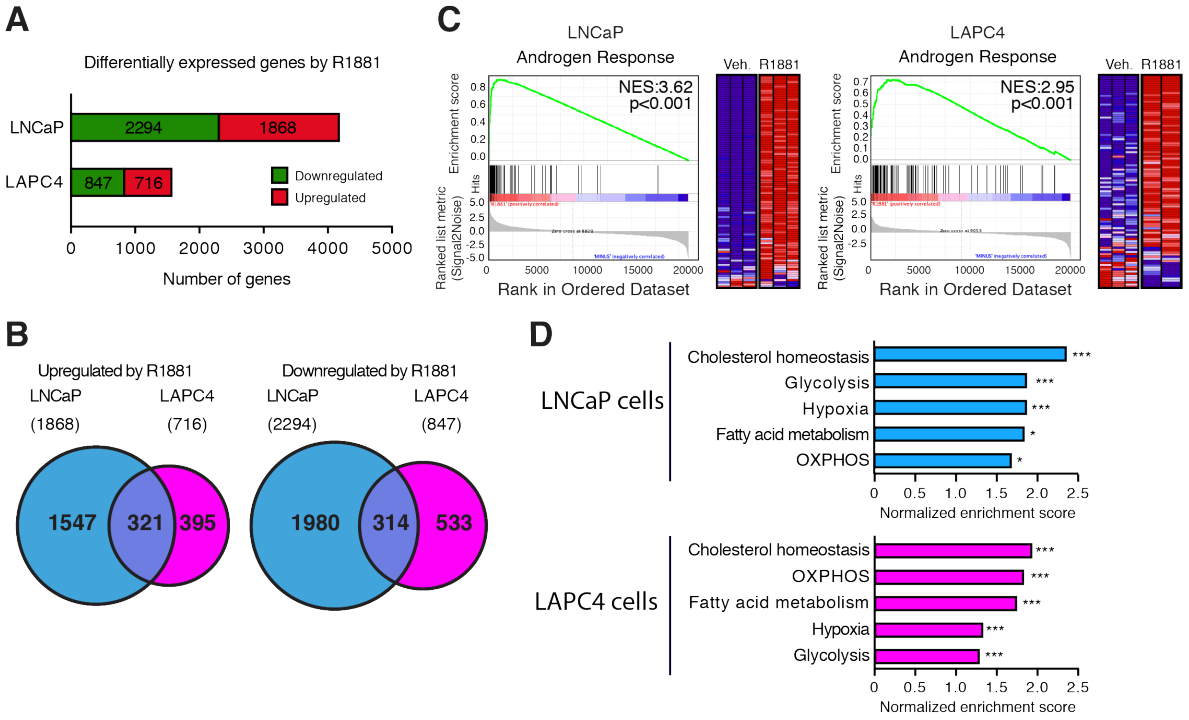
Figure Legends

Figure 1: Transcriptomic analyses of the androgen signaling pathway functions in human prostate cancer cells. A) Number of genes significantly up- or down-regulated following treatment with R1881 in LNCaP and LAPC4 cells. A FDR *q* value < 0.05 was used to identify differentially expressed genes. B) Venn diagrams showing the overlap between genes up-regulated (left) and down-regulated (right) by R1881 in LNCaP and LAPC4 cells. C) Gene set enrichment analysis (GSEA) plots for the “Hallmarks - Androgen Response” signature in LNCaP and LAPC4 cells. NES: normalized enrichment score. D) GSEA signatures enrichment scores for significantly enriched metabolic pathways in LNCaP and LAPC4 cells following 24h treated with R1881. OXPHOS: oxidative phosphorylation (mitochondrial respiration) * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Figures



Tables

Table 1: Number of reads for raw and trimmed sequences of PCa cells treated with androgens.

Cell lines	Treatment	Reads (Raw)	Reads (after trimming)
LNCaP	Control #1	9327182	7694732
	Control #2	11058265	9014786
	Control #3	10258931	8909738
	R1881 #1	7812714	6616969
	R1881 #2	9964804	8508748
	R1881 #3	10496859	8965255
LAPC4	Control #1	8744802	7390781
	Control #2	6528343	5443146
	Control #3	10146342	8641030
	R1881 #1	9384625	7940193
	R1881 #2	11134285	9474978

Chapitre 2. Genomic Deletion at *10q23* in Prostate Cancer : More than *PTEN* Loss ?

Résumé

Le gène *PTEN* code pour *la phosphatase and tensin homolog*; c'est un gène suppresseur de tumeurs qui est parmi les gènes les plus fréquemment inactivés dans le spectre des cancers chez l'humain. Les plus récentes approches de séquençage ont permis d'identifier des altérations génomiques du gène *PTEN*, notamment des délétions, des mutations ou des réarrangements dans environ 50 % des cas de cancer de la prostate (CaP). Il semble que les mécanismes menant à l'inactivation du gène *PTEN* sont spécifiques au cancer et ceux-ci incluent des mutations génétiques, des insertions/délétions, des altérations du nombre de copies (*CNA*), l'hyperméthylation du promoteur et l'interférence de l'ARN. L'examination de résultats disponibles publiquement provenant d'études de séquençage en profondeur de plusieurs types de cancer a montré que le CaP semble être le seul cancer dans lequel *PTEN* est perdu par *CNA*.

Contrairement aux mutations menant à l'inactivation du gène, tel qu'observé dans d'autres types de cancers, la délétion du locus *10q23* est la forme la plus courante d'inactivation de *PTEN* dans le CaP. En étudiant la région minimale supprimée au *10q23*, plusieurs autres gènes semblent être perdus simultanément avec *PTEN*. Les analyses d'expression indiquent que, comme *PTEN*, ces gènes sont également modulés à la baisse lors de la délétion de *10q23*. Ces résultats soulèvent la possibilité que *10q23* soit perdu en raison d'une pression de sélection pour non seulement inactiver *PTEN* mais aussi altérer l'expression des gènes environnants. Ainsi, plusieurs gènes de cette région supprimée, qui est d'environ 500 kb, peuvent également agir comme suppresseurs de tumeurs dans le CaP, soulignant la nécessité d'études supplémentaires pour comprendre leurs fonctions respectives dans ce contexte pathologique.

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Abstract

The *PTEN* gene encodes for the phosphatase and tensin homolog; it is a tumor suppressor gene that is among the most frequently inactivated genes throughout the human cancer spectrum. The most recent sequencing approaches have allowed the identification of *PTEN* genomic alterations, including deletion, mutation, or rearrangement in about 50% of prostate cancer (PCa) cases. It appears that mechanisms leading to *PTEN* inactivation are cancer-specific, comprising gene mutations, small insertions/deletions, copy number alterations (CNAs), promoter hypermethylation, and RNA interference. The examination of publicly available results from deep-sequencing studies of various cancers showed that PCa appears to be the only cancer in which *PTEN* is lost mostly through CNA. Instead of inactivating mutations, which are seen in other cancers, deletion of the *10q23* locus is the most common form of *PTEN* inactivation in PCa. By investigating the minimal deleted region at *10q23*, several other genes appear to be lost simultaneously with *PTEN*. Expression data indicate that, like *PTEN*, these genes are also downregulated upon loss of *10q23*. These analyses raise the possibility that 10q23 is lost upon selective pressure not only to inactivate *PTEN* but also to impair the expression of surrounding genes. As such, several genes from this deleted region, which represents about 500 kb, may also act as tumor suppressors in PCa, requiring further studies on their respective functions in that context.

Keywords: androgen; androgen deprivation therapy; androgen receptor; castration-resistant; neuroendocrine; steroid.

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Introduction

The *PTEN* gene on chromosome *10q23* encodes for the phosphatase and tensin homolog, a tumour suppressor gene that is among most frequently inactivated genes throughout the human cancer spectrum. Its lipid phosphatase activity allows PTEN to dephosphorylate phosphatidylinositol-triphosphate, therefore repressing the oncogenic PI3K/Akt/mTOR pathway. In prostate cancer (PCa), *PTEN* is frequently lost by deletion of the *10q23* region in tumours, which has been described several years ago [82, 100-102], and prostate-specific deletion of *Pten* in mice leads to PCa development [103]. The most recent sequencing approaches have allowed the identification of several types of genomic alterations of *PTEN*, including deletion, mutation, or rearrangement (including genomic inversions), and have further described *PTEN* alterations in about 50% of all PCa samples [87, 88, 104-110].

The inactivation rate of *PTEN* in PCa is similar to what has been described in other types of cancer, such as breast and endometrial cancers [111, 112]. However, mechanisms leading to *PTEN* inactivation appear to be cancer-specific, comprising gene mutations, small insertions/deletions, copy number alterations (CNA), promoter hypermethylation, and RNA interference [87, 88, 104-111]. For example, endometrial cancer is characterized by microsatellite instability that is associated with frameshift mutations, which are the most frequent inactivating alterations in *PTEN* in that type of cancer [111, 113]. In addition, patients with Cowden syndrome, who have a germline mutation in *PTEN*, also harbour a significantly higher risk of endometrial cancer [111, 114]. Cowden syndrome is a rare autosomal-dominant condition that leads to an increased risk of breast, thyroid, and endometrial cancers [111, 115]. However, patients with Cowden syndrome do not have increased risk of PCa, even though the loss of *PTEN* can be detected early in PCa patients [87, 100, 106, 109]. It is reported that in PCa, between 2 and 15% of primary tumours harbour a *PTEN* mutation, while between 30 and 40% exhibit an important deletion on chromosome 10q23 [88, 104, 106, 107, 109].

Importantly, many other genes are also present on the deleted region on *10q23*, most of which have not been investigated in the context of PCa. Moreover, because *PTEN* is more frequently deleted in PCa through large genomic deletions and at a higher frequency compared to other types of cancer instead of the gene-specific mutations that occur in most malignancies, it raises the possibility that genes lost at the same times as *PTEN* in PCa also display important tumour-suppressor functions. It is well accepted now that large genomic deletions can contain more than one important gene, but this concept was not investigated in the context of the loss of *10q23* in PCa. In this perspective article, we will discuss the genes that are lost along with *PTEN* upon deletion of the *10q23* locus that might well play a role in PCa development.

PTEN is more frequently altered through CNA rather than through a specific gene mutation in PCa

The *PTEN* genomic status was first screened through the cancer spectrum across the different cohorts available on the cBio Cancer Genomics Portal from *The Cancer Genome Atlas* (TCGA) group [116, 117]. Only cohorts with both mutation and CNA were kept for analysis. In most types of cancer, *PTEN* is often mutated (Figure 1, green color), with particularly high mutations rates in glioblastoma and uterine cancer, where the alterations rate is between 40 and 65% (Figure 1). Despite showing small CNA rates, gene mutations are the most common *PTEN* genomic alterations. Interestingly, the only type of cancer with high rates of *PTEN* genomic alterations particularly caused by CNA is PCa. In PCa, between 20 and 50% of all tumours exhibit *PTEN* alterations, with 60 and 90% of them being CNA instead of mutations (Figure 1). This suggests that CNA at the *PTEN* locus might affect more than *PTEN* itself and lead to the deletion of other tumour suppressor genes important to the aetiology of PCa.

We further analyzed the *PTEN* genomic alteration status between the different PCa cohorts (Figure 2A), which included the Michigan (MICH), Stand Up To Cancer (SU2C), and Fred Hutchinson Cancer Research Center (FHCRC) datasets, mostly comprising metastatic samples [88, 109, 118]; the Trento/Cornell/Broad dataset, comprised of metastatic neuroendocrine PCa (NEPC) [119]; the Broad/Cornell 2012, Broad/Cornell 2013, Memorial Sloan Kettering Cancer Center (MSKCC), and the two TCGA datasets, comprising mostly primary localized PCa [87, 107, 112, 120]. In cohorts mostly comprised of clinically localized tumours, *PTEN* genomic alterations, mostly CNA, ranged from 15 to 20%. This alteration frequency increased to 40–50% in cohorts of metastatic samples and castration-resistant PCa (CRPC) tumours, again mostly through CNA of *PTEN*. These results are consistent with previous reports linking *PTEN* loss to PCa aggressiveness as it is increased in more aggressive disease settings [121-124]. Further investigation of *PTEN* alterations in the CRPC/metastatic cohorts confirmed higher rates of CNA in these tumours compared to the TCGA cohort, which is mostly comprised of clinically localized tumours (Figure 2B). These data also confirmed that deletion of *PTEN* is the most frequent genomic alteration occurring at this locus in prostate tumours.

CNA at *10q23* leads to loss not only of *PTEN* but also of several additional genes

Interestingly, visualization of CNA at the *PTEN* locus in the various cohorts available on the cBioportal indicated that deletion of *PTEN* often results in the loss of a large (>500kb) genomic segment of chromosome *10q23* (Figure 3). Results from the metastatic cohort SU2C revealed that *PTEN* is commonly lost with other genes located at *10q23*, including *MINPP1*, *PAPSS2*, *KLLN*, and *ATAD1*. Moreover, deletion at *10q23* frequently occurs in one of the *RNLS* introns. By also investigating other cohorts with high coverage at *10q23* CNA status, we observed a similar deletion pattern, notably in the localized PCa cohort from the provisional TCGA dataset

(Figure 4, right). Again, the same similar minimal region seems to accompany the loss of *PTEN*, altering the same set of genes as in the more aggressive SU2C cohort, including the deletion breakpoint in the intronic region of *RNLS*. These results suggest that the loss of 10q23 in PCa cells does not solely to inactivate the tumour suppressor *PTEN*, but also that there is a selective pressure to lose other gene(s) at this particular genomic region in this specific type of cancer. Accordingly, CNA at *10q23* significantly altered mRNA expression of *PTEN,* and deletions of *PTEN* resulted in decreased mRNA expression in both the SU2C and the TCGA cohorts (Figure 4A and B, respectively). Genes surrounding *PTEN* that are located in the minimal deleted regions (Figure 3) also have a similar pattern, with a significantly decreased expression with either shallow or deep deletions in the two cohorts (Figure 4). The only exception was *PAPSS2*, which was not significantly altered by deep or shallow deletion (Figure 4). As reported previously [109, 112], various inactivating mutations of *PTEN* were also detected in both cohorts, but at lower frequency than CNA.

Future direction in PCa genomic alteration studies

Since the initial characterization of *PTEN* loss in PCa two decades ago [82, 100-102], most studies on CNA at *10q23* have focussed exclusively on *PTEN* as basically the only gene lost upon deletion at this specific locus, even in more recent deep-sequencing studies [87, 88, 104-110]. Given its known role as a tumour suppressor in most types of cancer and because mutations in this gene cause an hereditary syndrome with multiple cancer susceptibilities, *PTEN* is most certainly the main tumour suppressor gene lost with this deletion. However, other genes located in the minimal deleted region at *10q23* might play a significant role in PCa etiology because 1) CNA is the major genomic alteration in PCa, not a direct mutation of *PTEN*, which occurs in all other types of cancer; 2) a large region at *10q23* is deleted, comprising more than just *PTEN* and often including the loss of at least six other genes; and 3) some of these genes have already been associated with tumour-suppressing functions in PCa or other cancers. The question remains as to what roles these genes play in prostate biology and PCa development.

KLLN encodes for the KILLIN protein, which has been identified as a P53 target required for S phase checkpoint control to eliminate precancerous cells [125]. *KLLN* overexpression reduces PCa cell growth *in vitro* by decreasing the androgen receptor (AR) signalling, while its repression increases it; this is consistent with a tumour-suppressor function of this gene [126]. Interestingly, mutation in the promoter of *KLLN* is also associated with Cowden and Cowden-like syndromes, possibly by sharing its promoter with *PTEN* itself [127]. *ATAD1* depletion induces mitochondrial fragmentation and impairs respiration [128]. It is notable that increased mitochondrial respiration is a key metabolic phenotype associated with PCa development and progression [75, 93, 129-131]. *RNLS* encodes the renalase FAD-dependent metabolic enzyme [132], which has no currently known role in PCa. *PAPSS2*

encodes for PAPS synthase 2, which provides sulfate donors to sulfotransferase enzymes, including SULT2A1, which is a critical enzyme for dehydroepiandrosterone (DHEA) sulfation [133]. DHEA and its sulfate form (DHEA-S) represent the major adrenal androgen precursors and therefore are important sources for intra-tumour androgen synthesis. This is particularly relevant during PCa progression and is a therapeutic target used in the clinic (this pathway is inhibited using abiraterone acetate)[134-136]. The few patients identified with mutations in this gene are female, and heterozygous inactivation of *PAPSS2* has been associated with polycystic ovary syndrome, premature puberty, hyperandrogenic anovulation, very low DHEA-S levels, and increased androgen levels [137]. Even though *PAPSS2* is not significantly decreased by CNA, intra-tumour loss of *PAPSS2* could favour androgen excess and hyperactivation of AR, which is critical for tumour growth and cancer progression. *MINPP1* encodes a phosphatase linked to inositol-3-phosphate metabolism, similar to PTEN functions [138]. *CFL1P1* is cofilin pseudogene 1 and has no known function. However, based on mRNA expression profiles across tissues from the data presented by Fagerberg et al. [139], it shows high expression specifically in the testis, possibly reflecting a function in the male reproductive system.

Further experiments are now required to characterize the role of these genes located near *PTEN* and lost along with this important tumour suppressor in PCa. Only a few *in vitro* models of human PCa exist, some that exhibit complete loss of *PTEN*, such as in PC3 cells; some that exhibit mutation of *PTEN* and partial loss at *10q23*, such as LNCaP cells; and finally some that harbour wild-type *PTEN*, such as 22rv1 and DU145 cells [87, 102, 140]. These wild-type PCa cell lines thus represent potential *in vitro* models to study the impact on PCa cell proliferation of *PTEN* inactivation, with and without inactivation of one or more of the other genes located within the minimal deleted region at *10q23* (*KLLN*, *ATAD1*, *RNLS*, *PAPSS2*, *MINPP1*, and *CFLIP1*). Because in most tumours there is a single copy lost at *10q23* in most tumours, and not a complete loss of both copies, repression of these genes with RNA interference (RNAi) would mimic the gene expression decrease observed in tumour samples. The more recent genome editing technology using the CRISPR/Cas9 system would also allow the knockout of these genes along with *PTEN* to study their potential role as tumour suppressors of PCa *in vitro* [141].

Interestingly the mouse genome exhibits a similar gene architecture around *Pten*, with at least the presence of *Atad1, Rnls, Papss2,* and *Minpp1*. The prostate-specific loss of *Pten* PCa mouse model (*Pten*$^{flox/flox}$;PB-Cre4^{+}) is one of the most commonly used *in vivo* models to study PCa development and progression [103, 142]. In this model, *Pten* is inactivated by deletion of exon 5 [143] and other genes surrounding *Pten* are not altered. The single knockout of these genes in the *Pten*$^{flox/flox}$;PB-Cre4^{+} would allow the study of their function as tumour suppressors in this PCa mouse model *in vivo*. In addition, the development of a new PCa mouse model by inducing the loss of *19qC1,* the loci that contains *Pten* in the mouse genome, would mimic the human loss of *10q23* and would be highly informative on the biological functions as tumours suppressors of this region in comparison to the disruption of *Pten* only. This type of genetic engineering approach was successfully used to study oncogenic

chromosomal rearrangements in mouse models of human cancers, such as the *EML4-ALK* oncogene small-cell lung cancer [144]. Indeed, induction of this gene-fusion in adult mice promotes the development of non-small-cell lung cancers, clearly demonstrating the oncogenic properties of this chromosomal rearrangement. Inducing the loss of *19qC1* followed by rescue of *Atad1*, *Rnls*, *Papss2*, and *Minpp1* would also be an interesting approach to study the role of all the genes lost along with *PTEN* in human and their impact on PCa development and progression.

Concluding remarks

In summary, using publicly available results from deep sequencing studies of various cancers, PCa appears to be the only cancer in which *PTEN* is inactivated mostly through CNA. Large genomic deletions often contain more than one important gene, and this is a concept that needs to be revisited in the context of *PTEN* loss in human PCa. Instead of inactivating mutations as seen in other cancers, deletion at *10q23* is the most common form of *PTEN* inactivation. Investigation of the minimal deleted region at *10q23* revealed that several other genes appear to be lost in addition to *PTEN*. Expression data indicates that, like *PTEN*, these genes are downregulated upon CNA, and, together with the CNA profile, suggest that in PCa these genes represent potential novel tumour suppressor genes in PCa. Their potential function as PCa tumour suppressors thus remains to be determined using state-of-the-art genetic engineering approaches in *in vitro* and *in vivo* models of PCa.

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Figure legends

Figure 1. *PTEN* genomic alterations in the human cancer spectrum. Genomic alterations of the *PTEN* gene were visualized with the cBioPortal for Cancer Genomics [116, 117]. Only cohorts with data on both mutation and copy number alterations are shown.

Figure 2. Copy number alteration is the most frequent inactivation mechanism of *PTEN* in human prostate cancer. A) Genomic alterations of the *PTEN* gene in prostate cancer. Only cohorts with data on both mutation and copy number alterations are shown. NEPC: neuroendocrine prostate cancer; CRPC: castration-resistant prostate cancer. B) Specific alterations of *PTEN* in the MICH, FHCRC, SU2C, and TCGA cohorts. Note that the proportion of alterations is slightly different than in (A): all tumours are shown in (A) while all patients are shown in (B) (some patients had more than one sample sequenced).

Figure 3. The minimal deletion at 10q23 in PCa. Deletion status at the 10q23 locus in the SU2C (left) and the TCGA (right) PCa clinical datasets. Blue colour indicates copy number loss at this locus. Genes present in that genomic regions are shown (bottom panel).

Figure 4. Relationship between genomic alterations at the 10q23 locus and genes encompassed within this region. Expression of six genes located at the minimal deleted regions at the 10q23 locus in the SU2C (A) and the TCGA (B) PCa cohorts. *** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ in ANOVA with post-hoc Tukey's Honest Significant Difference (HSD). Note that for *RNLS* and *PTEN* expression data in the TCGA cohort (B), the sample with an amplification at *10q23* was not included for statistics.

Figures

Figure 1

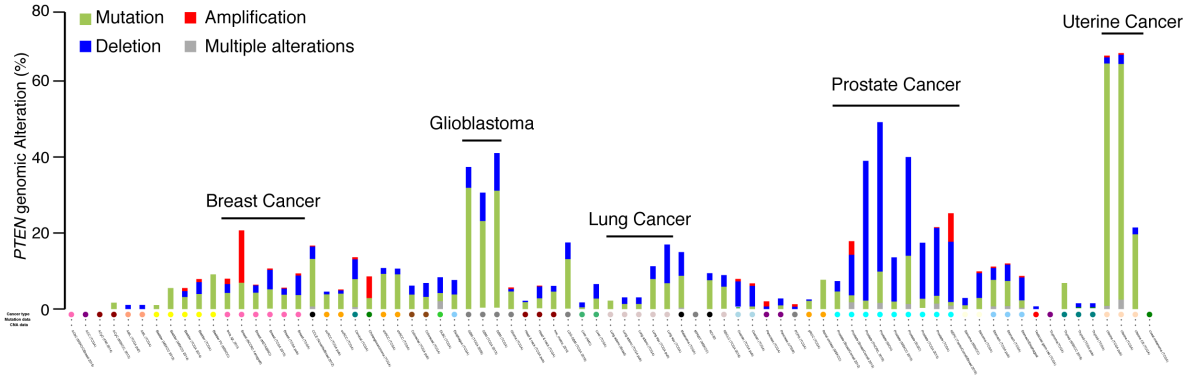


Figure 2

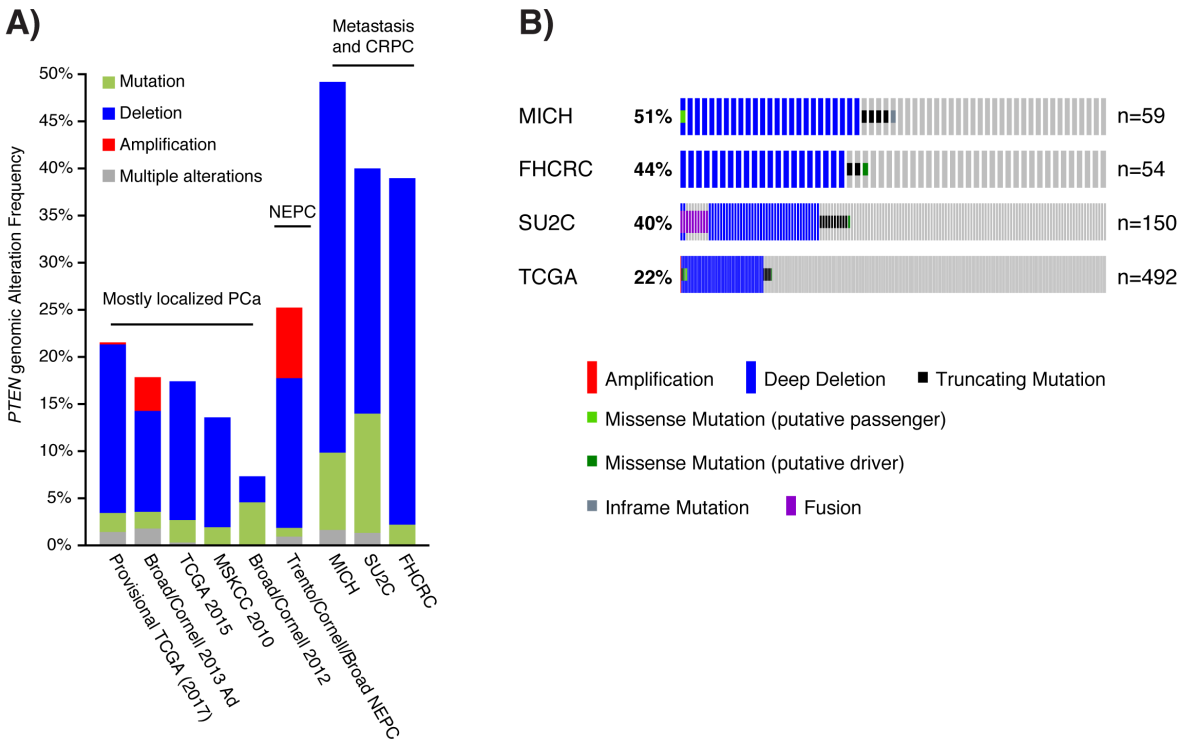


Figure 3

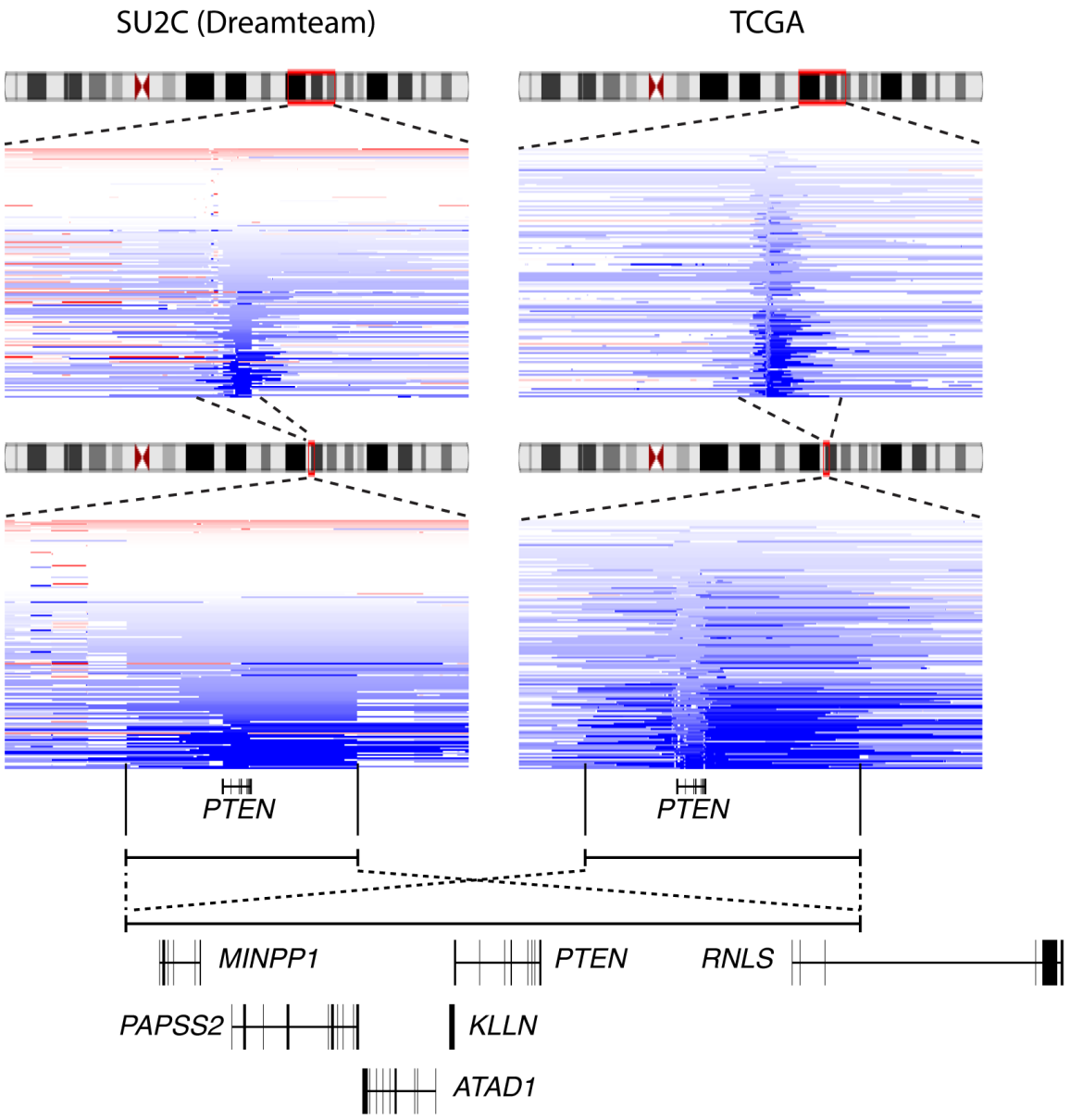
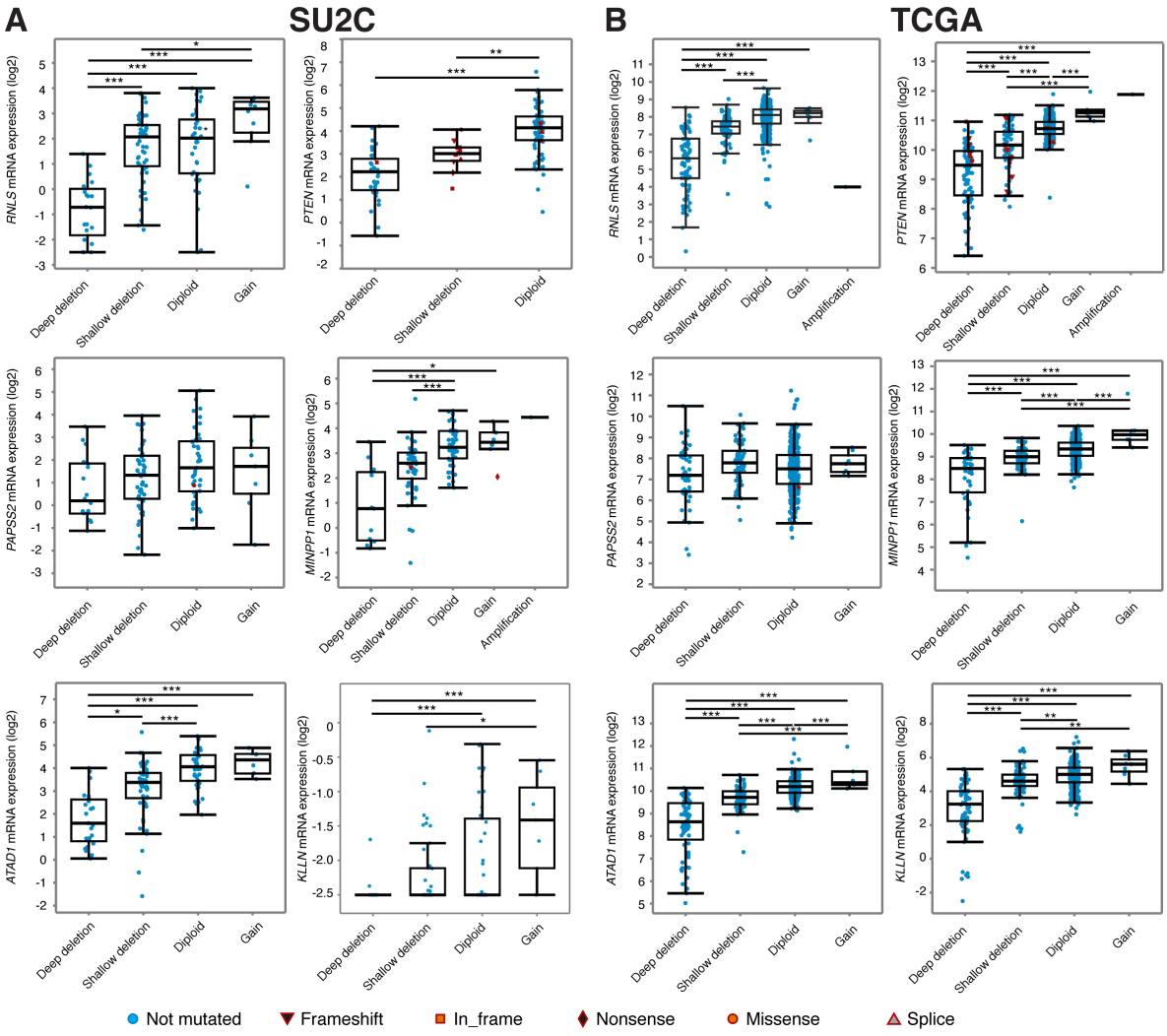

SU2C (Dreamteam)
TCGA
PTEN
PTEN
MINPP1
PTEN
RNLS
PAPSS2
KLLN
ATAD1

Figure 4


A
SU2C
B
TCGA
RNLS mRNA expression (log2)
PTEN mRNA expression (log2)
PAPSS2 mRNA expression (log2)
MINPP1 mRNA expression (log2)
ATAD1 mRNA expression (log2)
KLLN mRNA expression (log2)
Deep deletion
Shallow deletion
Diploid
Gain
Amplification
Not mutated
Frameshift
In_frame
Nonsense
Missense
Splice

Conclusion

The understanding of PCa biology has been drastically improved over the past decade. Bioinformatics has changed the ways how research is performed, and increasingly integrative approaches to biology seem inevitable. This new understanding of the disease has eventually impacted the life of patients by usage of new drugs and combinational therapies. Remarkable efforts are being put by researchers, and clinicians across the globe now better understand the critical role of AR in PCa progression as it has been highlighted in many studies already. Various researchs have been conducted using 'omics technologies to understand the PCa biology. Multiple functional studies have demonstrated the functions of AR and also shown that AR is the master regulator of cellular energy metabolism in PCa cells. Metabolic pathways and alterations play an influential role directly or indirectly on PCa development, progression, and resistance to therapy. AR has a direct impact on cell metabolism through pathways like aerobic glycolysis and mitochondrial respiration. This is shown to be having a direct impact on the clinical aspect of the disease, especially in metabolic reprogramming that occurs during the progression of PCa. Activation of metabolic pathways at cell level is very much appreciated by tumor cells to create cell membranes, nucleotides, energy and DNA. At the system level, alterations such as increased Body Mass Index (BMI), obesity, metabolic syndrome and diabetes can alter tumor metabolism and correlate with cancer progression. Most metabolic pathways such as glycolysis have been dissected thoroughly, and their relationship to cancer biology and novel metabolic signatures are being discovered in various subtypes of PCa. In spite of understanding the various functions of AR in transcriptional control of cell metabolism, many functions of AR remain unknown, which prevent the development of novel targeted therapies.

As shown in chapter 1, To understand the impact of androgens in much deeper sense to develop novel targeted therapies to treat PCa, an RNA-Seq bioinformatic pipeline has been built. This pipeline can be used to perform analyses on any RNA-Seq data. In this project, pathway analyses and differentially expressed genes have been identified and reviewed upon treatment with androgen in two AR positive cells lines, LNCaP and LAPC4. The data used in this project is publicly available to the research community to perform futher studies and comparative analyses. In this project, we have identified the metabolic pathways regulated upon androgen treatment by performing gene set enrichment analyses and pathway analyses. We have also shown that overlapped genes across the two cell lines, along with the differentially expressed genes in each cell line, are linked with metabolic pathways such as oxidative phosophorylation, cholesterol homeostasis, hypoxia and fatty acid metabolism.

For example, Fatty acid metabolism is linked to PCa development and progression through greater lipogenesis, high serum triglycerides, low serum high-density lipoprotein (HDL) cholesterol, and high total low-density lipoprotein (LDL)[145]. Risk of high-grade and aggressive PCa has been associated with hypercholesterolemia

(high levels of LDL), and, on the other hand, low risk of developing PCa is associated with high levels of HDL. Inconsiderate of circulating lipid levels, greater de novo fatty acid synthesis is a hallmark of PCa[146]. FA synthase (FASN) and lipid-modifying enzymes (ACAC, acetyl-CoA lyase, and SCD) are overexpressed in PCa[147]. Oncogenes and tumor suppressor genes impact the FASN activity. USP2a is an isopeptidase that stabilizes and prolongs the half-life of FASN, and low levels of this enzyme are associated with lower FASN concentrations and in turn with cell death[148]. AKT activity modulates fatty acid synthesis through the activation of SREBP and via mTORC1 activation. In normal cells, SREBP activates fatty acid synthesis. Its inhibition and overexpression in PCa has consequences in the progression of the disease[149].

AMPK is responsible for energy homeostasis and plays a central role in controling the switch between glucose and fatty acid metabolism. AMPK activation inhibits lipogenesis. Therefore, it is hypothesized that, in obese patients, AMPK function is impaired and FASN activity is restored, with a resultant increase in de novo lipogenesis[150]. AMPK regulates lipid metabolism via the phosphorylation of several proteins (ACC1, ACC2, 3-hydroxy-3-methyl glutaryl-CoA, SREBP1, SREBP2) involved in lipid and cholesterol synthesis and the inhibition of fatty acid oxidation[151]. Importantly, AR signaling in PCa upregulates lipid biosynthetic enzymes such as FASN and Acetyl, -CoA Carboxylase 1 (ACACA). Various studies have shown that FASN inhibitors, such as cerulenin, are effective in PCa tumors to decrease cancer cell proliferation and survival[152]. In recent pre-clinical models and in clinical trials, FASN inhibitors showed some positive results but not specifically related to PCa[153]. The link between AR and lipid metabolism suggests possible combined therapy with inhibitors of CPT1 such as teglicar (21270274) and etomoxir, in association with antiandrogens such as enzalutamide[154].

A study conducted by Gonthier et al. depicted that AR controls multiple isocitrate dehydrogenase (IDH) isoforms and IDH activity highlights a novel metabolic reprogramming necessary for prostate cancer growth. Citrate metabolism is impacted by various mutations of *IDH1* and *IDH2* in human cancer spectrum, including 1-3% of PCa[48]. In this study, relative expression levels of one cytoplasmic isoform (*IDH1*) and four mitochondrial isoforms (*IDH2, IDH3A, IDH3B, IDH3G*) have been demonstrated upon treatment with androgens in three AR positive cell lines LNCaP, LAPC4 and 22rv1. Overall, the main feature of this study stated that AR positively impacts the expression levels of *IDH1* when compared to the other isoforms and also experimentally demonstrated the reprogramming of PCa cell metabolism by the metabolic gene *IDH1.*

There is an abundance of evidence that AR positively impacts alternative splicing and alternative splicing pathway is essential for gene regulation in cells[155, 156]. The dysregulation of splicing is involved in many types of cancer, including prostate cancer. It is shown that AR is also expressed in the form of C-terminal truncated variants, termed as AR-variants (AR-Vs) or also known as AR3, through an alternative RNA splicing process[157-159]. AR-Vs lack the ligand-binding domain of full-length AR, constitutively activate AR-downstream transcription,

and promote tumor growth, even under castration conditions[160, 161]. The most common variant expressed and studied in context to PCa is AR-V7[162]. The process of production of *AR-V7* mRNA is still being studied and understood, but AR-V7 is well associated with resistance to ADT and CRPC development[162, 163]. It has been frequently detected in circulating tumor cells from patients with CRPC. Patients with higher AR-V7 expression in circulating tumor cells had a reduced PSA progression-free survival[164]. A recent meta-analysis of clinical studies reported that AR-V7 was positivity associated with higher PSA recurrence and poor prognosis in patients with CRPC treated with AR antagonists[165]. Although not every study has validated these relationships, these findings suggest that AR-V7 may serve as a prognostic and predictive biomarker for patients with metastatic prostate cancer[166]. Therefore, understanding the regulation of alternative splicing in the coming future should provide a new pathway for the development of novel therapeutic strategies.

In conclusion, the androgen receptor plays an important role in normal prostate cell growth and has been involved in almost all forms of prostate cancer. It is likely that constant androgen receptor activity underpins much of lethal CRPC, and promising targets are emerging to direct therapy in this regard. The concern is that prostate cancer cells are versatile and can draw on a spectrum of pathways to bypass the need for conventional androgen receptor binding with testosterone to survive and divide. As we discover more about the androgen receptor and its fundamental transcriptional role in maintaining key cellular processes such as metabolism and cell cycle, fresh targets are being identified by which the most common non-cutaneous cancer in men can be selectively brought under control.

With this project, we also believe that integration of bioinformatics analyses with traditional wet lab experiments data will add a new dimension to the process of decoding the PCa biology. Another blooming domain which could play an important role in the coming future is the integration of multi-omics data into clinical research. The popularity to understand and investigate the complex mechanisms across molecular layers is increasing by the performance of omics analyses. This approach is believed to help the process of discovering novel and actionable biological insights into pan cancer domain. Integration of multi-omics data remains challenging, however, and requires combination of several software tools and extensive technical expertise to account for the properties of heterogeneous data. Various tools are being developed by computational scientists with the collaboration of biologists to make the process of multi-omics easier and user friendly.

As aforementioned, when clinical data is integrated with omics profiles, it leads to an improved prognostic performance over the use of clinical variables alone. Sharing of omics data in the public databases like TCGA will help the research community to grow forward and faster. Example of the usage of publicly available data is the project titled « *Genomic Deletion at 10q23 in Prostate Cancer : More than PTEN Loss ?* » which is described in chapter 2. From various studies conducted previously, we know that *PTEN* is a tumor suppressor protein and

is very commonly lost in PCa. Identifying and understanding the *PTEN* loss in PCa has a great potential to improve our understanding of the progression of the disease and improve patient care. In this project, we have shown that genomic alteration frequency of *PTEN* is mostly in the form of deletion than mutation in PCa, and the deletion is much higher in CRPC when compared to localised PCa. We have also identified six other genes which are being deleted along with *PTEN* at *10q23*. These six genes were not much studied in context of PCa, but we believe further studies on these might play a huge role to better understand the progression of the disease. According to the literature, these six genes, *RNLS, PAPSS2, ATAD1, KLLN and MINPP1,* are believed to have tumor suppressor characteristics in human cancer. Further investigation, beyond the scope of the current study, will be needed to test the possibilities of these genes lost along with *PTEN.*

According to a study conducted by Yue S et al., the deletion or mutation of *PTEN* impacts metabolic reprogramming, which plays an important role in promoting prostate cancer tumorigenesis. The altered metabolism of cancer cells can provide abundant biomaterial and bioenergy for malignant proliferation and can confer a selective advantage for the survival and proliferation of cancer in the unique tumor microenvironment[167]. Other studies have also demonstrated that oncogenes and tumor suppressor genes control metabolic reprogramming through regulating the expression or activity of metabolic enzymes[168]. It has also been demonstrated that upon loss of *PTEN,* aerobic glycolysis is promoted in prostate epithelial cells[169]. Another study using metabolomics analyses revealed that the loss of *PTEN* promoted metabolic reprogramming by enhancing glycolysis, glutaminolysis, fatty acid synthesis and β-oxidation, and branched-chain amino acid catabolism in PCa cells. The metabolic reprogramming induced by *PTEN* loss provides biomaterials and bioenergy for the proliferation of PCa cells. This study implicates a potential therapeutic target for PCa cells with *PTEN* mutation or deletion[170].

Some of the lost genes like *KLLN* have been associated with tumor suppressor characteristics in context to PCa. A study in 2013 by Yu Wang et al. has been done to analyze and understand the relationship between *KLLN* expression and PCa, and the potential tumor suppressor impact on the disease[126]. In this study, it was shown that KLLN inhibits AR activity and decreases PSA levels in AR-positive prostate cancer cells. In addition to suppressing AR expression, KLLN could also act as a co-transcription factor for AR-targeting genes or could indirectly regulate AR function through KLLN-targeting genes. Further investigation to identify KLLN target genes will provide further insight of KLLN function in the context of novel drug development.

Another lost gene, *PAPSS2*, has been correlated with PSA recurrence. Its influence on PSA recurrence may be due to its role in the regulation of DHEA, which is a precursor of testosterone (Fig.4). *PAPSS2* gene encodes the 3'-phosphoadenosine 5'-phosphosulfate synthase 2, which catalyzes the formation of PAPS (3'-phosphoadenosine-5'-phosphosulfate) via a 2-phase biosynthetic process[171]. Although the alteration of *PAPSS2*

gene has been clinically associated to colon cancer[172], there have been no strong justifications stating that *PAPSS2* is strongly associated to PCa. However, a study hypothesized that the copy number loss of *PAPSS2* gene may lead to non-functional PAPSS2, which is unable to generate PAPS resulting in the accumulation of DHEA[137, 173]. An increased level of DHEA is predicted to increase the rate of androgen biosynthesis and a potential increase of PSA levels. PSA is a direct product of androgen receptor activity[174]. Therefore, it is endorsed that the molecular function and translational implication of *PAPSS2* loss in PCa needs further investigation.

By using bioinformatics analyses on transcriptomics data, my study provides a perspective of PCa that could facilitate the development of novel therapeutic targets and biomarkers for this disease. Further and deeper research are needed to validate the findings in large number of patients and investigate their potential clinical utilities in PCa.

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