CHEMOPREVENTIVE EFFECTS AND PROTEOMIC ANALYSIS OF MYO-INOSITOL TREATMENT ON HUMAN PROSTATE CANCER CELL LINE (DU-145)

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by

MOHAMMAD JAHIDUL ISLAM

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DEDICATIONS

This thesis is primarily dedicated to

My parents*, who are so precious to me,*

My kids, Efaz and Esham*, who have filled my life with love, joy, and happiness,*

&

My respected professor (Dr Azman) and mentors*, who are always there for me,*

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5-ARI	5α-reductase inhibitors
ADT	Androgen deprivation therapy
AR	Androgen receptor
AREs	Androgen response elements
ATCC	American Type Culture Collection.
BPH	Benign prostatic hyperplasia
CAD	Caspases-activated DNase
CID	Collision induced dissociation
COL6A3	Collagen type VI 3
CRPC	Castration-resistant prostate cancer
CSS	cancer-specific survival
CT	Computerized tomography
DAG	1,2-diacylglycerin
DAVID	Database for Annotation, Visualization and Integrated Discovery
DBD	DNA binding domain
DDR	DNA damage repair error
DHT	Dihydrotestosterone
DRE	Digital rectal examination
EDTA	Ethylenediaminetetraacetic acid
ESI	Electrospray ionization
FACS	fluorescent-activated cell selection
FADD	*F*as-*a*ssociated *d*eath *d*omain
FBS	Fetal Bovine Serum
FDR	False Discovery Rate
FGFR2	Fibroblast growth factor receptor 2
FITC	fluorescein isothiocyanate
FSH	Follicle-stimulating hormone
GnRH	gonadotropin-releasing hormone
GO	Gene Ontology

GST	Glutathione-S-Transferases
HGF	Hepatocyte growth factor
hPAP	Human Prostatic Acid Phosphatase
HPG	Hypothalamic-pituitary-gonadal
HPO	Human Phenotype Ontology
HR	Homologous Recombination
HRPC	Hormone-refractory prostate carcinoma
IPA	Ingenuity Pathway Analysis
IPA	Ingenuity Pathway Analysis
KEGG	Encyclopaedia of Genes and Genomes
KLF6	Kruppel-Like Factor 6
KLK3	kallikrein related peptidase 3
LBD	Ligand-binding domain
LH	Luteinizing Hormone
LHRH	Luteinizing hormone-releasing hormone
LUTS	Lower urinary tract symptoms
mCRPC	Metastatic Castration-Resistant Prostate Cancer
MEM	Minimum Essential Medium
MFS	Metastasis-Free Survival
MRI	Magnetic resonance imaging
MSDB	Mascot Protein Database
NE	Neuroendocrine
NEAA	Non-essential Amino Acids
NTD	N-terminal domain
OSCC	Oral squamous cell carcinoma
PAP	Prostatic acid phosphatase
PBS	Phosphate-Buffered Saline
PCa	Prostate cancer
PI	Propidium Iodine
PIKE	Protein Information and Knowledge Extractor
PKC	Protein Kinase C
PSA	Prostate-specific antigen
PSM	Peptide-Spectrum Matches

PTMs	Posttranslational modifications
SNPs	Single nucleotide polymorphisms
T	Testosterone
TA	Transit-amplifying Cells
TBE	Trypan Blue Exclusion Method
TLB	Thiourea lysis buffer
TSH	Thyroid-stimulating hormone
TUIP	Transurethral incision of the prostate
UGE	Urogenital sinus epithelium
UGM	Urogenital sinus mesenchyme
UGS	Urogenital sinus
USA	United States of America

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KESAN PENCEGAH-KIMIA DAN ANALISA PROTEOMIK OLEH RAWATAN MYO-INOSITOL PADA SEL KANSER PROSTAT (DU-145)

ABSTRAK

Kanser prostat masih menjadi kanser yang kedua paling kerap didiagnos pada lelaki dan merupakan punca ketiga paling utama bagi kematian yang dikaitkan dengan kanser, walaupun terdapat banyak pilihan rawatan. Terapi sedia ada yang tidak cekap dan kesan buruknya telah mendorong saintis mencari rawatan baharu bagi penyakit ini. Pengenalpastian dan pembangunan agen antiproliferatif baharu untuk kanser prostat adalah inovasi utama dalam merawat penyakit yang boleh membawa maut ini. Walaupun sebatian sintetik Myo-inositol digunakan secara meluas dalam penyelidikan kanser, fungsi antikanser sebenar bagi bahan kimia ini masih belum difahami dengan baik. Oleh itu, tujuan kajian ini adalah untuk mendedahkan kesan kemopreventif Myo-inositol terhadap sel kanser prostat (DU-145). Kesan rencatan pertumbuhan dan nilai IC_{50} telah ditunjukkan oleh Myo-inositol pada 0.06mg/ml ($p<0.05$). Kematian sel disyaki ada kaitannya dengan aruhan apoptosis dan kitaran sel yang terhenti. Rawatan dengan menggunakan Myo-inositol telah dikenal pasti selanjutnya melalui aruhan pada peringkat awal dan akhir apoptosis ($p<0.01$). Apoptosis juga boleh dikesan melalui penyerpihan DNA dan analisis pewarna pendarfluor Hoescht 33342. Tambahan pula, Myo-inositol menyebabkan perubahan pengawalaturan kitaran sel pada titisan sel DU-145, masing-masing pada fasa G_0/G_1 dan S ($p<0.01$). Keputusan pengenalpastian protein melalui elektroforesis 2D dan analisis LC-MS/MS menunjukkan 19 dan 23 protein telah dikenal pasti, masing-masing dalam sampel kawalan dan sampel terawat Myo-inositol. Analisis penganotasian protein

memberikan lebih penjelasan untuk sampel terawat Myo-inositol dan memperlihatkan pengelasan famili protein, proses biologi, fungsi molekul dan penyetempatan sel. Analisis pengayaan laluan mengenal pasti kemungkinan lebihan perwakilan bagi beberapa laluan, iaitu laluan pengisyaratan apoptosis (40.80%) ($p<0.01$), dan laluan p53 gelung suap balik 2 (20.80%) ($p<0.01$) yang diketahui terlibat dengan kemopreventif kanser prostat. Kesimpulannya, semua keputusan yang diperoleh dalam kajian ini menunjukkan bahawa sel DU-145 mengalami apoptosis yang signifikan dan kitaran sel yang terhenti disebabkan kepekatan perencat dos IC_{50} Myo-inositol memperlihatkan kadar kemopreventif yang agak tinggi. Tambahan pula, kajian Proteomik mendedahkan terdapat 12 protein yang memaparkan perubahan ekspresi yang signifikan selepas menerima rawatan Myo-inositol ($p<0.01$), dan antaranya ialah protein TNF1, TRAF2, APAF1, CCNDBP1, KRT8 dan CDKN1B yang menyumbang kepada perubahan yang signifikan pada kedua-dua tahap sel dan protein dalam kemopreventif kanser prostat; manakala protein COF1A, ANXA2, TUBB4B, LMNA, FIBB dan ACTC1 menunjukkan lebihan ekspresi pada sampel kawalan. Keputusan yang diperoleh daripada analisis proteomik menunjukkan bahawa semua protein yang dikenal pasti dalam sel DU-145 terawat Myo-inositol boleh menjadi parameter yang berpotensi untuk dinilai selanjutnya sebagai agen kemopreventif.

CHEMOPREVENTIVE EFFECTS AND PROTEOMIC ANALYSIS OF MYO-INOSITOL TREATMENT ON HUMAN PROSTATE CANCER CELL LINE (DU-145)

ABSTRACT

Prostate cancer remains the second most frequent diagnosed cancer in men and is the third leading cause of cancer related death, despite many available treatment options. The inefficiency of existing therapies and their adverse effects have led scientists to seek new treatments for this disease. The identification and development of new anti-proliferative agents for prostate cancer is a major innovation in treating this deadly disease. Although the synthetic compound Myo-inositol is widely used in cancer research, the actual anti-cancer function of these chemicals is still not well understood. This study was therefore intended to unveil the chemopreventive effects of Myo-inositol on prostate cancer cells (DU-145). The growth inhibitory effect and the IC_{50} value were demonstrated by Myo-inositol at 0.06 mg/ml (**$p<0.05$). It is suspected that cell deaths are related to apoptosis induction and cell-cycle arrest. Treatment with Myo-inositol has been further identified by induction of early and late apoptosis (***$p<0.01$). Apoptosis can also be detected using DNA fragmentation and Hoechst 33342 fluorescent dye stain analysis. Myo-inositol caused an alteration to the cell cycle regulation on DU-145 cell line at G_0/G_1 and S phase, respectively (***$p<0.01$). Protein identification results via 2D electrophoresis and LC-MS/MS analysis showed 19 and 23 proteins identified in control and Myo-inositol treated samples, respectively. Protein annotation analysis presented more explanations for Myo-inositol treated sample reveals protein family's classifications, biological process, molecular functions, and the cellular localization. Pathway enrichment

analysis identified over-representation of several pathways likely, apoptosis signalling pathway (40.80%), p53 pathway feedback loops 2 (20.80 %), which were known to be involved in the prostate cancer chemoprevention. In conclusion, all results obtained in this study, indicated that DU-145 cells underwent significant apoptosis and cell cycle arrest by the inhibitory concentration IC_{50} dose of Myo-inositol exhibited a considerable degree of chemoprevention. Furthermore, Proteomic study revealed 12 proteins which displayed significant changes in expression following Myo-inositol treatment (***$p<0.01$), among these proteins TNF1, TRAF2, APAF1, CCNDBP1, KRT8, CDKN1B contributed significant transformation at both the cellular and protein levels in prostate cancer chemoprevention; whereas, COF1A, ANXA2, TUBB4B, LMNA, FIBB, ACTC1 proteins were overexpressed in control treated samples. The results obtained from the proteomic analysis showed that all proteins identified in the Myo-inositol treated DU-145 cells can be potential candidate for further evaluation as a potential chemopreventive age

CHAPTER 1

INTRODUCTION

1.1 Research background

The prostate gland is extremely susceptible to illness in older adults, with the most common disorders being benign hyperplastic growth lesion of the prostatic gland (BPH), infection of prostate gland or prostatitis, and prostate malignancy (Risbridger, 2018). BPH is considered as a chronic diseases, most commonly occur in older men over 50-55 years of age characterized by the noncancerous enlargement of the prostate gland with an increased number (hyperplasia) of prostatic stromal and epithelial cells (Monib et al., 2018). Prostate hyperplasia is frequently correlated with the gradual development of incontinence of micturition and urinary bladder capacity related Lower Urinary Tract Symptoms (LUTS); as for illustration, recurrent hesitancy, intermittent stream, incomplete bladder evacuation, irregular straining, dribbling, then incomplete evacuation of the bladder. Irritative clinical features such as frequency, urgency, urge incontinence, and nocturia would undoubtedly reduce the patients' life quality significantly (McVary et al., 2019).

Several risk elements of distal urinary tract manifestations associated outcome can be categorized as conditional risk factors such as age, diet, cigarette smoking, excessive alcohol intake, and genetic predisposition. There are various deteriorating risk factors for LUTS, likely obesity, hypertension, diabetes, hormones, inflammation, and depression (Calogero et al., 2019).

Prostate cancer frequency is significant among men aged >65 years, suggesting that the advancing age is a considerable detrimental condition for prostate cancer occurrence, categorized as a non-modifiable risk factor. Moreover, positive family history is essential, but known genes presently explain only 35% of the familial risk, those patients present with regards to a positive family history of PCa; they are at intensified 60% possibilities of developing PCa (Cuzick et al., 2014). Race, diet, inflammation, and hormonal components are all other risk factors for prostate cancer (Ha Chung et al., 2019). As for the illustration, African American men are 1.6 occasions bound to be determined to have prostate malignancy and are twice as liable to terminate from the sickness as white men in the USA (Clouston et al., 2019).

Medical treatment preferences for prostate malignancy count on whether the disease is clinically confined or has spread to distal destinations (Uemura et al., 2016). Localized tumours remain in an idle state, not showing any symptoms. Most of these tumours are slow-growing (Infante et al., 2013). However, 15% of these cases are known to be aggressive, requiring some form of immediate treatment (Chang et al., 2014a). Different treatment alternatives for potent prostate malignancies include radical prostatectomy, radiation treatment, androgen deprivation treatment, and chemotherapy (Chang et al., 2014b).

Both benign and malignant prostate cells require androgens to arbitrate the prostate's development and function by stimulating the androgen receptor (AR) (Singh et al., 2014). There are various methods to decrease overall androgen levels, including orchiectomy (surgical castration) and chemical castration (Lehmusvaara et al., 2012). The gold standard for metastatic prostate cancer has been bilateral orchiectomy. By

this method, 95% of serum testosterone levels are eliminated (Waltering et al., 2012). AR signalling is the crucial determinant factor for transformation of prostate gland malignancy primarily by mesenchymal-stromal proliferation and cell survival, which usually respond well through androgen-deprivation treatment are signifies as castration-resistant prostate cancer (CRPC) (Shiota et al., 2011).

The advantage of radical prostatectomy may shift by the patient unexpectedly, no overall advantage of surgery was observed, although bone metastasis-free survival was higher in the radical prostatectomy group (Wilt et al., 2012) and existing malignant disease treatments regularly incorporate surgical expulsion and radiation therapy of the enormous biomass of malignancy, normally after that foundational most suitable chemotherapy for curative or remedial therapy (Magrini et al., 2019). Chemotherapy's major drawbacks include cancer recurrence, severe anaemia, drug resistance, bone marrow suppression, diarrhoea, and baldness, all of which are substantial side effects that might limit the use of synthetic chemotherapeutic medications while increasing the economic burden (Alabraba et al., 2019).

1.2 Problem statement

The global burden of cancer is increasing at an alarming rate, putting enormous strain on the general public and health systems at all levels of the economy. Cancer is the greatest cause of illness and death worldwide, independent of geographic diversity or human developmental status, primarily because of the extended period curative and palliative treatment measures, which can be rather expensive, together with the important misfortune due to morbidity and mortality (Cohen, 2017).

For a long time, scientists and doctors have been putting a lot of effort into figuring out how to detect cancer in its early stages before it progresses to the point where it becomes malignant and hence incurable or fatal (Lee et al., 2019). Explaining and understanding cancer's aetiology and pathways included in the initiation and further progression, the proliferation of this malignant disorder at each morphologic, inherited, molecular, and cellular level can considerably assist devise an approach for early detection, prevention, intervention, and initiate the targeted therapy (Rai et al., 2017).

According to the WHO Global Cancer Observatory (GLOBOCAN, 2020) there are around 10.0 million cancer deaths and 19.3 million new cancer cases reported in 2020. Prostate cancer is the second most common cancer among males and the fifth major cause of cancer mortality in 2020. Worldwide, an estimated 1.4 million new cases and 55,000 deaths have been reported. Furthermore, the incidence rate varies across the region, ranging from 6.3 to 83.4 per 100,000 males, with the highest rates in Northern and Western Europe and the lowest in Asia and North Africa (Sung et al., 2021b). According to the GLOBOCAN database published in 2020 (Sung et al., 2021a), the cumulative prevalence observed from 2007 to 2020 was 2146 (9.3%) instances. In Malaysia, prostate cancer led to the deaths of 900 men, accounting for 3% of all deaths, with incidence and mortality rates of 13.15% and 5.4%, respectively. In addition, the Malaysian National Cancer Registry reported that Chinese (50.73%), Malaysians (37.13 %), and Indians (37.13 %) accounted for more than half of the cases (6 %). The majority of affected men (99.2%) are over 45 years old, with only a minor percentage (0.8%) between the ages of 15 and 44 (Ismail, 2019).

Androgens coupled to the androgen receptor (AR) are responsible for the initiation and progression of prostate cancer (CaP). With a variety of androgen deprivation therapy (ADTs), this signalling cascade can be observed with initial effectiveness (Fujita and Nonomura, 2019a). However, after androgen deprivation, 10-20% of cases advance to a more invasive disease stage called castration-resistant prostate cancer (CRPC) (Chandrasekar et al., 2015). AR point mutations, AR overexpression, androgen biosynthesis modifications, structurally active AR spice variations without ligand binding, and changes in androgen cofactors are all examples of the pivotal mechanism involved (Kirby et al., 2011, Bluemn et al., 2017). Also, approximately a third of prostate cancers that recur after endocrine therapy contain an amplification of the AR gene. Amplification is not detected in any untreated prostate tumour, implying that gene augmentation is most likely linked to therapeutic failure (Zhang et al., 2015, Aurilio et al., 2020).

Nearly one third (31-33%) of all malignancies are compelled via dietary practices, subsequently tobacco smoking (21-29%) and hereditary defects (6-9%). However, residual is caused by specific environmental influences and lifestyle variation (Parascandola and Xiao, 2019). Numerous epidemiological reports have exhibited the association between staple foods and malignancy, primarily because of animal products intake and cooked meat (Key et al., 2020). Presently, the therapy for malignant growths which encloses medical invasive procedure such as surgery, radiotherapy, chemotherapy, and hormonal management. Thus, conventional management doesn't display any remarkable development, and the prognosis of cancer is still feeble (Wang et al., 2019). Therefore, the novel therapeutic innovation is extremely desirable to expand the quality of wellbeing of the patients.

Dietary factors, a significant factor that can modify cancer risk in a few assorted manners at multiple stages of the carcinogenic course. Several foods, nutrients and lifestyle have been associated to the inhibition of development of prostate cancer by the compounds with antioxidant action, arresting cell cycle or by inducing apoptosis, an essential role in the prevention of PCa (Lin et al., 2019, Focht et al., 2018, Van Hoang et al., 2018, Kobayashi et al., 2002). Dietary natural chemopreventive agents are divided into two broad sections, which is in view of their likelihood of efficiency and mode of action likewise, blocking agents and suppression agents. Furthermore, blocking agents act simultaneously among carcinogen exposure by avoiding carcinogens' metabolic activation from reducing the possibility of cellular DNA and proteins impairment. Conversely, suppression agents overwhelm cellular proliferation or induction of apoptosis pathway by inhibiting the activation of cellular transcription factors such as CFL1-A and ANXA2 (Shahabipour et al., 2017, Huang et al., 2008, Wang et al., 2017b).

Extensive studies relating to molecular mechanisms of chemopreventive natural products display several cellular targets in controlling diverse signalling pathways with fewer side effects than traditional chemotherapeutic pharmaceutical products (Zhang et al., 2017). "Fibre assumptions" denotes that excessive refining of grains and lack of dietary fibre can play a causative role in carcinogenesis of the colon and breast (Amawi et al., 2017).

1.3 General objective

The general objective of this study is to investigate the chemopreventive effects of Myo-inositol on the human prostate epithelial DU-145-cell line at cellular and protein levels.

1.4 The specific objectives of the present study

1. To investigate the inhibitory and antiproliferative properties of Myo-inositol compound on human prostate epithelial DU-145 cell line.

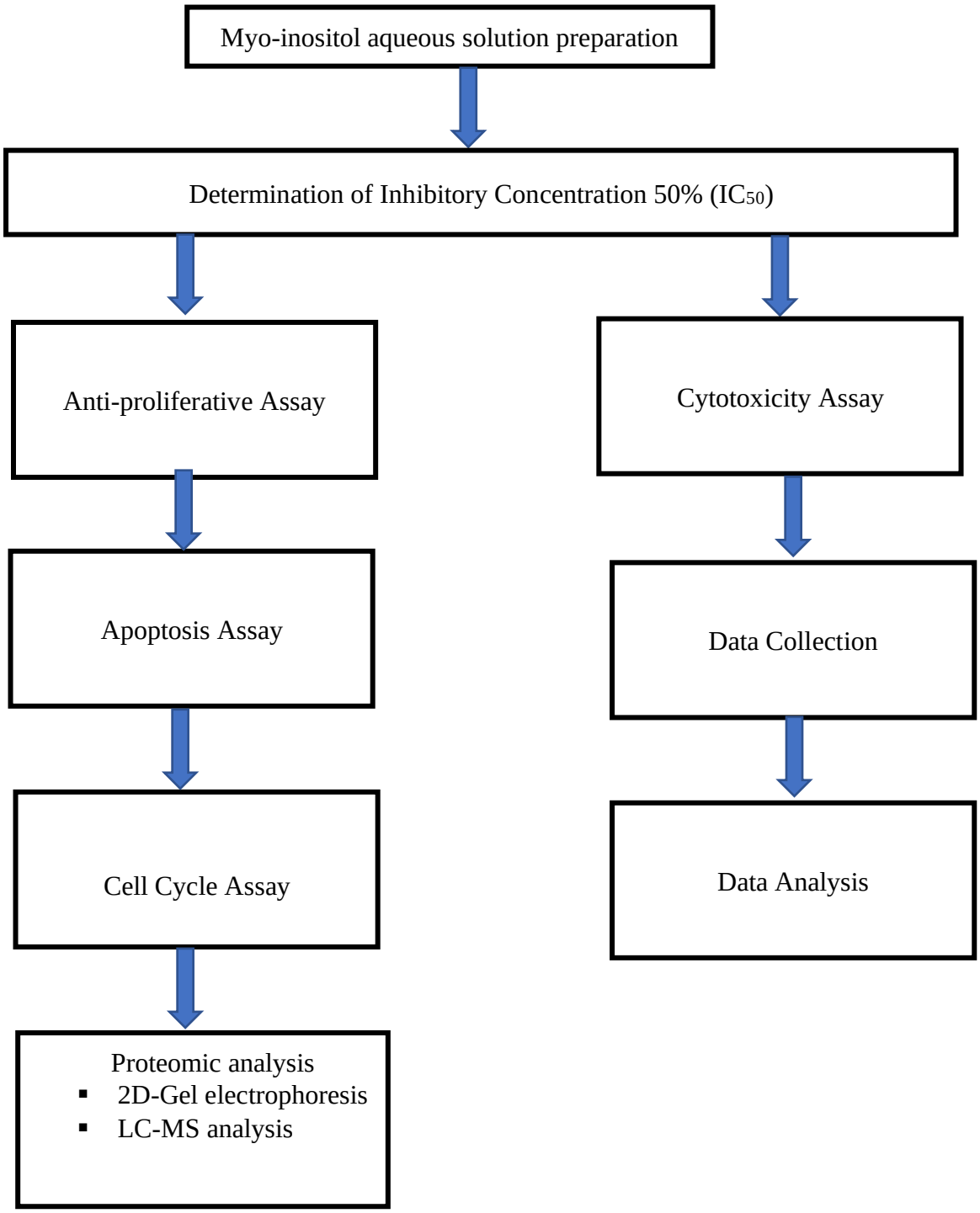
2. To determine the apoptosis induction and cell cycle arrest effect of Myo-inositol compound on human prostate epithelial DU-145 cell line.

3. To identify and corelate the proteins expression associated with apoptosis and cell cycle mechanism following Myo-inositol treatment on human prostate epithelial DU-145 cell line.

1.5 The hypothesis of the study

Myo-inositol shows inhibitory, antiproliferative, and chemopreventive effects on the androgen-independent DU-145 prostate cancer cell line. This may potentially suppress, delay, or reverse carcinogenesis at the cellular and protein level.

1.6 Flow chart of the study



CHAPTER 2

LITERATURE REVIEW

2.1 Prostate gland

2.1.1 Gross anatomy of the prostate gland

The prostate is the unpaired fibromuscular accessory gland of the male reproductive system covering the urethra in the pelvic cavity. It continues adjacent to the urinary bladder, the pubic symphysis behind it, and the rectum to anterior. The prostate is formed alike an inverted rounded cone with a wider base and a narrower apex resting below the pelvic floor, which is continuous above the neck of the bladder. The prostate's inferolateral surface is in contact with the levator ani muscles that frame the prostate together (Aronson and deKernion, 2007, Aaron et al., 2016).

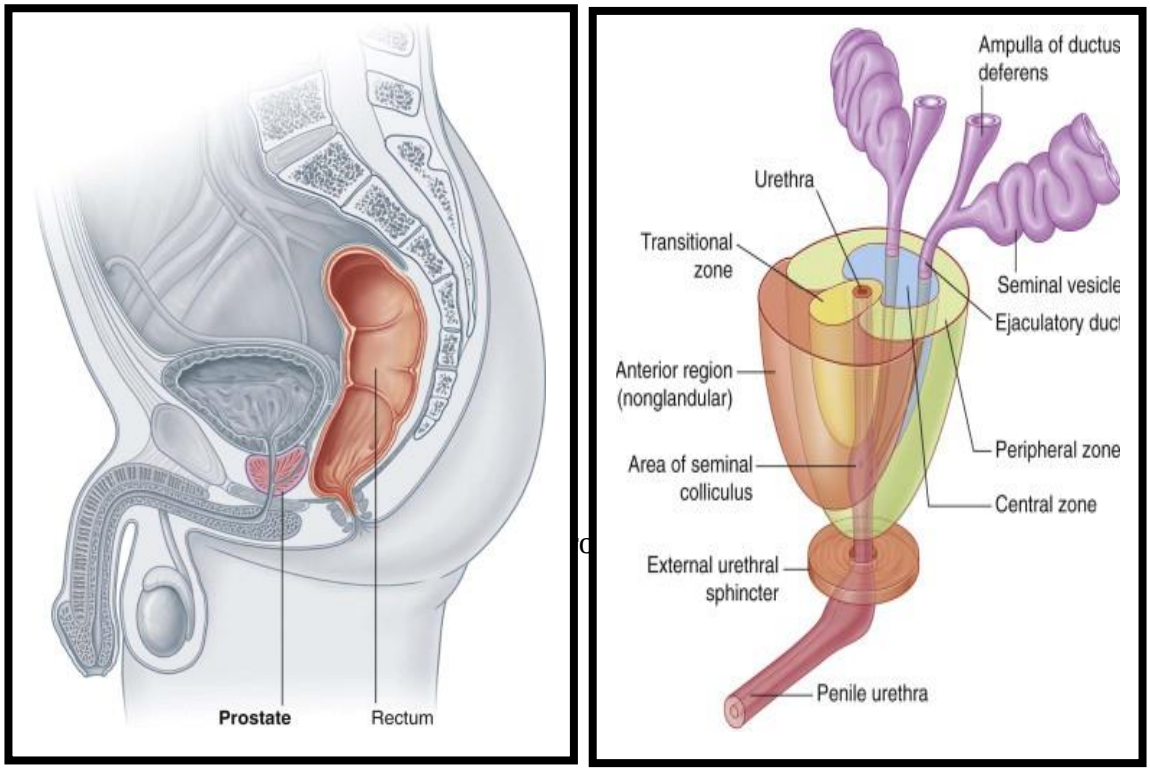


Figure 2.1: (A) Anatomical position of the prostate gland and (B) Zonal anatomy of prostate (Standring, 2016).

Anatomically, three distinct zones can be subdivided into prostate glandular tissue: peripheral (70% by volume), central (25% by volume) and transitional, respectively (by volume, 5%). The central region of the conical form around the ejaculatory ducts behind the periprostatic urethra (Zhang et al., 2018). Nearby the distal portion of the periprostatic urethra and the ejaculatory ducts, the transitional zone situated; it embraces the periprostatic urethral wall's smooth muscle. The ducts enter is the prostatic urethra just underneath the periprostatic sphincter and just above the ducts of the peripheral region. The central transitional zone and the periprostatic urethra are surrounded by the cup-shaped peripheral region of the prostate, excluding the anterior area where the anterior fibromuscular stroma fills the vacuum. In addition, the anteromedial region of the prostate, centrally located in relation to the prostate urethra and responsible for the gland's anterior convexity, consists solely of a fibromuscular stroma, the non-glandular portion of the prostate (Ricke et al., 2018).

The prostate's zonal anatomy, since most carcinomas in the peripheral region occur (Aaron et al., 2016) is clinically significant. Benign prostatic hyperplasia (BPH), on the other hand, affects the transitional zone (Bhat et al., 2021) that may grow to make up the bulk of the prostate. It produces the appearance of 'lobes' on both sides of the urethra as the transitional zone propagates. These lobes may compress or twist the periprostatic and prostatic sections of the urethra and produce symptoms (Henry et al., 2018).

2.1.2 Microscopic structure of the prostate

Histologically, numerous epithelial acini, which are surrounded by fibro-muscular stroma cells, form the prostate gland. Two major cell types are present in the human prostate gland: stromal and epithelial cells through a epithelial to stromal ratio of 2:1 (Abou-Elhamd et al., 2013). As glandular acini, the epithelium is organized to secrete into a lumen, which further intersects into a duct, and into the urethra. The epithelial portion is structured into a uniform layer consisting of four distinct cell subgroups: luminal secretory (50-59%), neuroendocrine (NE), basal, then transit amplifying (TA) components (comprising the rest 30-39% of entire epithelial (Ittmann, 2018).

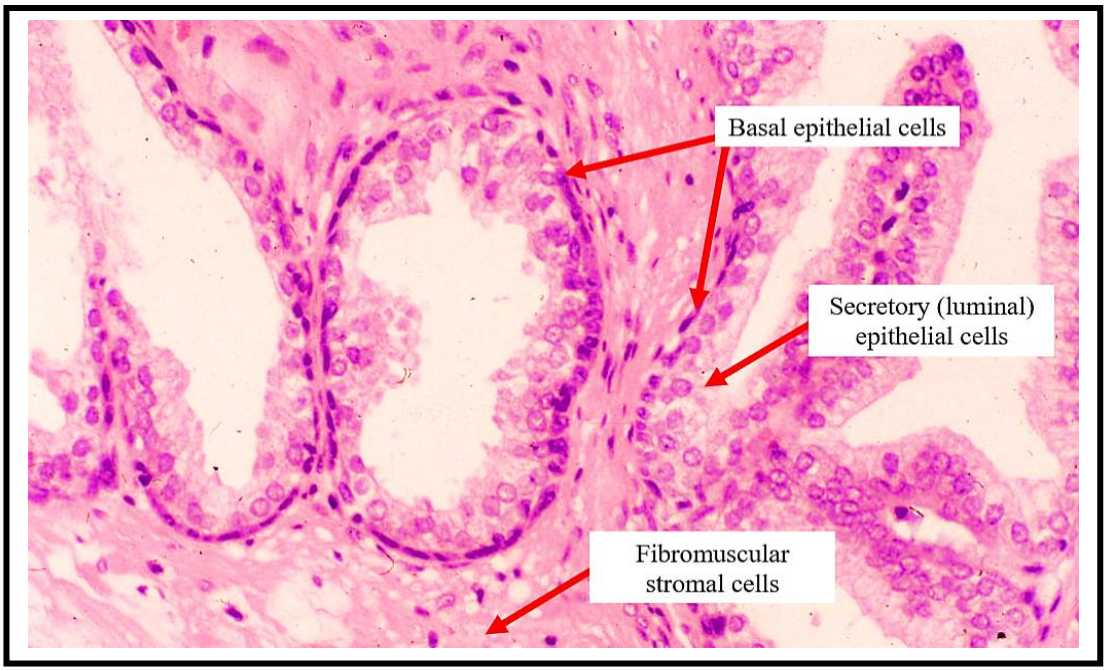


Figure 2.2: Prostate gland consists of parenchyma (tubulo-alveolar glands) and a characteristic fibromuscular stroma. Glandular parenchyma: formed by irregular prostatic alveoli with wide lumen. Secretory lining of alveoli varies from cuboidal to columnar depending upon activity (Ittmann, 2018).

As the essential cell type of the normal prostate, luminal cells are considered. Typical luminal cells are simple columnar in form with transparent cytoplasm that displays secretory potential. Luminal cells express the exocrine component of the epithelium, which secretes the prostate-specific antigen (PSA) protein into the lumen in addition to prostatic phosphatase (PAP). In addition, luminal cells express multiple molecular biomarkers such as CK8 and CK18 cytokeratins, CD57 (cell adhesion glycoprotein), p27Kip1 (cell-cycle inhibitor) and elevated AR concentrations (Zhang et al., 2018).

The basal layer, which is arranged above the basal acinar membrane, is made up of flattened cells. In basal cells, several molecular markers are expressed: p63 (an adherent to the p53 suppressor gene family), CK4, CK5 cytokeratin, and Bcl-2 (anti-apoptotic coefficient factor) and hepatocyte growth factor (HGF). They have low to undetectable AR expression relative to luminal cells, allowing their survival independent of androgen levels (Henry et al., 2018).

NE cells, solitary or in small clusters reside in the basal and luminal layers. These cells seldom enter the acinus lumen, but there are thinly dispersed apical processes directed to the lumen in the open type, while no apical extensions are present in the closed type. Chromogrenin A (neuron-specific analogues) is the key neuroendocrine markers. However, other distinct neuropeptides, potentially somatostatin, neurotensin, peptide-like thyroid stimulating hormone, serotonin, calcitonin, can also be formed by cells (Rybak et al., 2015).

Between the basal and luminal cells lives a small population of transit amplifying cells (TA), with no distinct morphological characteristics; however, the basal and luminal cell markers, namely CK5, CK8, CK14, CK18, AR and PSA, were released simultaneously (Timofte and Căruntu, 2018, Prajapati et al., 2013b, Wang et al., 2001, Guma, Prajapati et al., 2013a). Stroma fibroblasts, myofibroblasts, smooth muscle cells, vascular endothelial cells, nerve cells, and inflammatory cells make up these connective cells (Shiota et al., 2015, Tyekucheva et al., 2017).

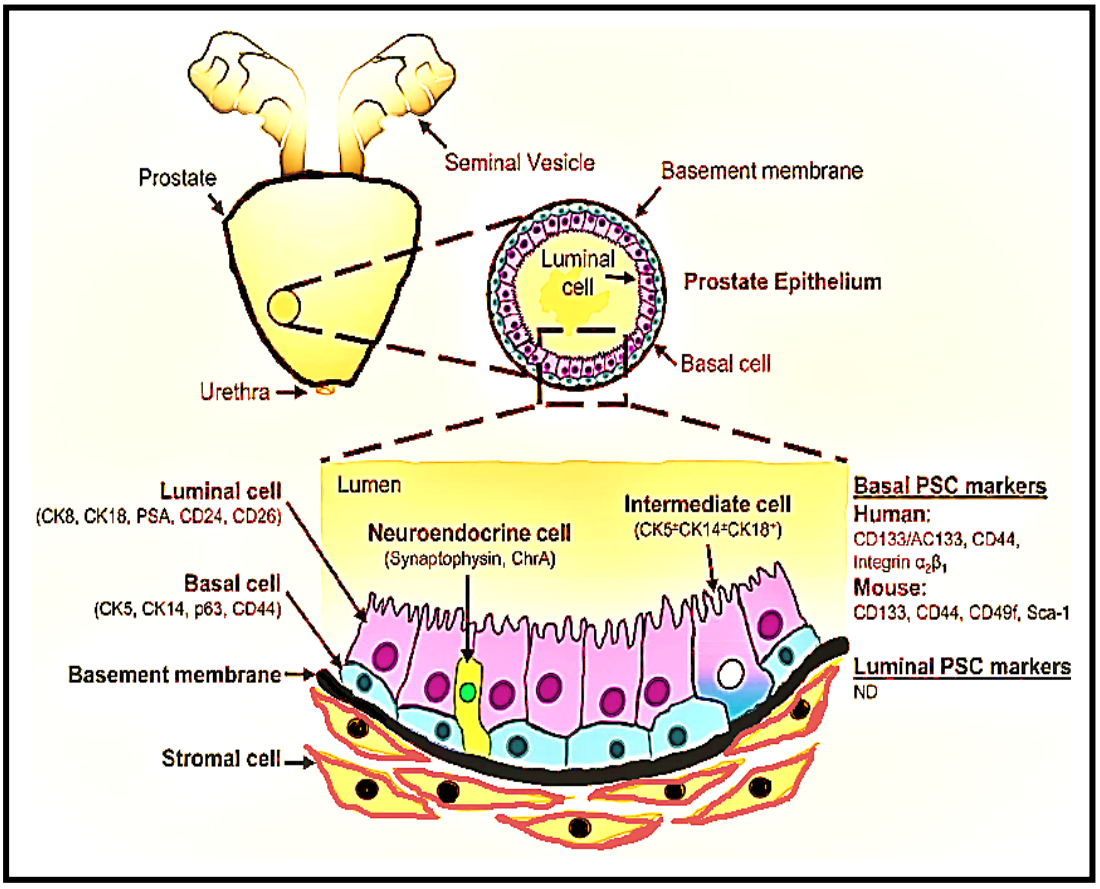


Figure 2.3: Showing the cellular architecture of the prostate epithelium, the prostate epithelium consists of an inner layer of secretory luminal cells. Basal cells form a continuous layer of cells around the luminal cells and in contact with the basement membrane (Henry et al., 2018).

2.1.3 Physiological roles and age-related Prostate gland changes

The central efficient and anatomical section of the normal human prostate gland is the peripheral zone (Costello and Franklin, 2009). The prostate functions are its involvement in the male ejaculate's reproductive aspects (its procreative purpose) and its participation in the orgasmic ecstasy (its recreative function). The key utility of the prostate gland is to produce and secrete prostatic fluids and, further precisely, to secrete enormous citrate levels in the prostate fluid (the sum contained in blood plasma is 400-1500 times). For the purpose of citrate growth, the tubular glandular epithelial cells have evolved to achieve this functional ability. Citrate is an essential metabolic mediator in normal mammalian cells as an important source of cytoplasmic acetyl Co-A for the production of the Krebs cycle for the development of ATP and allied synthetic pathways as well as for cholesterogenesis and lipid metabolism. In the prostate, the citrate is the end result of metabolism (Costello and Franklin, 2009). For bioenergetic and biosynthetic activities, epithelial cells in the prostate therefore sacrifice the normal metabolic use of citrate. In such conditions, the majority of human cells will not survive. However, by amalgamating metabolic and cellular adaptative relationships that allow survival and functional activity, the successful development of prostate epithelial cells for citrate manufacture and elimination has been accomplished (Costello and Franklin, 2009).

The prostate develops in the developing embryo in a series of ducts that are wrapped in a stroma that forms most of the gland. Duct epithelium, colliculus seminalis, and prostatic utriculum hyperplasia and squamous metaplasia occur prior to birth, most likely due to maternal oestrogen in the foetal blood (Chen et al., 2012). Following childbirth, it subsides and is followed by a quiet period of 12-14 years. The

prostate gland starts a maturation period at puberty and it grows more than twice as large during this time. Development is based on both the growth of follicles and the progression of ductal branch alteration, and likely duct end-buds. By the age of 17-18 years, morphogenesis and gland differentiations are completed. Through irregular proliferation of epithelial infoldings into the lumen of the follicles, the glandular epithelium develops during the third decade (Cooke et al., 2013). The volume remained virtually unaltered until 45-50 years after the third decade, once the epithelial folds begin to vanish, follicular outlines become more normal, and the number of amyloid bodies increases. All these changes are manifestations of prostatic involution. If a man lives long enough, it is an age-related illness that is inevitable, but it is not always symptomatic (Bonilla et al., 2016).

2.1.4 Clinical significance of the Prostate gland

In the prostate, cellular changes can occur that can be cancerous or non-cancerous. In older men, the prostate is too vulnerable to disease, with benign hyperplasia of the prostate (BPH), prostatitis, and prostate malignancy being the most prevalent conditions. Particularly relevant are the prostate zones, as diverse prostatic diseases appear from distinctive zones (Vargas et al., 2012).

Prostatitis is a common condition associated with prostate gland inflammation (Risbridger et al., 2010). In their lifetime, up to 25% of men develop a prostatitis. It is possible to classify prostatitis as acute and chronic. It is more likely that men at risk for acute prostatitis will have diabetes, cirrhosis, and suppression of the immune system (Khan et al., 2017). As it typically occurs in those with infections of the urinary tract, *Escherichia coli* (60%), other *enterobacteria* (5% to 10%), *Pseudomonas*, *Ceretia* and *Klebsiella* are usually the main cause (10% to15%) (Bostwick, 2020).

Sudden fever, chills, burning symptoms in the oesophagus, back, anus and perineum discomfort and pain are seen in patients with acute bacterial prostatitis. In severe bacterial prostatitis, about 10% ultimately improve and 10% in chronic pelvic pain syndrome. Most forms of acute prostatitis have an antibiotic sensitive to present therapies (Naide et al., 2006). A common cause of re-infection due to urinary tract infection is chronic bacterial prostatitis (Bostwick, 2020). As common causative agents, *Chlamydia*, and *Trichomonas* have been suggested. Chronic inflammation, including both BPH and malignancy, may increase or intensify glandular pathology (Xia et al., 2012). The theory is that, because of the cell-mediated immune response, chronic prostatic inflammation leads to damage to the tissue and endless healing of the wound, which leads to prostatic development. T cells can be the first line of protection against luminal foreign agents that enter the prostate through the prostate part of urethra. The increased frequency of penetration of CD4, T-lymphocytes into cancer tissue is distinctive and associated with poor outcomes (Wesch et al., 2020).

Prostate enlargement is the most common pathological condition of the prostate, also known as benign nodular hyperplasia or BPH. Nodular hyperplasia occurs in three stages: nodule development, regions of transformation and periurethral tissue, and nodular growth is large. The spread rises in males under 70 years of age (Bostwick, 2020). Lower urinary tract (LUTS), nocturnal (waking up at night), urinary frequency, urgency, urinary incontinence, recurrent urinary incontinence, and frequent urinary incontinence are collectively referred to as complications of the symptoms most frequently reported in men with BPH (Coleman, 2018, Rosario and Bryant, Beardsley et al., 2016).

2.2 Prostate cancer

2.2.1 Key Principles of carcinogenesis

Cancer development is a multi-step mechanism known as cellular and genetic mutation-related carcinogenesis. At the heart of carcinogenesis, nonlethal genetic damage lies. The initial damage (or mutation) may be caused by exposure to the environment, spontaneous, or likely inherited from the germline. Proto-oncogenes promote growth, tumour suppressor genes limit development, genes govern programmed cell death (apoptosis), and genes involved in DNA repair are the most common targets of cancer-causing alterations (Murshed, 2019). Mutant allele frequencies of proto-oncogenes are considered dominant because, given the existence of a typical counterpart, they transform cells. In general, by contrast, until mutations may occur, two common tumour suppressor gene alleles have to be disrupted. It is conceptually possible to classify carcinogenesis into four stages: tumour initiation, malignant transformation, tumour proliferation, and progression of the tumour (Sonnenschein and Soto, 2016). Consequently, mutations can be gained during cell proliferation by miss-repair of damaged DNA, resulting in spontaneously initiated (mutated) cells. There is less time for cells to proliferate and eliminate cooperative bonds with their DNA to restore damaged DNA (Tanaka et al., 2013).

The formation of carcinogen-DNA adducts is essential to chemical carcinogenesis theories, and may be a required but not appropriate prerequisite for the initiation of tumours. As a tumour-initiating event, it is possible to identify DNA adduct formation that activates proto-oncogenes or inactivates tumour-suppressing genes. If the indicated cells live without repair for weeks, months, or years, they may grow independently and in a clonal manner. By forming two new initiated cells, cell

division remains symmetrical through the initiation process. Mitogenic stimulation by intrinsic and extrinsic factors. This leads to an increase in the number of new cells and to apoptosis prevention (Torgovnick and Schumacher, 2015, Maner et al., 2020).

The "promotion" of tumour is functionally defined as a process that accelerates and enhances tumour formation after initiation. It is the selective multiplication of initiated cells that is the essence of promotion. It requires the growth of the initiated cell(s) into a focal lesion. Promotion often involves an extended period of action that can take weeks, months, or years to complete. After the termination of therapy, the influence of promotion can disappear again. The promotion of tumours is also associated with the induction of enzymes, in the affected organs, or with tissue damage. The tumour promotion method, however, is not a direct DNA reactive or harmful mechanism, but involves gene expression modulation, resulting in an increase in cell number through cell division and a decrease in apoptotic cell death (Maner et al., 2020, Rundhaug and Fischer, 2010).

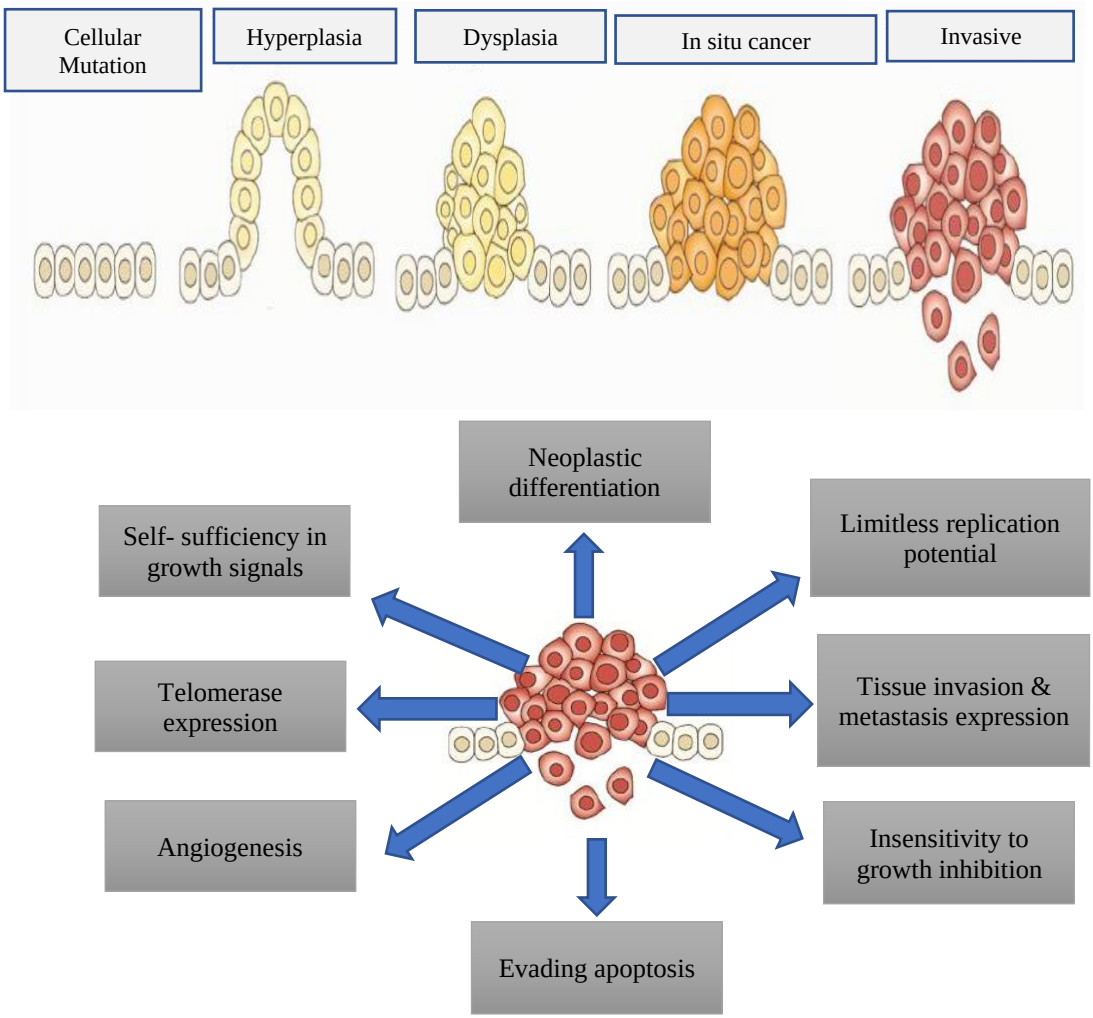


Figure 2.4: Multistep cancer development. (a) Cancer begins when a single cell experiences a mutation. This mutation causes the cell to divide more rapidly than normal cells. Malignant tumours develop as a result of mutations that render them capable of invading neighbouring tissues or metastasizing to distant tissues in the body. (b) The mutations that occur during carcinogenesis may alter eight fundamental cellular properties that lead to the development of the aggressive cancer phenotype (Kulesz-Martin et al., 2018).

A tumour promoter can often cause pre-neoplasms and neoplasms with exposure to long-term and high-dose, even without initiation stimuli. In response to the insult, for example, genotoxicity, which results in a lack of repair, or can spontaneously develop the starting cells. Not all cells in contact with a tumour promoter are in the propagation process, and only the cells pass to the next level of "progression" promoting apoptosis to separate and prevent apoptosis (Khor et al., 2018).

Only preneoplastic lesions and benign tumours are created by the sequence initiation for promotion. Their transformation to malignant lesions is called progression. Thus, multiple genetic lesions seem to be necessary for malignancy (Osada and Takahashi, 2002). In most cancers, genotypic and phenotypic changes do not meet a fixed endpoint, but demonstrate increasing autonomy and the risk of metastasis. The development of the tumour involves the expression of the malignant phenotype and the ability of malignant cells over time to develop more aggressive features (Jiang et al., 2015). Pre-neoplasm and/or benign neoplasm are characterized as the classification of lesions between initiation and propagation measures recognized by histopathological investigation. Their transformation into malignant lesions (with metastasis) is the last stage in the carcinogenesis process called "conversion." During the progression phase, a neoplastic or malignant phenotype is detected through genetic and epigenetic processes. At this point, expansion is independent of the presence or absence that is important to development. Irreversibility, genetic instability, growth factor development, invasion, metastasis, neoangioogenesis are the concepts of progression for neoplastic progression (Lison, 2016).

2.3 Pathogenesis of Prostate Cancer

2.3.1 Mutations in prostate cancer

Inherited mutations in the BRCA gene have been linked to an increased risk of breast, ovarian, prostate, and other cancers (Mehrgou and Akouchekian, 2016, Ruiz de Sabando et al., 2019, Nyberg et al., 2020). BRCA1/2 is a protein encoded by the primary oncogene associated with breast, ovarian, and prostate cancer susceptibility. It is a critical component of the homologous recombination (HR) system, cooperating with multiple enzymes to protect the genome from double DNA strand breaks (Paul and Paul, 2014, Page et al., 2019). DNA damage repair error (DDR) accounts for 25% of these modifications in metastatic castration-resistant prostate tumors (mCRPC), owing to the prevalence of BRCA2 mutations. In mCRPC patients, the BRCA2 mutation is a major negative prognostic factor linked to short metastasis-free survival (MFS) and cancer-specific survival (CSS) (Privé et al., 2021). DDR genes are implicated in the mechanisms underlying genomic stability; they participate in the repair process of DNA disruption during the cell cycle, ensuring proper mitotic cell division and distribution of genetic material to daughter cells. Furthermore, BRCA2 is an independent prognostic factor linked to poor outcomes. The CSS and MFS in localized PCa were considerably shorter in BRCA2 carriers than in non-carriers after 5 years (Messina et al., 2020). The rate of DDR gene mutations in males with metastatic PCa ranges from 11 to 33 %, which is much greater than the incidence in men with localized Pca (Nombela et al., 2019).

Prostate specific antigen (PSA), also known as kallikrein related peptidase 3 (KLK3), is one of the most prevalent proteins in normal human prostate epithelial secretion and seminal plasma. PSA is normally excreted outside the body in the urine or sperm. If PSA returns to the body and is found in large concentrations in the blood, there is a problem with the prostate. PSA readings can be adjusted to total prostate volume using the PSA (ng/ml) / Prostate Volume (cc) indication as PSA levels rise with prostate enlargement (Gupta et al., 2017, Downing et al., 2003, Wysock, 2020). Prostatic enlargement is more common as men become older; therefore, it is conceivable for the prostate to grow larger. This method can be used to determine PSA levels based on age. PSA values in men under 50 should be between 0-2.5 ng/ml (Atan and Güzel, 2013). The typical PSA level for men between the ages of 50 and 59 is 0-3.5 ng/ml. The PSA level for men aged 60 to 69 should be 0-4.5 ng/ml. PSA values of 0–6.5 ng/mL are considered normal for men aged 70–79 (Ilic et al., 2018, Atan and Güzel, 2013). The tumor suppressor gene P53 (Tp53) is thought to play a key role in cell cycle control. Tp53 has a number of roles that are well understood, including prostatic apoptosis and DNA repair tissue. Tp53 mutations are the most common mutations identified in human neoplasia so far, and they're thought to be risk factors in a variety of cancers, including urogenital cancer. In PCa, p53 mutations are linked to metastatic disease, androgen independence, and a poor prognosis. The p53 tumor suppressor protein induces PSA gene transcription, and loss of p53 function (tumor suppression) may contribute to higher PSA levels during prostate cancer progression (ECKE et al., 2010, Wan et al., 2018).

2.3.2 Ligand/Androgens in Prostate Cells

Androgens play a major role in the prostate's growth and function. Androgens are a group of steroid hormones that are most prominent in males with testosterone (T) (Messner et al., 2020). Testosterone is generated mainly by the Leydig cells in the testes (90%), although the adrenal glands often produce small quantities (10%). Typically, testosterone is metabolized and converted by the enzyme 5 alpha-reductase into its more active equivalent, 5 alpha-dihydrotestosterone (DHT) (Azzouni et al., 2012). DHT has a 10-fold higher AR binding affinity than testosterone in the prostate. Androgens, as androgen receptor (AR) based ligands, usually exert their effect on cell biology by activating AR signalling (Feng and He, 2019).

2.3.3 Brief Description of Androgen receptors (AR)

Receptor of AR nuclear steroid hormone is a transcription factor depending on the released upstream ligand. On the X chromosome, the human AR gene is (Xq11-12). Eight coding outgrowths encode a 110-kDA protein AR gene with four functional distinct domains. There is an N-terminal domain (NTD) consisting of two units of transcriptional activation (AF-1 and AF-5), a domain of DNA binding (DBD) with two domains of zinc binding four-cysteine, and a domain of ligand binding (LBD). AF-2 is a transcription activation unit and an LBD and LBD-connected hub region. Two trans-activation domains are found in the AR protein, in NTD, hormone-independent activation function 1 (AF1) and in LBD, hormone-independent activation function 2 (AF2) (Fujita and Nonomura, 2019b). AR is the major mediator for the genomic behaviour of androgens, being the predominant receptor for androgens. Not all forms of cells inside the prostate gland are AR-positive, however. At the same time, high levels of AR are expressed via the luminal secretory cells (Davey and Grossmann, 2016).