INHIBITORY EFFECTS OF ANDROGRAPHOLIDE IN PC-3 CELL LINE AND THE INDUCTION OF APOPTOSIS VIA THE INVOLVEMENT OF CASPASES 3, 8 AND 9

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by

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LIST OF SYMBOLS

μ	micro
∑	Summation
ºC	Degree celcius
M	Molar
±	Plus-minus
nm	Newton-metre
%	percentage

LIST OF ABBREVIATIONS

PC	Prostate Cancer
FBS	Fetal Bovine Serum
PBS	Phosphate Buffer Saline
PSA	Prostate Specific Antigen
FDA	Food and Drug Administration
NCI	National Cancer Institute
WHO	World Health Organization
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
DMSO	Dimethyl Sulfoxide
DMEM	Dulbecco's Modified Eagle Medium
WST	Water Soluble Tetrazolium Salt
LC	Latent Concentration
dH_2O	Distilled water
Q	Quadrant
SEM	Standard error of mean
IPS	Institut Pengajian Siswazah
USM	Universiti Sains Malaysia

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KESAN PERENCATAN ANDROGRAPHOLIDE DALAM TITISAN SEL PC-3 DAN INDUKSI APOPTOSIS MELALUI PENGLIBATAN CASPASES 3, 8 DAN 9

ABSTRAK

Andrographolide merupakan labdane diterpenoid yang diasingkan daripada sejenis pokok herba yang bernama *Andrographis Paniculata*. Kompoun ini mempunyai pelbagai kegunaan dalam bidang perubatan, terutamanya kesan anti-kanser. Satu kajian terdahulu telah mendedahkan bahawa andrographolide menghalang pertumbuhan sel-sel kanser paru-paru, otak, kolon, dan payudara. Oleh kerana kajian yang terhad, pengetahuan tentang kesan anti-kanser andrographolide terhadap sel-sel kanser prostat tidak terperinci. Dalam kajian semasa, andrographolide dinilai atas PC-3, titisan sel kanser prostat yang agresif dan berbahaya. Analisis sitotoksisiti adalah penting dalam penyelidikan terhadap perubatan baru untuk menilai biokompatibiliti kompoun tersebut sebagai ubat yang bakal digunakan pada sel-sel kanser. Kerja ini menggunakan ujian WST-1 untuk menentukan kadar kelangsungan hidup sel sel kanser PC-3 dan sel normal Hs27 yang terdedah kepada dos andrographolide yang berbeza iaitu (0-200 μM). Hasilnya menunjukkan bahawa dos andrographolide memberi kesan toxisiti yang tinggi terhadap sel PC-3 tetapi bukan pada sel Hs27. NCI menetukan nilai LC50 sebanyak 26.42 μM (selepas 48 jam inkubasi) boleh diterima dalam penyelidikan perubatan. Buat pertama kalinya dalam kajian ini, tiga dos andrographolide yang berbeza telah digunakan iaitu kawalan, separuh LC50, dan LC50 (0, 13.21, dan 26.42 μM) dalam semua analisis berikutnya. Metastasis adalah penting untuk penyakit ini berkembang; oleh itu, ‘scratch assay’ dan ‘transwell invasion assay’ digunakan untuk menguji kebolehan andrographolide untuk

menghalang pertumbuhan sel PC-3. Hasilnya menunjukkan bahawa andrographolide menghalang kedua-dua serangan dan migrasi berbanding dengan kawalan. Kehadiran ekor komet telah mendedahkan bahawa rawatan andrographolide 26.42 μM menghasilkan kerosakan DNA maksimum pada sel tunggal, diikuti dengan 13.21 μM dan 0 μM. Di samping itu, aktiviti kematian sel maksimum dilihat pada dos 26.42 μM andrographolide, diikuti dengan 13.21 μM dan kawalan. Aktiviti caspase 3 (caspase executor), 8 (laluan intrinsik), dan 9 (laluan ekstrinsik) dalam pengantara apoptosis meningkat dengan ketara. Separuh LC50 (13.21 μM) menunjukkan lebih banyak aktiviti dalam caspases ini daripada LC50 (26.21 μM), sesuai dengan penemuan baru yang berkaitan dengan senario ribut caspase. Ini membolehkan kita mengenal pasti 13.21 μM sebagai dos andrographolide yang menghampiri julat ideal untuk pengaktifan aktiviti caspase. Keupayaan andrographolide untuk mencegah perkembangan kanser dalam sel-sel kanser PC-3 telah terbukti melalui peraturan apoptosis berperantara caspase, penindasan metastases, dan induksi kerosakan DNA.

INHIBITORY EFFECTS OF ANDROGRAPHOLIDE IN PC-3 CELL LINE AND THE INDUCTION OF APOPTOSIS VIA THE INVOLVEMENT OF CASPASES 3, 8 AND 9

ABSTRACT

Andrographolide is a labdane diterpenoid isolated from the plant Andrographis paniculata. This substance has numerous medicinal uses, notably anticancer effects. A previous study has revealed that andrographolide inhibits the growth of lung, brain, colon, and breast cancer cells. Due to a lack of research, the knowledge of andrographolide's anti-cancer effects on prostate cancer cells is relatively poor. In the current study, andrographolide was assessed on PC-3 cells, an aggressive androgen-independent prostate cancer cell line. Cytotoxicity analysis is vital in drug discovery research for assessing the biocompatibility of the drug being used on cancer cells. This work used the WST-1 assay to determine the cell survival of PC-3 cancer cells and Hs27 normal cells exposed to varying doses of andrographolide (0-200 µM). The results indicate that andrographolide dose-dependently suppresses the viability of PC-3 cells but not Hs27 cells. The NCI considers the LC_{50} value of 26.42 µM (after 48 hours of incubation) is acceptable. For the first time in this study, three different concentrations of andrographolide were used: control, half LC_{50}, and LC_{50} (0, 13.21, and 26.42 µM) in all subsequent analyses. Metastasis is essential for the disease to progress; hence, the scratch assay and the transwell invasion assay were used to test andrographolide on PC-3 cells. The results showed that andrographolide inhibits both migration and invasion compared to the control. The presence of a comet tail has revealed that a 26.42 µM andrographolide treatment produces the maximum DNA damage to single cells, followed by 13.21 µM and 0 µM. In addition, the maximum

cell death activity was seen at 26.42 µM andrographolide concentration, followed by 13.21 µM and the control. The activity of caspases 3 (executor caspase), 8 (intrinsic pathway), and 9 (extrinsic pathway) in mediating apoptosis increased significantly. The half LC_{50} (13.21 µM) demonstrates significantly more activity in these caspases than the LC_{50} (26.21 µM), in accordance with the new finding related to the caspase storm scenario. This enables us to identify 13.21 µM as the andrographolide dosage that approaches the ideal range for caspase activity activation. The ability of andrographolide to prevent the progression of cancer in PC-3 cancer cells is proven through the regulation of caspase-mediated apoptosis, suppression of metastasis, and induction of DNA damage.

CHAPTER 1

INTRODUCTION

1.1 Introduction

The occurrence of cancer has been a worrying health dilemma encountered by humans for decades. Cancer is defined as the uncontrolled growth or cell division in a specific part of the human body. The severity of cancer in the human body is divided into several stages, indicating the seriousness of that disease. The stage of cancer is relatively different in all the cancer types due to the biomarkers and organs involved. Statistically, the reported data has shown a significant increase in the incidence of cancer and has been speculated to have higher numbers in the coming years. To be specific, in Asia in 2018, the region was predicted to have had 8.2 million new cancer cases and 5.2 million cancer-related deaths, representing over half of the global cancer burden (Xia, 2022). There are multiple types of cancer and the common ones include breast, prostate, brain, ovarian, cervical, and colorectal cancer.

Prostate cancer refers to cancer of the prostate gland. In men, a tiny gland in the form of a walnut generates the seminal fluid that nurtures and carries sperm. Cancer of the prostate constitutes one of the most prevalent kinds of cancer. Numerous prostate cancers are slow growing and restricted to the prostate gland, where they are unlikely to be harmful. However, while some kinds of prostate cancer develop slowly and may require minimal or no therapy, others are invasive and can rapidly spread. Almost all prostate malignancies are adenocarcinomas (Earnest, 2019). These tumours arise from glandular cells (the cells that make the prostate fluid that is added to the semen). Small cell carcinomas, neuroendocrine tumours (different from small cell carcinomas), transitional cell carcinomas, and sarcomas are some of the other prostate cancer forms. These further kinds of prostate cancer are uncommon. If an individual

is diagnosed with prostate cancer, it is usually adenocarcinoma. In addition, the significance of androgen in prostate cancer also contributes to the division of these cancers into different categories (Smith, 2022). Usually, prostate cancers are androgen-dependent, meaning they require androgen to sustain the growth of the cancer. This scenario is different for androgen-independent cancer cells.

Androgen-independent prostate cancer cells are considered more aggressive as they have the ability to survive in the absence of androgen. These types of prostate cancer cells are resistant to androgen deprivation therapy. The treatment options for prostate cancer have always been limited to targeting the androgen receptors in the prostate cells to inhibit the growth of prostate cancer. Hence, the urgency of discovering more therapies, which hinder prostate cancer development, has increased (Jose, 2004). In this study, the PC-3 cancer cell line has been used, as it is one of the candidates representing androgen independent prostate cancer cell lines. The PC-3 cancer cell was purchased from ATCC Company, which has isolated and maintained this cell from a 62-year old male who has stage 4 prostate cancer.

Factors that contribute to the development of prostate cancer are crucial to be understood to curb the further progression of this disease. The risk factors that are commonly studied relating to prostate cancer development are age, race or ethnicity, geography, family history, and the genetics that occur in an individual's body. A study has reported that low-grade prostate cancers may go into a condition of dormancy and reawaken as the individual ages, whereas high-grade prostate cancers are invasive from the start (Roderick Clark, 2022). Besides, another study has concluded that family history and genetics have a major contribution to the increase in prostate cancer development (Nair-Shalliker, 2022). Other components that have an effect on prostate cancer disease are diet, vasectomy, and obesity (Baboudjian, 2022). Statistics indicate

that prostate cancer contributes to the highest number of cancers diagnosed in the male population, which requires efficient treatment to curb the number of individuals diagnosed with this cancer type.

Early diagnosis is crucial for prostate cancer treatment as well as the inhibition of its growth. Prostate-specific antigen (PSA) levels and the Gleason Score are well-established diagnostic parameters used to assess the severity of prostate cancer disease. There are numerous advancements that have been in trial phase for the early detection of prostate cancer disease, yet these are the valid markers that have been used until date to measure the severity of prostate cancer (Mahmood Barani, 2020). Higher PSA levels, in particular > 4.0 ng/mL, indicate a higher risk of prostate cancer, whereas the Gleason score, which is higher than 3, indicates an elevated risk of the presence of prostate cancer. Although there are additional tests required to ascertain the presence of prostate cancer in an individual, the fundamental parameters, which are used to evaluate the risk of prostate cancer, are both PSA levels and the Gleason Score.

There are various treatment options for prostate cancer, such as surgery, radiation therapy, hormonal therapy, and targeted therapies. Surgery is still the primary treatment that has been used for decades in modern medicine. The procedure that is relatively used is radical prostatectomy, which is the removal of certain parts or the entire prostate gland (Costello, 2020). Although surgery is the primary treatment in treating prostate cancer, the advancement of research on various pharmaceutical therapies has contributed to novel and improved treatment therapies such as targeted therapy. Targeted therapies are tested for their efficiency in inhibiting the progression of prostate cancer. Targeted therapy is a form of cancer treatment that uses medications to target and destroy cancer cells while causing minimal harm to healthy cells. These medicines target the programming that distinguishes cancer cells from conventional,

healthy cells; i.e., the cancer cells' inner workings. Each type of targeted therapy works in a different way, but they all change how cancer cells grow, divide, heal, or interact with each other.

The most common types of targeted therapy used in cancer research include small-molecule drugs and monoclonal antibodies. Small-molecule drugs are efficiently able to enter cells; hence, they are used for intracellular targets. Monoclonal antibodies, often known as therapeutic antibodies, are made in a laboratory. These proteins are intended to bind to certain cancer cell-associated targets. Some monoclonal antibodies tag cancer cells so that the immune system can find them and kill them more easily. As for small molecule drugs, these molecules are able to target the receptors or enzymes which are involved in the initiation and progression of prostate cancer. The efficiency of the drug target is due to the extremely small size of the compound used to combat the progression of a particular disease. Andrographolide, the compound of interest in this study and is one of the many potential small molecule drug that is actively tested against the cancer disease.

Andrographolide is a bioactive compound used as a candidate for small-molecule drugs in this study. This compound is found in a plant species called *Andrographis Paniculata*. This plant is composed of various bioactive compounds, but the andrographolide is one of the most crucial compounds, which is made up of the labdane diterpenoid structure that is found in that plant. This compound upholds various pharmaceutical benefits, namely, anti-inflammation, anti-viral, anti-bacterial, anti-oxidant, as well as anti-cancer properties. The efficiency of andrographolide has been evaluated in numerous cancer cells, for instance, ovarian cancer, cervical cancer, colorectal cancer, lung cancer, and brain cancer.

In this study, the andrographolide compound is tested on the PC-3 cell line to determine its effect on varying aspects of the progression of the cancer. As metastasis is crucial in the development of cancer, this compound has been used to determine the inhibition ability of andrographolide on the invading aggressive PC-3 cell line. Besides, control of cell proliferation as well as increased cell death are important in order to curb the advancement of the PC-3 cancer cell line. The DNA damage of single cells has been evaluated in order to determine the extent of the damage exerted by the andrographolide compound on the PC-3 cancer cells. Caspases are key proteins that influence the rate of apoptosis as they regulate the pathway with their presence. Hence, the rate of caspases is also analysed on the PC-3 cell line upon the treatment of andrographolide.

1.2 Problem statement

The treatment for cancer, and specifically prostate cancer, has been observed to focus on the inhibition of the progression of the disease. The ability of the current treatment to contribute to the regression of aggressive prostate cancer has not been identified or observed. Hence, it is very crucial for the advancement of drug discovery to treat this aggressive cancer as well as contribute to the regression of this disease by studying different potential bioactive compounds. Andrographolide is one of the bioactive compound which is being actively studied in cancer inhibition. This compound can be found in a plant species called Andrographis Paniculata. This plant has been used in traditional Chinese and Indian medicine due to its various pharmaceutical values, which include anticancer properties. The major bioactive compound in this plant is andrographolide and this compound is the only stable diterpanoid found in this plant. Active research is still going on to understand the mode of action of andrographolide exhibited in the inhibition of cancer progression. The mechanism of action of andrographolide on various cancer cells such as colorectal cancer, ovarian cancer, breast cancer, and cervical cancer has been reported in recent studies. However, the mechanism of action of andrographolide on prostate cancer cells is vaguely understood due to a lack of research. Hence, in this study, the andrographolide compound was tested on the PC-3 cancer cell line, which is a form of androgen-independent cancer. This cancer cell line represents an aggressive form of prostate cancer, which is able to survive without androgen receptors. The potential of andrographolide in prostate cancer inhibition could be evaluated when it is studied on this PC-3 cancer cell line.

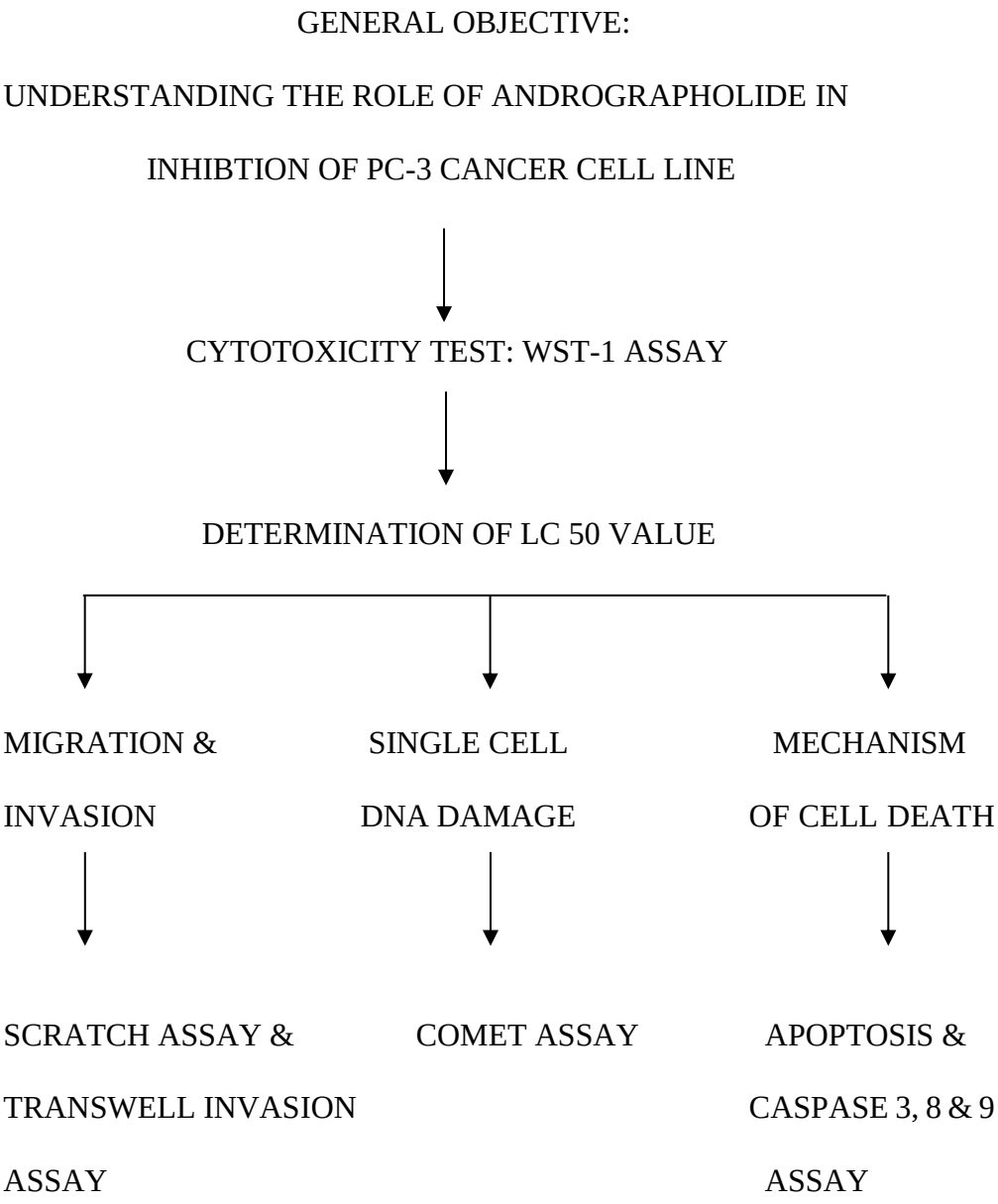
1.3 General Objectives

To understand the role of andrographolide in inhibiting prostate cancer progression.

1.3.1 Specific Objectives

1. To evaluate the inhibition of andrographolide in cell proliferation of PC-3 cells upon andrographolide treatment
2. To determine the DNA damage and it's intensity in PC-3 cell line once the cells undergo andrographolide treatment
3. To investigate the rate of apoptosis and caspase 3, 8 and 9 activity in PC-3 cell line after andrographolide treatment.

1.4 Research flowchart



CHAPTER 2

LITERATURE REVIEW

2.1 Cancer

Cancer is one of the non-communicable diseases that is both the most difficult to treat and the most common throughout the world. In most cases, cancer is brought on by genetic alterations that lead to the unregulated replication and cellular proliferation of human cells. These processes are excessive compared to those that occur in healthy cells (Hanahan D, 2000) . Besides, cancer also refers to a vast category of conditions that can affect any organ or tissue in the body. Additional terms include malignant tumours and neoplasms, which are used to address this disease (Hanahan D, 2000A defining characteristic of cancer is the fast proliferation of aberrant cells that invade neighbouring tissues and migrate to other organs. This phenomenon, known as metastasis, is the leading cause of cancer-related mortality (Buske, 2017). Since cancer is caused by a change in the genes, it is important to know everything there is to know about how tumours start and grow.

DNA contains every piece of genetic information. Different proteins govern all of a live organism's physiological activities, and proteins are composed of amino acids. Any mutation within a protein's amino acid sequence can alter its form and composition, making it hyperactive or hypoactive (De Matteis, 2018). The configurations of amino acids in proteins are encoded in specific regions of DNA, known as genes. Moreover, any change to the gene sequence might modify the proteins and result in aberrant behaviour. This modification of the genetic sequence is known as a mutation. Malignancies happen when cells grow out of control, so genes that control cell growth are very important to the development of cancer (Saha, 2022).

Mutational activation of genes that promote cellular proliferation might result in uncontrolled growth of a specific cell. This gene type is known as a proto-oncogene. When a proto-oncogene undergoes a mutation that results in oncogenesis, it is termed an oncogene (Weinstein IB, 2006). A second kind of gene that limits cell growth and proliferation is intended to regulate uncontrolled growth. If the mutation leaves these genes inactive, the cell proliferates and grows uncontrollably (Burkhart DL, 2008). These regulatory genes are often known as tumour suppressor genes, and they are critical to carcinogenesis. Cancer is a multistep process involving the activation of one or more oncogenes and the inactivation of one or more tumour suppressor genes. Although there are exceptions to this norm, malignant alterations are often caused by the sequential activation and silencing of numerous oncogenes and tumour suppressor genes (Velez, 2022).

Although cancer disease is considered detrimental among humans, specifically in later stages, WHO has reported a tendency to scale down the incidence and mortality caused by this disease. In addition to that, this can be done through a discrete approach, for instance, banning smoking, limiting vendors supplying alcohol, encouraging healthy weight loss f..or obese people, and finally, diet consumption with all nutritional factors taken into account (Society, 2016). The above stated methods are general and brief. However, the factors that contribute to this disease progression in general are physical carcinogens, chemical carcinogens, and biological carcinogens.

2.1.1 Carcinogens

Cancer-causing agents are known as carcinogens, which could be chemical, physical, or biological entities. Chemical carcinogens comprise chemical components present in a range of compounds, such as tobacco smoke and certain pesticides; physical carcinogens are insoluble in fluid particles of soft or hard matter. They exist in a solid

state. UV light and medical x-rays are examples of radioactive carcinogens that emit ionising radiation (Bruni, 2020). The capacity of carcinogens to cause cancer has been determined by determining cancer growth in humans or by conducting animal tests in the research lab. These compounds may cause cancer in laboratory animals and have a tenuous association with human tumours (Das, 2020).

Physical carcinogens consist of fibres, particles, hard and soft synthetic fibers, and gels. Some physical carcinogens arise naturally, while others are manufactured. The chemical composition of physical carcinogens is exceedingly varied, and many of them are understudied. The exact mechanism through which physical factors induce cancer has not yet been identified (Ledda, 2020). A number of distinct mechanisms most likely triggers Cancer. Some physical carcinogens interact with genetic factors and other environmental agents to cause cancer, further complicating matters. Asbestos, for example, may cause cancer on its own, but its carcinogenic potential is significantly enhanced when coupled with cigarette smoke (Langevin, 2020).

Chemical carcinogens comprise the most diversified category of carcinogenic agents, and they have high binding effectiveness to the human DNA of living systems (JHJ, 2009). This makes them a potential cause of cancer. They may form bonds with DNA, RNA, and proteins in either a covalent or noncovalent manner. They are tremendously effective in mutating genes and altering the mechanisms that control the transcription of genomes during the early stages of the development of cancer (Martincorena, 2015). They have nothing in common in terms of structure, and most of them become active through one of three metabolic changes: alkylation, oxidation, or de-alkylation (Pon, 2015).

The chemical compounds themselves do not normally directly trigger the growth of cancer; rather, it is the constituents of the chemical compounds that are often

accountable for carcinogenesis inside the organisms. This depends on the location inside the live cell in which the chemical component is susceptible to an enzymatic reaction. Either these compounds undergo a progressive transformation into electrophilic reagents and are thereafter exposed to the calcification or corrosion processes. This results in the formation of chemical carcinogens. Several chemical classes, such as polycyclic aromatic hydrocarbons (PAHs), aromatic amines and nitro compounds, alkylating agents, and N-nitroso amines and amides, can be used to classify chemical hazards (SM., 2004). The N-nitroso compounds, often known as NOCs, are not considered to be carcinogenic in the workplace; rather, they are classified as investigational carcinogens. In addition, occupational exposure to inorganic agents such as nickel, chromium, arsenic, and asbestos dust has been shown to significantly increase the risk of developing human cancer (Berenblum I, 2011).

A biological carcinogen is any biological material that both directly and indirectly contributes to the occurrence of cancer. Plants, animals, bacteria, fungi, or viruses may be the source (Baba A, 2007). Direct or indirect carcinogens can be found among biological agents. Certain viruses, including HPV, EBV, HTLV-1, and KSHV, are direct carcinogens that integrate their genomes or engage with cellular proteins to induce oncogenes, which eventually inhibit apoptosis (Kutikhin AG, 2013). They turn normal cells into malignant cells. Also, some biological factors cause the breakdown or metabolic change of substances in the digestive system, which turns molecules into cancer-causing substances.

Infectious pathogens may also affect the expression or activities of certain metabolising enzymes (Birkett, 2019). Cancer and inflammation have a close association. By generating proinflammatory chemokines and cytokines (Pei, 2020), infectious pathogens such as H. pylori, HBV, and HCV produce chronic inflammation

and ROS-induced DNA damage. Inhibition of the activity of immune cells by viruses like HIV-1 can manifest their carcinogenicity as immunosuppression raises cancer risk (Banik, 2020). Owing to their carcinogenicity, bacteria and protozoa can also create or release toxins that damage many biological processes due to their carcinogenic properties.

2.2 Statistics of Cancer Disease

Globally, cancer is the main cause of mortality and a significant impediment to extending the average lifespan (Xia, 2022). Cancer is the primary or second leading cause of death before the age of 70 in 112 of 183 countries, and the third or fourth leading cause of death in 23 countries. In many countries, stroke and coronary heart disease death rates have gone down a lot compared to cancer death rates. This has caused acquired cancer to become the leading cause of death.

Internationally, the cases and deaths of cancer are increasing at an alarming rate. This is due to the ageing and increase of the populace, as well as shifts in the frequency and distribution of the major causes of cancer. Many of which are connected with economic growth (Rumgay. 2022). In 112 countries, prostate cancer is the most common cancer in men while in 36 countries, lung cancer is the most common cancer in men. In addition, when 11 countries are taken into count, colorectal cancer is the most common cancer in men.

In terms of mortality Figure 2.2, lung cancer is the main cause of cancer-related death in males in 93 countries, due partly to its high mortality rate, followed by prostate cancer (48 countries) and liver cancer (23 countries). In opposition to men, the most intermittently diagnosed cancers among women are breast cancer (159 nations) and cervical cancer (23 of the remaining 26 countries) according to Figure 2.1. In 110 and

36 countries, respectively, breast and cervical cancer are the main cancer-related causes of death, followed by lung cancer in 25 nations.

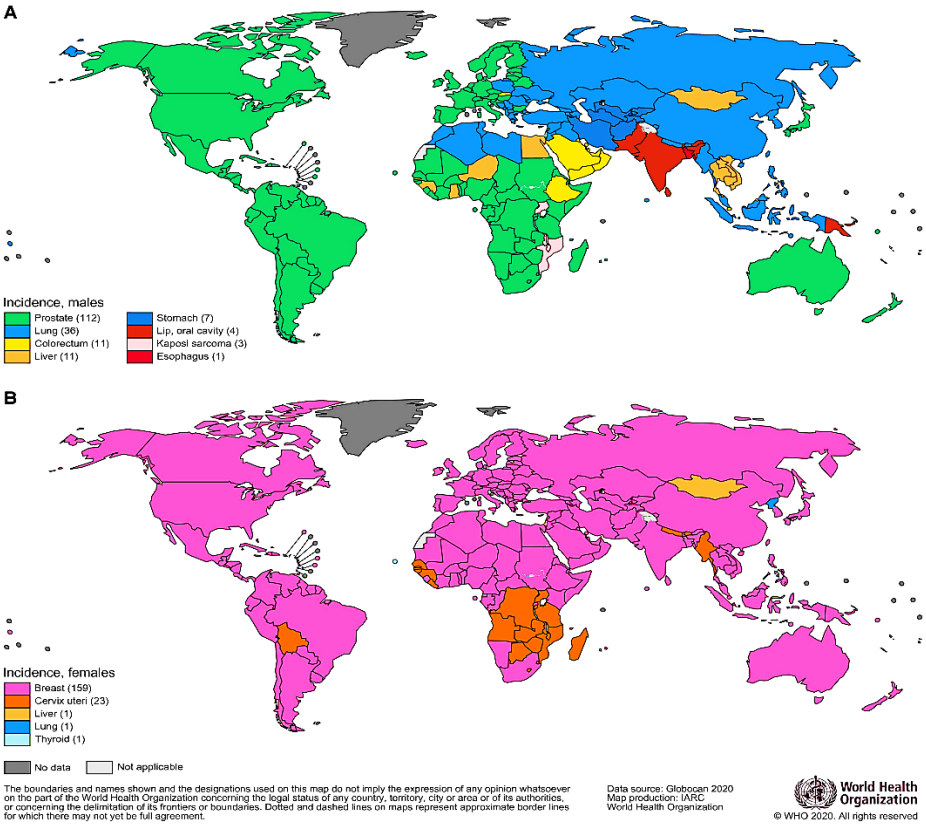


Figure 2.1 Most Common Type of Cancer Incidence in 2020 in Each Country Among (A) Men and (B) Women. The numbers of countries represented in each ranking group are included in the legend. Source GLOBOCON 2020

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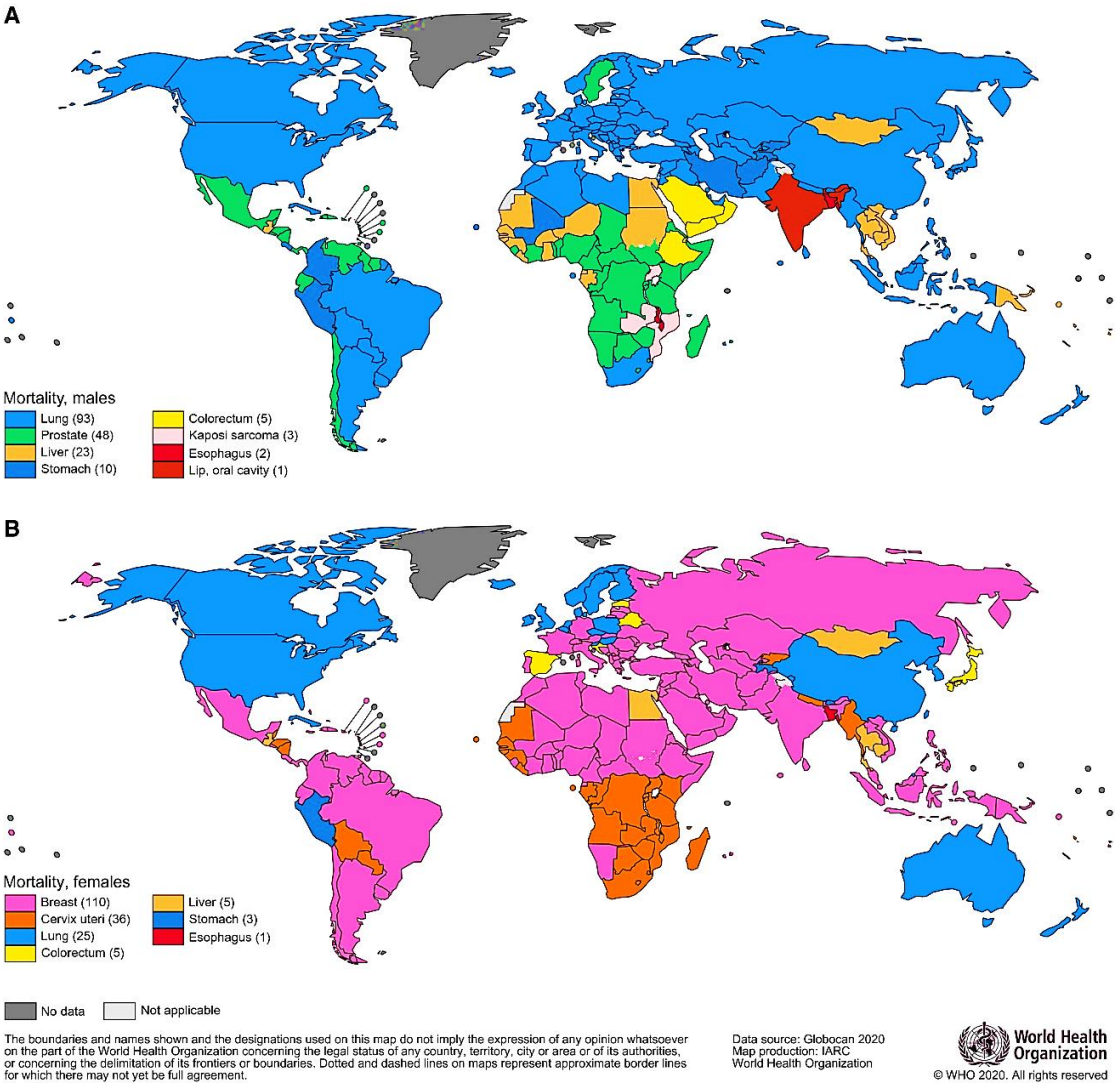

Figure 2.2 Most Common Type of Cancer Mortality by Country in 2020 Among (A) Men and (B) Women. The numbers of countries represented in each ranking group are included in the legend. Source: GLOBOCAN 2020

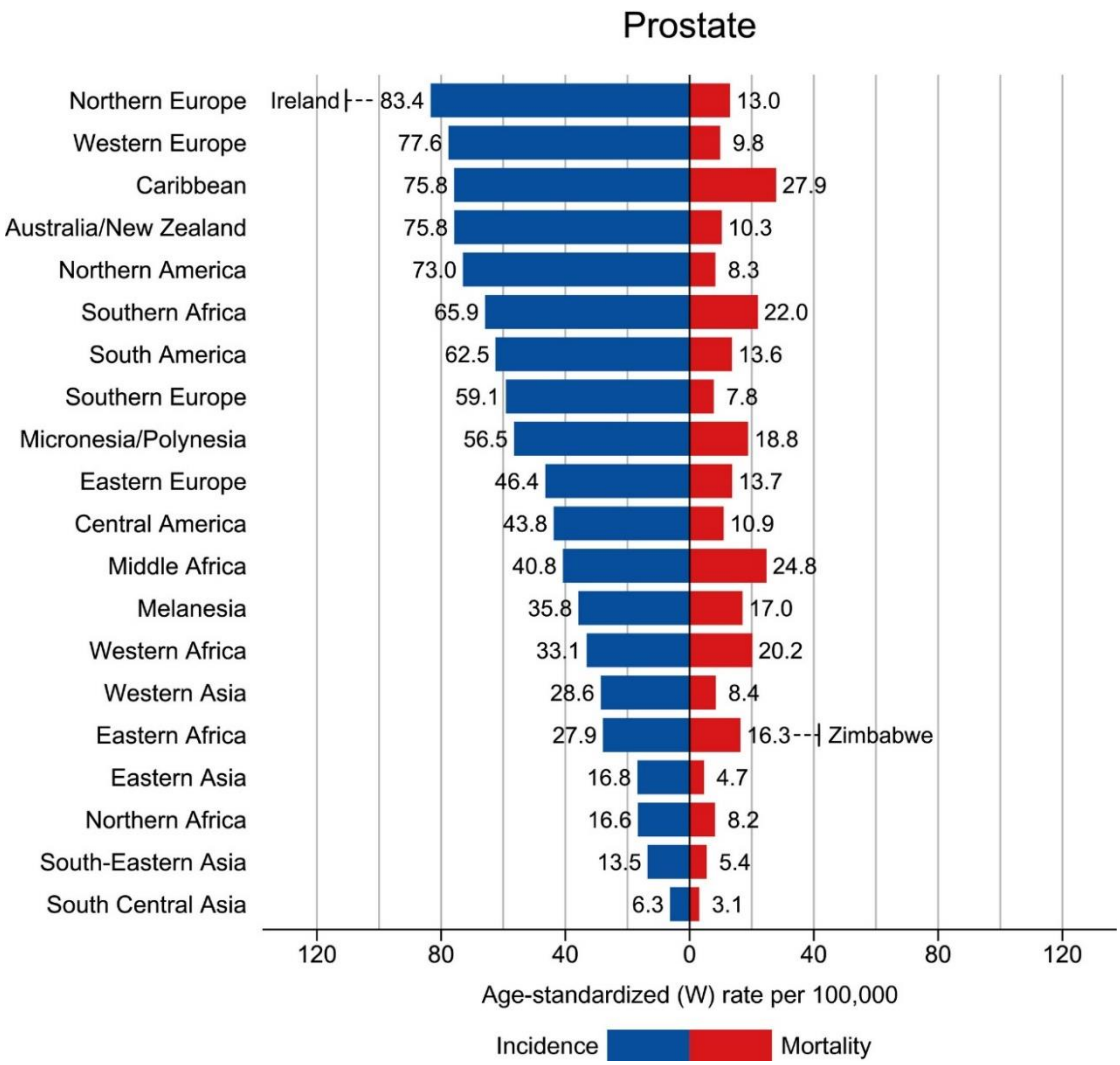

Figure 2.3 Region-Specific Incidence and Mortality Age-Standardized Rates for Prostate Cancer in 2020. Rates are shown in descending order of the world (W) age-standardized incidence rate, and the highest national age-standardized rates for incidence and mortality are superimposed. Source: GLOBOCAN 2020

2.3 Prostate Cancer

As cells in the prostate gland begin to proliferate excessively, prostate cancer occurs. The prostate is an exclusive gland that is found in males. It produces a portion of the fluid that comprises sperm. Below the bladder (the hollow organ that stores pee) and in front of the rectum lies the prostate (the last part of the intestines). Just beneath the prostate are glands known as seminal vesicles that produce the majority of the semen's contents. The urethra, the channel that transports urine and sperm out of the body via the penis, passes through the prostate's core (Hammerick, 2009).

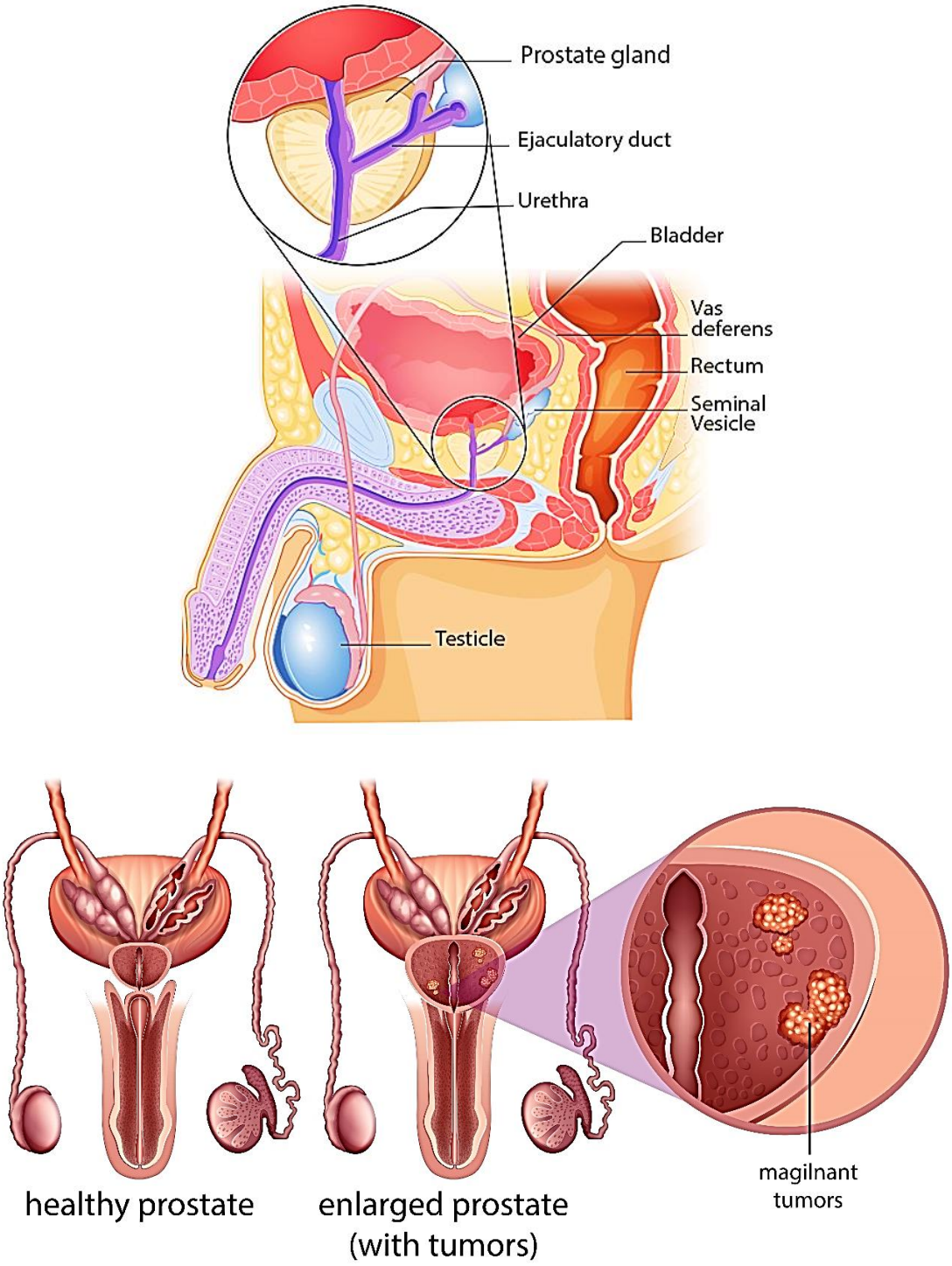

Figure 2.4 Figure above depicts the healthy prostate and prostate that has malignant tumors; picture courtesy VecteezyImages.

Prostate cancer represents the most commonly diagnosed non-skin cancer and the second greatest cause of cancer-related mortality among males. PSA monitoring is an essential prostate cancer screening method. However, histopathological prostate cancer is prevalent in older men. Slow-growing, well-differentiated prostate cancer, which can be detected using current histological parameters, tends to increase with age, affecting approximately 40% of men over the age of 50. However, the majority of histological tumours are slow growing and offer minimal threat to those affected. Only about 11% of them manifest clinically, and 3% are fatal to patients (Dong, 2005). In addition, a study reported that, one man in nine over the age of 65 is afflicted with prostate cancer, making it the most often diagnosed cancer in males (Grossman, 2018). To date, men suffering from advanced prostate cancer are lacking an effective treatment, despite the fact that early identification with blood screening of prostate specific antigen (PSA) with advanced procedures for medical intervention and radiation therapy has significantly reduced the number of fatalities.

In its early stages, when it is contained within the prostatic capsule, prostate cancer is treatable with synergistic effect of surgery and radiation therapy. In reality, most cases of prostate cancer do not cause any symptoms, and most men have distinct cause of mortality (Thalgott, 2013). On the contrary, prostate cancer can develop into phases characterised by local invasion of the seminal vesicles accompanied by spreading mostly to the bone, which is generally lethal if not diagnosed early. When this disease spreads to other parts of the body, it results in the body not requiring the androgens. This is often caused by androgen-ablation treatment. A lot of research has been done on important parts of the clinical development process that affect how patients do. These include: (1) recognition of prognostic factors that differentiate rare, aggressive forms of prostate cancer from the vast number of indolent cancers; (2)

comprehension of the mechanisms that lead to androgen independence; and (3) comprehension of what causes prostate cancer to predominantly metastasize to bone (Denmeade, 2010).

On the other hand, little emphasis has been placed on the processes behind prostate cancer's beginning and the molecular features of a cancer growth route. Analysis of the chromosomal abnormalities typically detected in prostate cancer has proven to be a particularly lucrative field of study. Patterns of persistent allelic loss in prostate cancer are most likely caused by the reduction or loss of activity of putative tumour suppressor genes (Xue, 2022). Losses of heterozygozity on chromosomes 8p, 10q, 13q, and 17p are particularly prevalent. Additionally, there have been losses of chromosomes 6q, 7q, 16q, and 18q, but the mechanism behind this is unknown. Furthermore, while chromosomal gains tend to be less frequent than chromosomal losses, gains at 8q and 7 are somewhat prevalent. (Li, 2020).

2.4 Androgen in Prostate Cancer

Since the prostate is an androgen-regulated organ, researchers have been interested in the involvement of androgens in prostate cancer for decades. Androgens are needed for the formation, growth, and survival of the organ. The link between androgen levels and prostate cancer has been thoroughly elucidated. The intracellular androgen receptor (AR), which pertains to the superfamily of ligand-dependent transcription factors, mediates androgen activity (Feldman, 2001). In addition, AR interacts with testosterone to activate the transcription of androgen-responsive genes and control both the healthy prostate gland and prostate cancer development. Targeting the androgen-signaling axis is the standard treatment for patients with prostate cancer that has spread (Pienta, 2006).

Extensive research conducted over more than a decade suggests that the inadequacy of androgen withdrawal therapy may not be due to a lack of androgen signalling but instead to genetic alterations that lead to abnormal stimulation of the androgen-signaling axis (July, 2002). In hormone-refractory carcinomas, there are two main types of genetic changes in the androgen receptor (AR): somatic mutations that cause less selective ligand binding. Next is the false stimulation of receptors by estrogens, progestins, adrenal androgens, glucocorticoids, and/or AR antagonists; and a genomic amplification of the AR genotype. This could make it possible for the androgen-signalling axis to stay active even when androgen levels are very low (Scher, 2005). Germline SNPs in the repeat sequence of the AR gene, which likely affect how the AR gene works, have been linked to a higher risk of getting prostate cancer, but other germline mutations are still rare.

The most frequently used treatment for advanced prostate carcinoma is androgen-ablation therapy, which causes tumour downregulation in the short term due to widespread death of androgen-dependent cancer cells (Wang, 2015). Unfortunately, in the vast majority of cases, such treatment fails to effectively halt the recurrence of androgen-independent, very aggressive metastatic prostate cancer. This change toward androgen independence is thought to occur because of selection favouring the growth of androgen-independent cells. Prior to androgen suppression, these cells coexisted in an androgen-dependent population (Gingrich, 2017). Due to its response to this treatment, a lot of research has been done to find out how cancer cells avoid being dependent on androgen right away and what the status of the androgen receptor (AR) is (Koivisto, 2008).

At first, it was assumed that androgen-independent tumour development was induced by the absence of AR mRNA and protein in extremely hostile and/or metastatic

rat and human cell lines (Culig, 2008). Contrary to predictions, it was later demonstrated that AR protein is produced very uniformly in original tumours, recurrent local tumours, and even metastases (Sweat, 2019). These findings suggest that carcinoma cells circumvent the need for androgens via a mechanism that does not entail the suppression of AR expression. Androgen independence may be caused by changes in the activity, function, and/or sensitivity of the androgen receptor (AR).

One theory is that the AR becomes hypersensitive to residual androgens at low concentrations. Indeed, the hormone-binding region of AR is frequently changed in both cell lines and tumours, making AR more tolerant of binding other steroid hormones and therefore losing its particular requirement for androgens (Elo, 2015). AR alterations are seen in primary tumours and hormone-resistant diseases not only in the hormone-binding regions but also in their coding regions (Tilley, 2016). Duplication of CAG repeats, whose length is inversely related to androgen activity (Hakimi, 2016), and amplification of the whole AR gene, which may be common in diseases that keep coming back, are two other changes that change how the AR works. When androgens are restricted (as during androgen-ablation therapy), it has been suggested that AR's combined activities with growth factors such as IGF, FGF, and/or EGF may overcome hormone restrictions. (Culig, 2008).

2.5 Caspase Mediated Apoptosis Pathway

The development of castration-resistant metastatic prostate cancer is owing to the upregulation of survival mechanisms, including apoptosis suppression and anoikis resistance, and enhanced neovascularization. Therefore, targeting apoptotic players is of crucial relevance in prostate cancer therapy, as loss of apoptosis and resistance to anoikis are critical in aberrant malignant development, metastasis, and imparting

therapeutic failure (Shin, 2020). To induce apoptosis, the majority of therapeutic drugs work via intrinsic mitochondrial, extrinsic death receptor or endoplasmic reticulum stress pathways. Current treatment modalities aim to restore regulatory molecules that govern pro-survival channels, such as PTEN, which controls the activity of AKT. Other techniques focus on reactivating the apoptotic pathways either by down-regulating anti-apoptotic components like BCL-2 or by up-regulating pro-apoptotic protein families, most notably the caspases (Jiang, 2020). Caspases are a class of cystine proteases that play crucial roles in apoptotic and inflammatory signal transduction pathways. During carcinogenesis, the major loss or inactivation of key members of the caspase family impairs apoptosis induction, generating a drastic imbalance in the growth dynamics and, eventually, the development of malignant tumors (Carneiro, 2020). Recent exploitation of apoptosis pathways to reinstate apoptosis induction via caspase reactivation has provided new molecular platforms for the development of effective treatment interventions against advanced prostate cancer and other solid tumors (Heitzer, 2020).

2.5.1 Extrinsic Pathway

The extrinsic mechanism is activated when external ligands such as TNF, Fas-L, and TRAIL bind to the extracellular domain of the DR (transmembrane receptors), i.e., the type 1 TNF receptor (TNFR1), Fas (also known as CD95/Apo-1), as well as TRAIL receptors. The FasL/FasR and TNF/TNFR1 models accurately describe the sequence of events during the extrinsic phase of apoptosis (Goelz, 2021). This activation of DRs by specific death ligands (DLs) results in the formation of a signaling complex that induces cell death (DISC). This disc is made up of the Fas-associated death domain (DD) as an adaptor molecule, procaspase-8, procaspase-10, and the cellular FLICE inhibitory proteins (c-FLIPs) (Neophytou, 2021). The prodomain of caspase 8 persists at the DISC, while the active caspase 8 dissociates from the DISC to