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EXPRESSION ANALYSIS OF PCBB AND IVD GENES IN METASTATIC PROSTATE CANCER

Safa Ahmed A ABU DAYA

Master's Thesis

Supervisor

Asst. Prof. Dr. Yalda HEKMATSHOAR

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The thesis titled EXPRESSION ANALYSIS OF *PCBB* AND *IVD* GENES IN METASTATIC PROSTATE CANCER prepared by SAFA AHMED A ABUDAYA and submitted on 19/01/2026 has been **accepted unanimously** for the degree of Master of Science in Biomedical Sciences.

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I hereby declare that all information/data presented in this graduation project has been obtained in full accordance with academic rules and ethical conduct. I also declare all unoriginal materials and conclusions have been cited in the text and all references mentioned in the Reference List have been cited in the text, and vice versa as required by the abovementioned rules and conduct.

Safa Ahmed A ABU DAYA

Signature

DEDICATION

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ABSTRACT

EXPRESSION ANALYSIS OF PCBB AND IVD GENES IN METASTATIC PROSTATE CANCER

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Prostate cancer is a leading cause of cancer-related mortality among men worldwide. It demonstrates variable biological behavior ranging from localized indolent forms to highly metastatic and treatment-resistant stages. Understanding the molecular mechanisms responsible for this progression is essential for improving diagnosis and therapeutic strategies. The present study focused on the expression analysis of *PCBB* and *IVD* genes in prostate cancer cell lines representing different stages of disease progression. Human prostate cancer cell lines PC3 (CRL-1435™, metastatic, androgen-independent) and LNCaP (CRL-1740™, androgen-dependent) were used as experimental models. Total RNA was extracted, quantified, and converted into complementary DNA (cDNA) for quantitative real-time polymerase chain reaction (qRT-PCR) analysis. The comparative gene expression analysis revealed that *PCBB* expression was significantly reduced in PC3 cells compared to LNCaP, indicating a potential relationship between *PCBB* downregulation and the metastatic phenotype of prostate cancer. In contrast, *IVD* expression showed no statistically significant difference between the two cell lines, suggesting that its transcription remains stable regardless of disease stage. Statistical analysis was conducted using GraphPad Prism and significance was determined at $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$. The findings demonstrate that *PCBB* may play a role in the metabolic or molecular changes associated with prostate cancer metastasis, while *IVD* expression appears unaffected. These results contribute to the growing understanding of gene-specific regulation in prostate cancer and may guide further studies exploring their diagnostic and prognostic potential.

Keywords: Prostate Cancer, *PCBB* Gene, *IVD* Gene, Metastasis, Gene Expression, qRT-PCR.

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ABBREVIATIONS

ADT	:	Androgen Deprivation Therapy
AR	:	Androgen Receptor
ATM	:	Ataxia Telangiectasia Mutated Gene
BCAA	:	Branched Chain Amino Acid
BRCA1/2	:	Breast Cancer Gene 1 and 2
cDNA	:	Complementary Deoxyribonucleic Acid
CRPC	:	Castration-Resistant Prostate Cancer
Ct	:	Cycle Threshold
DMEM	:	Dulbecco's Modified Eagle Medium
DNA	:	Deoxyribonucleic Acid
FBS	:	Fetal Bovine Serum
IVD	:	Isovaleryl-CoA Dehydrogenase
mRNA	:	Messenger Ribonucleic Acid
PBS	:	Phosphate-Buffered Saline
PC3	:	Prostate Cancer Cell Line (Androgen-independent)
PCBB	:	Prostate Cancer Binding/Metabolic-Related Gene (target gene in this study)
PCR / qRT-PCR	:	Quantitative Real-Time Polymerase Chain Reaction
PSA	:	Prostate-Specific Antigen
RNA	:	Ribonucleic Acid
RPMI	:	Roswell Park Memorial Institute Medium
SD	:	Standard Deviation
scRNA-seq	:	Single Cell Ribonucleic Acid Sequence

LIST OF SYMBOLS

p	:	Probability Value Indicating Statistical Significance
± SD	:	Mean Value ± Standard Deviation
μL	:	Microliter (10^{-6} liters)
ng/μL	:	Nanograms Per Microliter, Concentration Unit for Nucleic Acids

1. INTRODUCTION

Prostate cancer (PCa) is the second and third greatest cause of cancer deaths among American and European males, respectively. It remains as one of the top causes of cancer death based on the development of advanced and metastatic stages of the cancer (Dong et al., 2019). Even when the systemic therapy develops to enlarge, metastatic prostate cancer has not been well cured, thus it is associated with low survival in case of advanced cancer in the bones.

The emerging evidence of molecular profiling research indicates that the normalcy of the re-establishment of the metabolic process and malfunctioning of the mitochondrion are the characteristic behaviors of the aggressive prostate cancer, especially at the castration-resistant and metastatic stages. The normal metabolic conditions relief to abnormal energy production and redox maintenance in these tumors, encouraging the growth, survival and metastasis dispersal. (Wanjari et al., 2023)

The most established gathering of information is that mitochondria-related genes, e.g. those related to metabolism of branched-chain amino acids, oxidative metabolism, play a role in disease pathogenesis and sensitivity to treatment, though canonical drivers, such as androgen receptor signaling, and changes in DNA repair pathways, are also well established. It has also been possible to find out that mitochondrial gene signatures are also associated with drug resistance, and the presence of those genes as *PCBB*, is one of the candidates that have their role to play in prostate cancer that can be verified during the experiment.

1.1 PROBLEM STATEMENT

Prostate cancer is a serious issue in the globe and a major cause of cancer morbidity and mortality amongst men and especially with the advancement to the advanced and metastatic stages. Despite the successful treatment of localized prostate cancer, adverse clinical outcomes and insufficiency of survival are associated with the spread of the disease to a more severe state of metastatic and castration-resistant prostate cancer (mCRPC) (Sayegh et al., 2022). However, despite the advances in systemic treatment, including taxane-based therapy, androgen receptor-targeted therapy, resistance to treatment is a recurring event in a

large segment of patients and demonstrates a major deficiency in the currently implemented treatment options.

The most recent molecular studies have demonstrated that prostate cancer development cannot be explained only by the alterations of the androgen receptor signaling or even the DNA repair mechanisms. Instead, the process of tumour evolution is steered by the advanced re-programming of the molecules that entail metabolic rearrangement, mitochondrial collapse, and changed cellular bioenergetics (Maekawa et al., 2024). These changes permit the survival of cancerous cells under therapeutic pressure and permit a spread of the cancer to a metastasis. However, although there is a current trend of metabolic reprogramming being considered a hallmark of aggressive prostate cancer, the role of single metabolic genes in mitochondria has not been adequately reported.

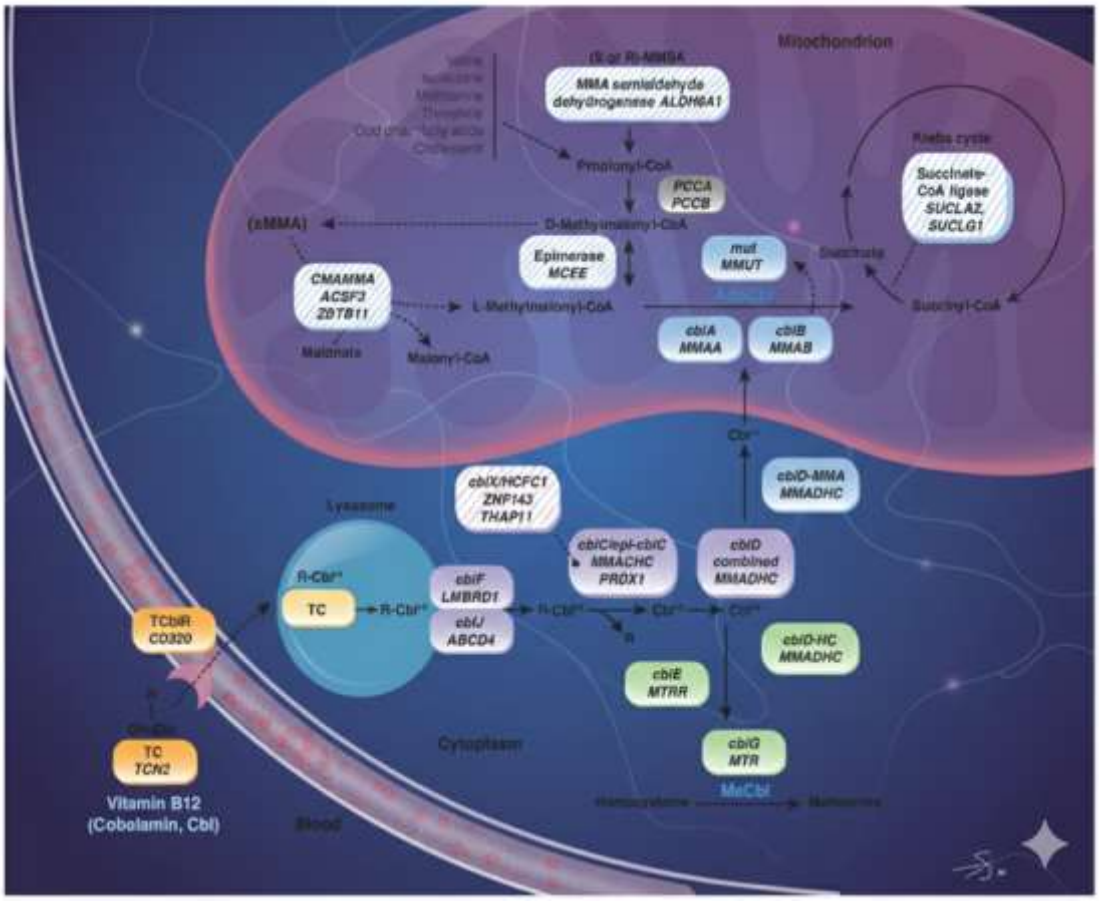

Figure 1.1: Major Pathway of the Conversion of Propionyl-CoA Into Succinyl-CoA.

(Modified from Manoli et al., 2016)

Mitochondrial branched-chain amino acid and acyl-CoA genes have been in the spotlight as critical to the maintenance of energy homeostasis and redox balance. *PCBB* and *IVD* encode the enzymes in the mitochondria that regulate the key processes in these metabolic pathways, yet the contributions of these enzymes have not been experimentally recapitulated in metastatic prostate cancer. The absence of laboratory-based expression data of these genes in models of metastatic prostate cancer is an enormous gap in the knowledge, and hence limits the understanding of metabolic mechanisms contributing to treatment resistance.

1.2 AIM OF THE STUDY

The primary goal of the study will be to analyze how the expression level of the mitochondrial metabolic genes *PCBB* and *IVD* differs in cases of metastatic prostate cancer through the quantitative molecular techniques. The research will attempt to determine whether the aggressive disease behavior is associated with the dysregulation of these genes by examining their relative expression in prostate cancer cell lines that are representative of the various phenotypes of metastasis and responses to androgens.

In addition to that, the research will contextualize the changes in gene expression within the larger context of the progression and resistance to chemotherapy in prostate cancer, as expressed in the recent literature in the field of molecular and clinical biology. The research is expected to provide a mechanistic insight into the probable role of mitochondrial metabolism in metastatic prostate cancer through a combination of experimental results and the existing biological concepts.

1.3 IMPORTANCE OF THE STUDY

The molecular essence of the metastatic prostate cancer is significant to improve the prognostic evaluation and decision-making in therapeutic areas. Genetic susceptibility, androgen signaling dysfunction and malfunction of DNA repair processes have long been studied (Vietri et al., 2021).

The ATP production, oxidative stress, and apoptotic sensitivity processes- processes, which cause direct effects on cancer cell survival to stress cases induced by chemotherapy, are regulated by the mitochondrial metabolic enzyme. Given that this is prostate cancer that is in its advanced stage, such pathways have been linked to dysregulation that is correlated

with heightened tumour plasticity and resistance to cytotoxic agents (Sayegh et al., 2022). There are, however, not many experimental tests that prove certain mitochondrial metabolic genes.

The research will provide the quantitative data of *PCBB* and *IVD* in PC metastasis models to introduce new laboratory-based data into an area of research where bioinformatics and pathway research have largely conformed. The findings are applicable in the process of identifying the metabolic biomarkers of aggressive disease and provide an underpinning for future studies that dwell on the mitochondrial metabolism as a treatment.

1.4 RESEARCH GAP

Still, even after the tremendous progress of characterizing the genomic and molecular localization of prostate cancer, gaps remain in the relevance of the mitochondrial metabolic regulation towards developing the disease and therapeutic reaction. Recent articles highlight how metabolic and mitochondrial pathways play a role in the development of advanced prostate cancer, with most works covering global metabolic signatures, but not the role of single genes (Mirzaei et al., 2022).

One of the pathways, the mitochondrial amino acid metabolism, involves both *PCBB* and *IVD*, and both are involved in the pathways that regulate cellular energy balance and oxidative stress. Even though these genes are biologically important, they have not been directly addressed in metastatic prostate cancer, and no such experimental evidence has been presented to demonstrate that the expression of these genes is correlated with aggressive phenotypes. The existing literature consists largely of indirect correlations made on the basis of transcriptomic or pathway-wide studies, and has not been verified on a functional or quantitative basis.

This research gap will be addressed in this research paper, which will generate experimentally acquired qRT-PCR data on *PCBB* and *IVD* expression of metastatic prostate cancer cell models. The particular value of the study to the outcomes of the research on mitochondrial metabolic regulation of prostate cancer progression and treatment resistance lies in demonstrating the gene-specific alterations of the expression and the potential biological value of these alterations.

2. GENERAL INFORMATION

2.1 PROSTATE CANCER AND ITS CLINICAL SIGNIFICANCE.

The clinical significance of prostate cancer (PCa) is that it is very heterogeneous, with low-growing and minimally invasive treatment tumours on the other hand and aggressive tumours that may result in metastasis and death. The artificial variability of the effect is a serious diagnostic and therapeutic issue that has to be emphasized several times in modern literature and the development of complex risk stratification systems. There is the urgency of the need to distinguish between clinically significant prostate cancer (csPCa) and the low-risk disease following the impossibility of prostate-specific antigen (PSA) to be a specific screening technique. They hold that new biomarkers, including genomic and molecular signatures, are promising in the area of improvement in the diagnostic accuracy, and they also observe that, as a discipline, much of the biomarkers have not been adequately validated to be applied in daily clinical practice, which points to a gap in the translation of the discoveries to daily clinical practice (Eyrich et al., 2021).

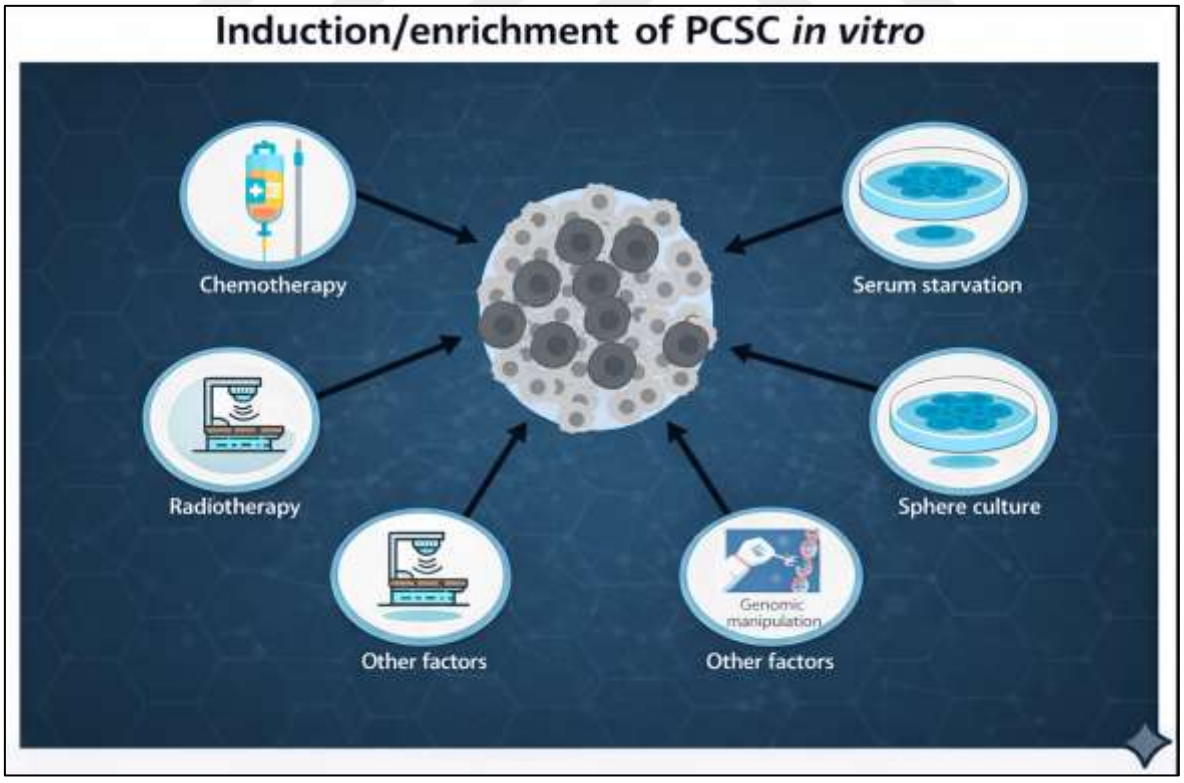

Figure 2.1: Differentiation of PC Cell Lines to PCSCs or Stem-like PC Cells by Chemotherapy.

(Modified from Yang et al., 2021)

In order to supplement this diagnostic point of view, modern paradigms in the diagnosis and treatment of prostate cancer are based on the combination of approaches, specifically, the use of both imaging (in the case of multiparametric MRI) and biomarkers and risk-adapted treatment (Williams et al., 2022). Their review demonstrates that multiparametric MRI is clinically useful to reduce overdiagnosis and overtreatment, it is focused more on clinical decision-making on the systems level rather than molecular innovation. This analogy reveals one of the key paradoxes of the literature: whether the creation of molecular biomarkers in the treatment of prostate cancer or better integration of the current diagnostic models through the improved use of the clinical tools should be regarded as the top priority.

In one of the molecular aspects, it is discussed genetic malpractices in the repair of DNA, and specifically on DNA genes such as BRCA1 and BRCA2 or ATM. Their research lays stress on the idea that they are not just what has indirectly contributed to the aggressiveness of the disease, but they also have therapeutic implications for the responsiveness to PARP inhibitors. This molecular interpretation adds greater importance to the argument that clinical management of prostate cancer extends beyond the detection stage to a one-to-one treatment plan. However, despite the convincing arguments that genetic defects can be treated with specific therapies, they themselves admit that these defects affect only a part of the patients and not the entire population, and thus cannot be applied to all patients (Ghose et al., 2021).

The conceptual framework provided by the research is more expansive since it concentrates on the biological and clinical complexity of prostate cancer, including tumour microenvironment interactions, metabolic reprogramming, as well as treatment resistance (Wasim et al., 2021) . Their synthesis is key since it makes use of both molecular, cellular and clinical evidence to provide a case that the development of prostate cancer cannot be described based on single pathways or biomarkers. However, at the cost of the depth of separate mechanisms, although it is a trade-off between the breadth and specificity of the literature, compared to the more limited scans (Eyrich et al.,2021; Ghose et al., 2021).

The epidemiological and etiological factors in the population-based view, which substantiate the clinical burden of prostate cancer on a global scale and imply age, ethnicity, genetics, and lifestyle as some of the key contributors to the risk of the disease. Their outcomes placed the molecular and clinical research into the large-scale context of the public health

frameworks (Al-Ghazawi et al., 2023). However, as a narrative review, their article is excessively reliant on secondary data and lacks an explicit analysis of methodological disagreements among epidemiological research, which limits their capacity to inform particular clinical interventions.

All these studies have culminated in one, which is the fact that the clinical significance of prostate cancer is its heterogeneity, the existence of diagnostic ambiguity, and also variable behavior to therapy. Even though there have been more advances in the area of biomarkers, imaging, and molecular genetics, validation, availability, and integration in clinical practice remain inconsistent. The literature hence warrants the need to conduct additional research that can fill the gap or transfer between molecular knowledge and clinical application, particularly in aggressive/metastatic diseases, and the necessity to embrace multidisciplinary modalities with the aim of promoting patient prognosis.

2.2 THE METASTASIS IN PROSTATE CANCER.

The most challenging stage of this disease, which has a poor prognosis, is not responsive to treatment, and is extremely affected by morbidity, is metastatic prostate cancer (mPCa). As a matter of fact, it is always known today that metastatic progression is a biological and clinically independent phenomenon and not a continuation of local disease. The summary of the modern treatment options presented by current research regarding metastatic prostate cancer is appropriate, as it addresses the ever-increasing treatment options that include androgen deprivation therapy (ADT), chemotherapy, androgen receptor pathway inhibitors, radiopharmaceuticals, and novel targeted therapies (Posdzich et al., 2023). Their review highlights the treatment progress, but at the same time, it also highlights a key limitation to the treatment because, despite a variety of treatment options currently available, metastatic prostate cancer is incurable, and treatment only prolongs its life but never achieves a lasting effect of managing the disease.

The recent clinical advances in the treatment of metastatic prostate cancer supports this weakness (Sayegh et al., 2022). Their contribution indicates the trend of treatment intensification in the hormone-sensitive and castration-resistant setting, and these tendencies are supported with the help of large randomized trials. However, despite the improved

survival rates in combination therapies, it is also mentioned that mechanisms of treatment are becoming more complicated, and toxicity accumulates (Sayegh et al., 2022).

The biological heterogeneity of metastatic prostate cancer at a more resolved level through the use of single-cell RNA sequencing (scRNA-seq), which is used to analyze the lymph node metastases. In their discoveries, they demonstrate the strong heterogeneity of the tumor microenvironment and an immunosuppressive environment of metastatic dissemination. This article offers good mechanistic information about metastatic behavior, showing that not only tumor-specific changes but also extensive immune remodeling are involved in the progression of metastases. But scRNA-seq can provide high-resolution data; the limitation of scRNA-seq lies in the few samples that the study uses to limit it to a small group of patients (Xin et al., 2023).

Accordingly, the targeting of metastasis-related molecular drivers, proving that tumour growth and metastasis in prostate cancer models can be inhibited with engineered exosomes by inhibiting SIRT6. This paper presents a very strong preclinical investigation between metastatic potential and epigenetic control. Nonetheless, it is unclear how it can be translated to clinical practice, with results obtained mostly in vitro and in animal models. Comparing it to the clinical trials, there has always been a gap between mechanistic discovery and clinical use in studying metastatic prostate cancer (Han et al., 2021).

It can be seen as a standard in clinical evidence because it highlights that darolutamide can significantly increase the overall survival of patients with metastatic hormone-sensitive prostate cancer. This is a strong study since it is a large, randomized controlled trial printed in a high-impact journal, which offers substantial credence to treatment intensification strategies. However, despite this historic trial, a major obstacle can be seen: better survival does not mean curing, and resistance is bound to happen. In that way, although It promotes the standard-of-care treatment, the biological factors that lead to the ultimate treatment failure are not mentioned (Smith et al., 2022).

Lastly, the results of metastatic prostate cancer in context through population-based cohort analysis of the causes of death. According to their results, prostate cancer itself has been the leading cause of mortality despite the developments in systemic therapy. This epidemiological finding supports the severity of the clinical metastatic disease, and it

highlights the low effectiveness of the current therapies to eradicate the disease in the long term. The study is, however, limited in terms of mechanistic insight due to its retrospective nature, which is unable to explain changing strategies in therapy outside the boundaries of the research (Elmehrath et al., 2021).

All these studies, as a whole, exhibit that metastatic prostate cancer is characterized by the complexity of treatment, the biological heterogeneity, and relentless mortality. Clinical trials have enhanced survival rates, preclinical studies have shown some promising molecular targets, but there is still a need to integrate biological knowledge and long-term clinical benefit. This has served as a reminder of the fact that there is a gap in research between the molecular mechanisms, including changes in gene expression and clinically relevant results in metastatic disease.

2.3 THE ROLE OF GENES IN PROSTATE CANCER

Genetic changes are of primary importance in the onset, progression, and response of prostate cancer and, as a result, produce the pronounced heterogeneity in the disease. Modern literature has continuously shown that prostate cancer cannot be analyzed using single-gene principles but rather based on intricate interactions of hereditary susceptibility, somatic mutations, epigenetic control, and transcriptomic reorganization. This review provides a general overview of the genetics landscape of prostate cancer, outlining common somatic alterations such as PTEN deletion, TMPRSS2–ERG fusion, and mutations in DNA repair genes. Although the review is a successful summary of the existing knowledge on genetic drivers, it is so broad that it does not allow a critical examination of how particular genetic changes can be converted into clinical decision-making, which once again highlights a systematic shortcoming of broad narrative reviews (Sekhoacha et al., 2022).

The presence of inherited determinants of prostate cancer is discussed more critically by, both of which highlight the clinical significance of the germline alterations in the genes BRCA1, BRCA2, ATM, and HOXB13. Vietri et al. pay attention to the consequences of hereditary prostate cancer in terms of targeted therapy and prevention, with special emphasis on the therapeutic importance of the DNA repair deficiencies. In their turn, it is a more population-genetic approach, critically evaluating the level of penetrance, variable expressivity and the issues associated with predicting risks in carriers. The combination of

these studies is complementary: The opportunities of the translational approach, but Ni Raghallaigh and Eeles warn against overgeneralization because of the absence of full knowledge on the gene-environment interaction and unstable clinical outcome of carriers of mutations (Vietri et al., 2021; Ni Raghallaigh & Eeles, 2022).

Beyond the information on protein-coding genes, a new element to the field of prostate cancer biology, namely, long non-coding RNAs (lncRNAs). Their detailed discussion attributes dysregulation of lncRNAs to pathways that are related to proliferation, metastasis, and resistance to therapy, which reveals the broadening of genetic possibilities beyond the well-known oncogenes and tumor suppressors. Nevertheless, the study heavily depends on bioinformatic and correlative data, though it is based on pathway analysis, and very little validation of its functionality is given. The limitation is indicative of a more general problem in transcriptomic research, in which clinical utility can too frequently be outpaced by molecular discovery (Mirzaei et al., 2022).

The growing complexity of prostate cancer genetics with genetic alterations placed in the framework of precision medicine. In a review, the authors combine the data of genomic, epigenomic, and transcriptomic studies to explain the way that molecular stratification may inform personalized therapy. A more modern synthesis of the knowledge compared to the previous reviews, which can be proposed to agree with the genetic findings and newly developed clinical applications. However, they go further to state that a lot of the suggested molecular biomarkers have not been prospectively validated, and this is the disconnect between the prospects of precision medicine theory and practice (Maekawa et al., 2024).

Current study reintroduces clinical relevance but with the main emphasis on the metastatic prostate cancer management, which the authors emphasize the relevance of genetic profiling in making a therapeutic choice, especially in the case of advanced disease. Their piece shows that the treatment choice is becoming more and more affected by molecular changes, particularly the changes in DNA repair pathways. This review has, however, been restricted by the clinical emphasis and, therefore, has not been able to venture into the upstream genetic mechanisms as a tool, but not a biological driver in their context (Sayegh et al., 2022).

Taken together, these studies indicate a great agreement that genetic manipulations form the basis of heterogeneity of prostate cancer and affect disease progression. Nevertheless, they

also demonstrate severe drawbacks: excessively associative data usage, lack of functional validation, and difficulties in reducing the genetic complexity into the conventional clinical practice. The literature underpins a change towards integrative strategies involving a combination of genomic, transcriptomic and clinical information even in advanced and metastatic disease. This overview reveals the importance of focused studies of gene expression to determine the functional and clinical significance of individual genes as a part of the complicated molecular environment of prostate cancer.

2.3.1 *PCBB* and *IVD* of Metastatic Prostate Cancer

The role of *PCBB* and *IVD* gene in the metastatic prostate cancer should be placed in the framework of cancer metabolism, metastatic phenotypes, and new emerging molecular drivers of aggressiveness. The *PCBB* and *IVD*, unlike well-characterized oncogenes or DNA repair genes, are not traditionally considered major prostate cancer genes, which is another significant gap in knowledge of the existing literature. The results of studies on prostate cancer that specifically target these genes are not easily available, which means that critical evaluation of their potential relevance is necessary in the context of their potential relevance, and not conclusive mechanistic interpretations.

A study does not explicitly examine *PCBB* or *IVD* on a molecular or genetic scale; rather, the article addresses the problem of optimization of real-time verification during high-dose-rate prostate brachytherapy. Although not mechanistically informative, this research has an indirect applicability since it highlights the growing clinical focus on precision and optimization of treatment in prostate cancer. Its insertion points to one shortcoming of the existing field: the technological and therapeutic updates have advanced more rapidly than the molecular knowledge of the less widely studied metabolic genes. Therefore, although the work by Ambrosi can lead to the enhanced delivery of clinical services, it does not focus on the biological factors that ensure metastatic development, and thus, supplementary molecular research is essential (Ambrosi., 2022).

A biologically pertaining understanding of the aggressiveness of prostate cancer by showing that inorganic pollutants that persistently manifest promote invasive and aggressive phenotypes. *PCBB* and *IVD* are not directly discussed, but this work is essential to putting the metabolic dysregulation in prostate cancer into context. The authors demonstrate that

environmental stressors reprogram cellular metabolism and tumor-related signal transduction. Such a discovery is specifically applicable to the case of *PCBB* and *IVD*, which are both linked with the work of mitochondrial and metabolic processes. Nevertheless, a more attention to signaling pathways and phenotypic consequences, neglecting the role of certain metabolic enzymes and genes. This does not allow direct attribution but goes hand in hand with the postulation that metabolic genes can be involved in metastatic behavior (Buñay et al., 2024).

An article employs a pan-cancer approach to identify prognostic metastatic phenotypes in different malignancies, such as prostate cancer. Their comparison upholds common molecular and phenotypic features of metastasis, which argue in favor of the concept that metastatic development involves common biological programs and not cancer-type-specific mutations exclusively. Even though there is no specific mention of *PCBB* and *IVD*, the strength of the study is that it is based on cross-cancer metabolic and transcriptional signatures related to metastasis. This pan-cancer perspective is useful in supporting the fact that even the metabolic genes which are not characteristic of prostate cancer may be contributory factors in metastatic competence. However, the extent of research limits the prostate cancer-mechanical resolution (Zaorsky et al., 2022).

A combination of these studies suggests that direct experimental or clinical evidence to validate the association between *PCBB* and *IVD* and metastatic prostate cancer is apparently lacking. This omission does not render their relevance defensive; instead, it creates a major gap in the literature. Recent research on prostate cancer has prioritized androgen signaling, DNA repair and immune modulation over metabolic genes, which can be engaged in mitochondrion functioning, cellular energy contents and establish metastatic niches. The indirect data of the works on the accuracy of cure, environmental interference with the metabolism and metastasis pan-cancer phenotypes point to the fact that *PCBB* and *IVD* are the genes to be studied in particular.

More to the point, the literature has not yet established the cause or the effect of the metastatic progression on the altered expression of *PCBB* and *IVD*. The outcome of such uncertainty is the fact that specific gene expression studies in metastatic prostate cancer tissues are required that determine functional relevance. Contextually, it is evident that the

present research has been supported by contextual evidence as opposed to direct evidence to testify to the scientific justification of the present study.

3. METHODOLOGY

3.1 CELL CULTURE PROCEDURES

Cell culture techniques are essential for providing constant and reproducible experimental conditions for molecular and genetic analyses. The usage of two widely recognized human prostate cancer cell lines, namely PC3 (CRL-1435™) and LNCaP (CRL-1740™), was made. Such models characterize different phenotypic and molecular subtypes of prostate carcinoma: PC3 cells are androgen-independent and sourced from the metastasis to bone, while LNCaP cells are androgen-sensitive and came from the metastasis to the lymph node (Kaighn et al., 1979; Horoszewicz et al., 1983). The two cell lines together give researchers an opportunity to study the differences in the expression of genes that are related to the regulation of the metastatic potential and molecular control, among others.

3.1.1 Removal of Cell Cultures From Stock

The frozen vials of the PC3 and LNCaP cells were taken out from storage at −80°C and carefully thawed in a water bath at 37°C until only a small crystal of ice remained. Rapid thawing ensures that cryo-induced osmotic stress is kept to a minimum and that the cell membrane remains intact. The resulting cell suspension was then poured into a sterile 15mL conical tube containing 5mL of RPMI-1640 medium (Gibco, USA) which was pre-warmed, supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, USA) and 1% penicillin–streptomycin (Thermo Fisher Scientific, USA) to neutralize residual DMSO. After that, centrifugation of the tube at 800 rpm for 5 minutes was done to form the cell pellet. Following the careful removal of the supernatant with a pipette, 1mL of fresh complete medium was put to the cell pellet to resuspend it. Then the suspension was moved to a 25 cm^2 culture flask that had 5mL of complete medium and was incubated at 37°C with a humidified atmosphere of 5% CO_2. This whole process resulted in the recovery of cells to the flask surface that were viable and had optimal adherence (Freshney, 2016).

3.1.2 Cell Line Used

The PC3 and LNCaP cell lines were grown in RPMI-1640 medium, which is suitable for the metabolic needs of carcinoma cells of epithelial origin. The medium was supplemented with 10% heat-inactivated FBS to provide the required growth factors and hormones and also

with 1% penicillin–streptomycin to avoid bacterial contamination. This mix of nutrients and antibiotics creates an environment that is ideal for the maintenance of growth characteristics that are physiologically typical. All the manipulations of the cultures were done in the laminar flow biosafety cabinet under sterile conditions to ensure the maintenance of asepsis.

3.1.3 Medium Change

The replacement of the medium on a regular basis is essential to keep the pH, the nutrients, and the metabolic waste products at the right levels. The moment the cells were firmly connected to the surface of the flask and showed good morphology under the inverted microscope, the spent medium was taken out with the help of a sterile pipette. The monolayer was then gently washed with phosphate-buffered saline (PBS, pH 7.4) to remove serum and non-adherent debris. When working with 25 cm^2 flasks, 2 mL of PBS to make sure that the washing was sufficient. After the PBS was removed, fresh complete medium that had been pre-warmed was added 5 mL for 25 cm^2 flasks. The cultures were then taken back into the incubator at 37°C and 5% CO_2. The medium was changed every 2-3 days based on the confluence of the cells, in order to keep the growth kinetics stable and stress on the cells to a minimum (Masters, 2000; Riss et al., 2019).

3.2 CELL CULTURE PASSAGING

Subculturing or passaging is a principal method to maintain populations of cells that are actively growing and genetically uniform. Continuous cell lines such as PC3 and LNCaP have to be subculture regularly, and otherwise, over-confluence, nutrient depletion and pH changes will occur that might influence the cells' morphology and behavior (Freshney, 2016). The passage of cells was done in this research under sterile conditions and with the help of the standard enzymatic detachment and resuspension technique to ensure both high viability and uniform growth.

Once the cell monolayer was about 80–90% confluent, the spent media was aspirated very carefully with a sterile pipette so as not to disturb the adhered cell layer. The cells were washed with 2 mL of Dulbecco's phosphate-buffered saline (DPBS, pH 7.4)—for 25 cm^2 flasks—once to get rid of serum that was left and that could influence trypsin activity. After washing, a 0.05% trypsin-EDTA solution (Gibco, USA) that was pre-warmed to 37°C was

added (1 mL for 25 cm² flasks) to make sure that the culture surface was fully covered. The flasks were kept in a 37°C, 5% CO_2 atmosphere for about 5 minutes until the cells started to detach, which was confirmed through inverted microscope observations. Gently tapping the flask helped free the remaining attached cells without exerting mechanical stress on them.

As soon as all the cells were detached, the neutralization of the enzymatic reaction was done by adding 10 mL of complete RPMI-1640 medium with 10% FBS. By stopping trypsin activity, membrane damage was consequently prevented. The resulting cell suspension was then put into a sterile 15 mL centrifuge tube, which was subsequently centrifuged at 800 rpm for 5 minutes at 21°C to get the cell pellet. Afterward, the supernatant was discarded, and the cell pellet was resuspended with care in 1 mL of fresh medium. The cells were then transferred to new culture flasks generally at a 1:3 to 1:5 split ratio, depending on the cell density to be given the ideal surface area for further growth. A fresh complete medium (5 mL for 25 cm² flasks) was added and the flasks were placed back for incubation at 37°C and 5% CO_2.

This passaging procedure was carried out every 2–3 days or according to the growth rate of the respective cell line. PC3 cells were more proliferative and less contact inhibited, so they required more frequent subculture than LNCaP cells, which have slower doubling times and higher sensitivity to handling. By ensuring consistent passing intervals and gentle mechanical manipulation, both lines' viability and genetic fidelity were preserved. Keeping cell cultures within their logarithmic growth phase is vital for reproducible molecular results; otherwise, prolonged confluence or stress might trigger unwanted differentiation or gene expression changes (Riss et al., 2019; Hughes et al., 2007).

3.3 COUNTING CELLS WITH A HEMOCYTOMETER

The precise measurement of live cells is obligatory for the upkeep of culture uniformity and the delivery of dependable experimental results in molecular analyses. The live cell count allows the experimenters to determine the seeding density for the subsequent assays of RNA extraction or transfection standardization (Strober, 2015). In this work, the trypan blue dye exclusion assay combined with a Neubauer hemocytometer was utilized to report both living and dead cell counts for PC3 and LNCaP cultures before the RNA isolation process.

After the detaching process, which was part of the subculturing, 10 µL of cell suspension was mixed with an equal volume of 0.5% trypan blue solution (10 µL) and left for 2 minutes at room temperature (Gibco, USA). Then, through capillary action, the mixture was gently introduced into the hemocytometer chamber. The trypan blue dye has the capability to selectively go through the membranes of dead cells that are already compromised and these dead cells are blue when viewed under the microscope, on the contrary, the live cells do not take the dye and are thus colorless (Stoddart, 2011). This technique enables the fast and easy distinguishing of living cells from dead ones without affecting the cell morphology significantly.

Cell counting was achieved by using a light microscope with 10× magnification and recording the number of living cells in five big squares of the hemocytometer grid. The concentration of viable cells per milliliter was calculated according to the following formula:

$$Cell\ concentration\ \left(\frac{cells}{mL}\right) = \frac{Number\ of\ viable\ cells \times 10^4}{Number\ of\ squares\ counted} \times Dilution\ factor \quad (3.1)$$

The dilution factor compensates for the equal volumes of cell suspension and trypan blue mixed, which is usually 2. The constant value of 10^4 is used to convert the volume of the chamber to one milliliter, considering that each large square corresponds to a volume of 0.1 mm^3 (0.0001 mL). This method provides a highly precise determination of the concentration of live cells in the cell suspension. The retrieved amount was utilized to establish the cell densities for seeding and to maintain the same number of cells in different experimental replicates of PC3 and LNCaP cell lines.

Regular viability checks helped to conduct all the analyses on the cell populations that were physiologically healthy and thus, the RNA quality wasn't affected by the confounding factors of cell death or over-confluence. The trypan blue assay was regularly used in cell culture research, and it was considered a widely accepted and validated approach for viability assessment because it is simple, cost-effective, and reproducible (Louis & Siegel, 2011; Riss et al., 2019).

3.4 RNA EXTRACTION

The extraction of high-quality RNA is the major prerequisite for the proper analysis of gene expression, since the purity and integrity of RNA govern successive applications like reverse transcription and quantitative PCR (Rio et al., 2010). In the current research, total RNA was extracted from prostate cancer cell lines PC3 and LNCaP using the TRIzol reagent technique, which is a mixture of phenol and guanidinium isothiocyanate that helps to break down the cells and concurrently preserve the RNA integrity during the extraction process. All the steps were done under the RNase-free conditions in order not to let the RNA degrade.

The cells growing to about 80 to 90 percent confluence were collected by sucking out the culture medium and washing with phosphate-buffered saline (PBS, pH 7.4) once to get rid of any serum. Then, TRIzol reagent (Thermo Fisher Scientific, USA) at a rate of 1 mL for every 1 million cells was poured up to the culture flask with the monolayer sandwiched right under it. The flask was gently shaken such that the cell lysis was done completely and the cell lysate was put into sterile RNase-free microcentrifuge tubes. The samples were given a 5-minute incubation at room temperature so that the nucleoprotein complexes dissociated completely.

Phase separation was carried out by adding chloroform (Merck, Germany) at a rate of 0.2 mL per 1 mL of TRIzol used. After that, there was a vigorous shaking for 15 seconds. The acidified samples were then incubated at room temperature for 3 minutes and afterward centrifuged at 12,000 × g for 13 minutes at 4°C (Figure 3.1). As a result of the procedure, the mixture was separated into three layers: the lower layer being red due to the phenol-chloroform phase and containing the proteins, the middle layer that is of DNA, and the upper layer which is clear and aqueous that contains the RNA. The clear aqueous layer was gently pipetted out and transferred to a new RNase-free tube while being careful not to disturb the middle layer.

Figure 3.1: Refrigerated Centrifuge.

For RNA precipitation, 0.5 mL isopropanol was added to every 1 mL of TRIzol reagent used, then samples were mixed gently and left to incubate for 10 minutes at room temperature. After conducting another centrifugation at 12,000 × g for 10 minutes at 4°C, a white or translucent RNA pellet appeared at the bottom of the tube. The supernatant was then removed, and the pellet was rinsed with 1 mL of 75% ethanol in order to get rid of salts and organic impurities left over from the previous steps. A quick vortex and a 7,500 × g centrifuge for 5 minutes at 4°C followed, the ethanol was removed, and the RNA pellet was air-dried in the room for 5-10 minutes so as not to dry too much because drying could hinder solubilization. The RNA was then made soluble in RNase-free distilled water at an approximately volume of 80 μL for PC3 and 60 μL for LNCaP samples and kept at -80˚C until further processing.

The spectral purity and concentration of the RNA were determined through NanoDrop spectrophotometer measurements of absorbance at both 260 and 280 nm wavelengths. A260/A280 ratios of the samples lying in the range of 1.8 to 2.1 were regarded as the criteria of being sufficiently pure for further applications (Wilfinger et al., 1997). The intact RNA of high quality from both PC3 and LNCaP cell lines was immediately used for cDNA synthesis.

The method of TRIzol-based extraction still occupies the position of a gold standard in molecular biology as the most effective, scalable, and universal means that yield total RNA recovery inclusive of small RNAs from various biological sources (Chomczynski & Sacchi, 2006). The application of this method yielded very high amounts of intact RNA that were suitable for quantitative expression analysis of the target genes in metastatic prostate cancer models.

3.5 CDNA SYNTHESIS

The synthesis of complementary DNA (cDNA) from purified messenger RNA take place in an instant and through that, the quantitative PCR analysis is based on this stable DNA template. The extraction of total RNA from PC3 and LNCaP prostate cancer cell lines and subsequent cDNA creation using the High-Capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, Cat. No. 4368814) made the basis of the current research. The method consists of the manufacturer's protocol slightly altered to ensure the yield and quality are consistent.

The 20 µL master mix of RT enzyme was prepared on ice for each reaction. The master mix was composed of 2 µL of 10× RT buffer, 0.8 µL of 25× dNTP mix (100 mM), 2 µL of 10× random primers, 1 µL of MultiScribe™ reverse transcriptase (50 U/µL), and 4.2 µL of nuclease-free water, plus 10 µL of template RNA. The input RNA for each reaction was set at 2 µg. In order to have equal input across all the samples measured, RNA concentrations were obtained via NanoDrop spectrophotometry and specific volumes of RNA and RNase-free water were adjusted per sample.

Mixing the components of the reaction was done in sterile, nuclease-free tubes and to make sure the mixture was homogeneous, it was gently mixed prior to centrifugation, which would bring the contents to the bottom. Thermal cycling was done with a Biorad T100 Thermo Cycler (Figure 3.2) under the specified conditions as indicated in Table 3.1.

Table 3.1: Thermal Cycle Program.

Settings	**Step 1**	**Step 2**	**Step 3**	**Step 4**
Temp	25 °C	37 °C	85 °C	
Temp	10 min	120 min	5 min	Hold

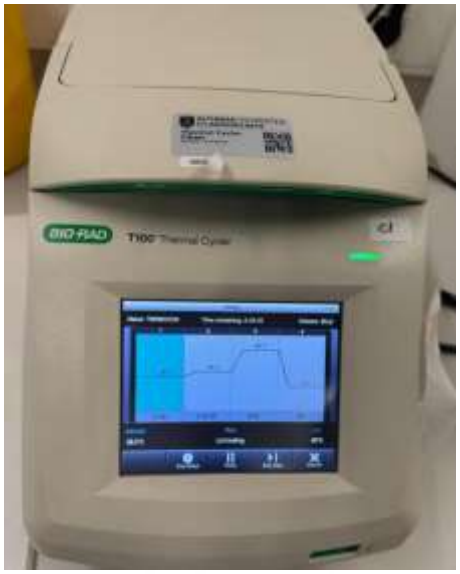

Figure 3.2: Thermal Cycler.

Once done, cDNA was stored at -20 °C until amplification. The reaction conditions were optimized so that there would be total reverse transcription and the RNA secondary-structure interference would be minimized. The High-Capacity kit utilizes both oligo(dT) and random hexamer primers, thus permitting the transformation of the total RNA populations, including the partially degraded transcripts, into representative cDNA libraries (Gibson et al., 1996) to be done efficiently.

The samples were immediately available for the subsequent quantitative PCR focusing on the *PCBB* and *IVD* genes, which guaranteed high fidelity and reproducibility of the template throughout the experiment's runs.

3.6 PRIMER PREPARATION AND HANDLING

Specific primer pairs were designed and validated for each target and reference gene to guarantee amplification specificity and efficiency. The primer sequences applied in this work were fabricated from the published mRNA sequences of the corresponding genes and checked for the non-existence of secondary structures or dimer formation using NCBI Primer-BLAST. The forward (F) and reverse (R) primer sequences for *PCBB*, *IVD*, and the reference gene *HPRT* are illustrated in Table 3.3.

Table 3.2: Primer Sequences Used for qRT-PCR Analysis.

Gene	Primer Sequence (5′–3′)	Direction
PCBB	AGGCAGTCTGTCAGGAGCACAT	Forward (F)
PCBB	CCAGAGTCATTCAGCCCAATCAC	Reverse (R)
IVD	ACACCATTCCCTACCTGCACGT	Forward (F)
IVD	ACATACTGCCGACACGCCATGA	Reverse (R)
HPRT	TGACACTGCAAAACAATGCA	Forward (F)
HPRT	GGTCCTTTTCACCAGCAAGCT	Reverse (R)

3.7 REAL-TIME POLYMERASE CHAIN REACTION (QRT-PCR)

The quantitative real-time polymerase chain reaction (qRT-PCR) is a method that is very sensitive and specific for determining the levels of gene expression through the detection of the corresponding DNA (cDNA) amplification in real-time. For this reason, qRT-PCR was used in the present study to measure the relative expression levels of the *PCBB* and *IVD* genes in PC3 and LNCaP prostate cancer cell lines, with normalization against the housekeeping reference genes. The amplification reactions were done using a SYBR Green-based detection system, which allows for the quantification of fluorescence proportional to the accumulation of double-stranded DNA during each PCR cycle (Bustin et al., 2009).

3.7.1 Reaction Setup

A total volume of 10 µL was prepared for each reaction in 96-well optical plates. The reaction mixture was composed of 5 µL of SYBR Green PCR Master Mix (Thermo Fisher Scientific, USA), 0.5 µL of forward primer (10 µM), 0.5 µL of reverse primer (10 µM), and 4 µL of diluted cDNA template. All components were prepared on ice to prevent nonspecific amplification, and master mixes were briefly vortexed and centrifuged to eliminate air bubbles. Negative control reactions (no-template controls, NTC) were performed for each gene to ensure the absence of contamination or primer-dimer formation.

The plates were covered with optical adhesive films and briefly spun down to make sure that all the reaction contents were at the bottom of each well. The amplification reactions were carried out on light cycler 96 roche Real-Time PCR System (Figure 3.3). The following thermal cycling protocol was followed:

a. Initial denaturation: 95 °C for 10 min

b. Amplification (40 cycles):

   i. Denaturation at 95 °C for 15 s

   ii. Annealing/extension at 60 °C for 60 s

c. Melt curve analysis: 60–95 °C, with a 0.3 °C increment per cycle to assess amplicon specificity.

The instrument software recorded the fluorescence intensity during each cycle and generated amplification plots automatically. Melt-curve analysis was used to confirm the existence of one specific amplification product, while the absence of additional peaks indicated that there were no primer-dimer artefacts (Livak & Schmittgen, 2001).

Figure 3.3: Lightcycler 96 Roche Real-Time PCR System.

3.7.2 Data Analysis

All statistical analyses were performed using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA). The results of the qRT-PCR assay were analysed using the unpaired two-tailed Student's t-test, with the purpose of comparing relative expression levels between differently treated groups. The significance of differences was determined at p-values of less than 0.05, 0.01, 0.001, and 0.0001, where appropriate. The data are shown as mean ± standard deviation (SD).

4. RESULTS

4.1 INTRODUCTION

The chapter describes the findings of the experiment that was performed due to the quantitative analysis of *PCBB* and *IVD* gene expression in human prostate cancer cells LNCaP and PC3. They were selected to reflect the various biological phenotypes of prostate cancer, with LNCaP cells being androgen sensitive and PC3 cells being a model of an androgen-independent metastatic variant. The data was also analyzed using qRT-PCR, which is a very sensitive and quantitative technique for detecting a minor change in the expression of the genes.

All the gene expression values were normalized using the housekeeping gene *HPRT*1 to correct the input variation of the cDNA and amplification efficiency. The relative differences in expressions were calculated, and the statistical significance was determined by the 2^(-ΔCt) procedure in which an unpaired two-tailed Student t-test was made of the 2^(-ΔCt). It is transparent and reproducible since the findings are in the form of standard deviation and mean.

4.2 QUALITY EVALUATION AND STANDARDS OF TEMPLATES WITH RNA

A strict check of the total RNA integrity and purity of PC3 and LNCaP cells was done prior to the downstream molecular analysis. The spectrophotometric analysis of NanoDrop has shown that the A260/A280 ratios of all the samples were within the optimal range of 1.8 to 2.1, indicating that proteins or phenolic compounds were not highly contaminated.

In order to get the desired standard comparative gene expression analysis, the cDNA generation was standardized with the mass of the RNA in the samples being equal. The reverse transcription reaction was repeated with the same 2 μg RNA amount, thereby eliminating the biasing effect of concentration by making the changes in expression as a result of the actual biological variation as opposed to technical artefacts.

4.3 ASSAY SPECIFICITY AND PERFORMANCE QRT-PCR

PCBB, *IVD* and *HPRT*1 gene amplification was performed in the best cycling conditions by qRT-PCR. The melt analysis revealed that all of the targets possessed one unique melting point, which revealed the specificity of the primer and the lack of non-specific products of the amplification. Amplification was not seen in the no-template controls, which is an indication that there was no contamination. The differences in cycle threshold (Ct) between technical replicates were low, and this is another validation of the reproducibility and accuracy of the assay.

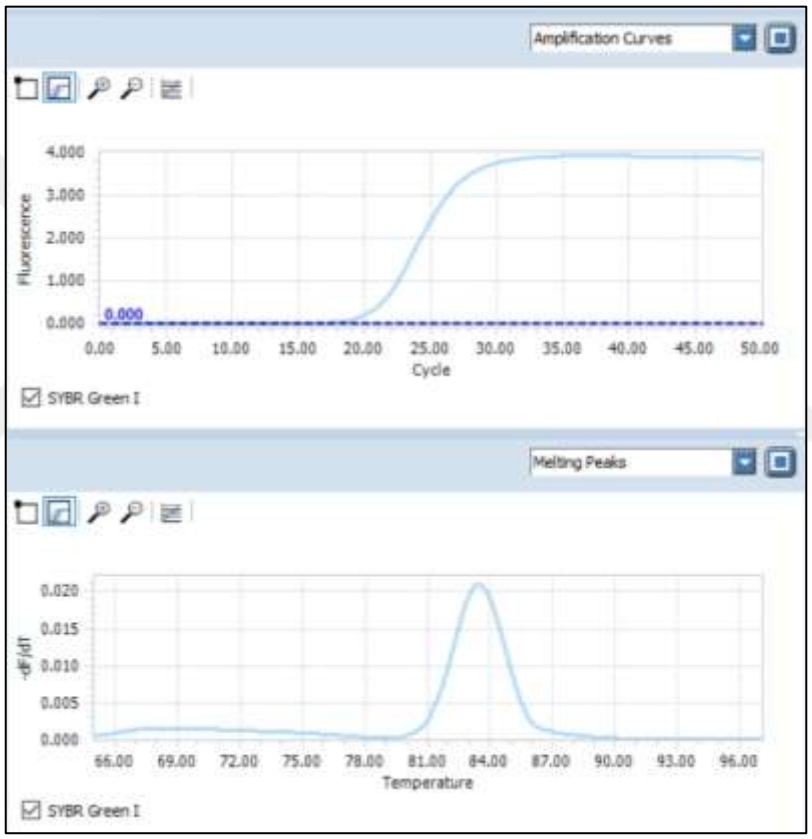

Figure 4.1: *PCBB* Gene Ct and Melting Curve.

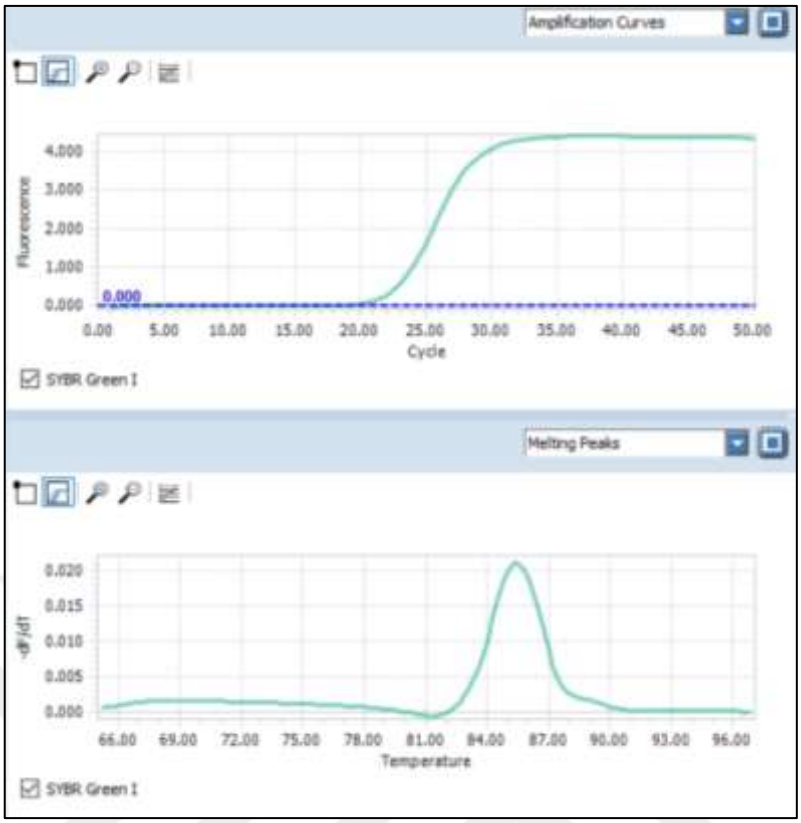

Figure 4.2: *IVD* Gene Ct and Melting Curve.

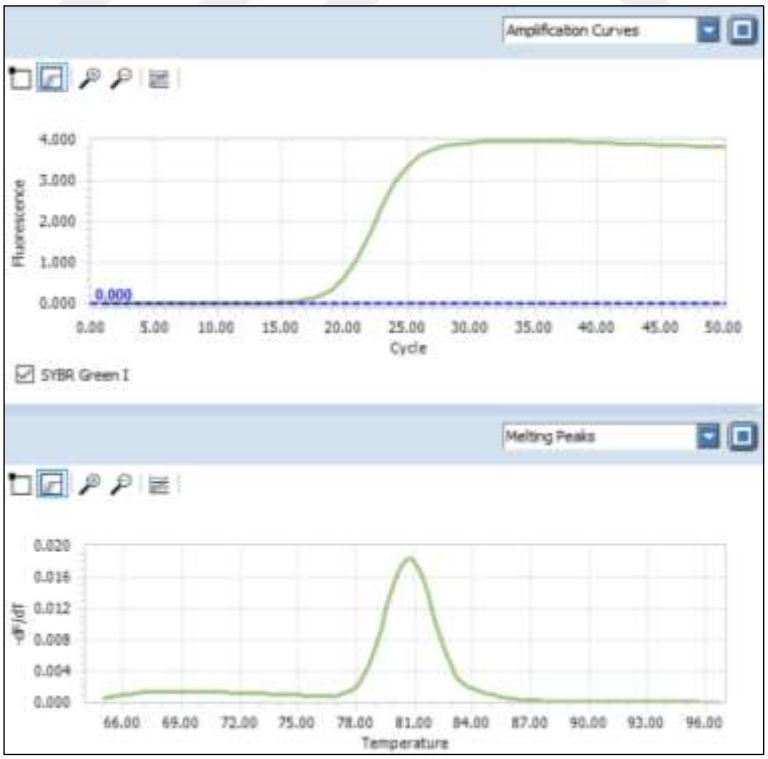

Figure 4.3: *HPRT* Ct and Melting Curve.

4.4 THE *PCBB* GENE EXPRESSION ANALYSIS

The quantitative method analysis revealed a significant decrease in *PCBB* mRNA in PC3 cells compared to the LNCaP cells. When normalized to *HPRT*1, all samples of PC3 had higher values of *PCBB* ΔCt, which shows that the transcripts were less abundant. Relative quantification of the 2^(-ΔCt) technique demonstrated that in comparison with the androgen-sensitive LNCaP control, *PCBB* was considerably down-regulated in the metastatic PC3 cell line.

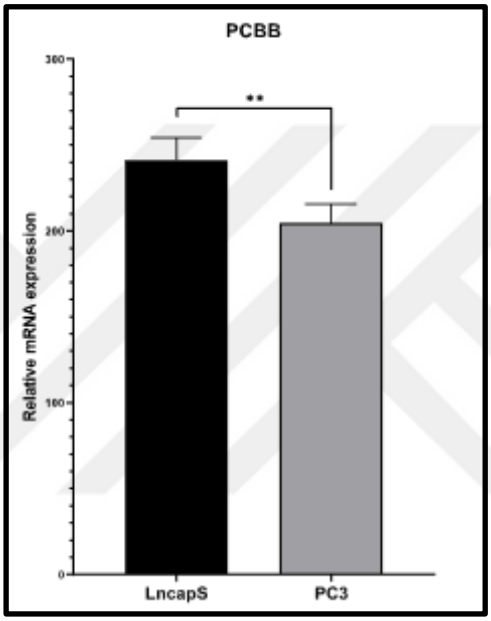

Figure 4.4: Relative mRNA Expression Levels of *PCBB* Gene in PC3 and LNCaP Cell Lines (mean ± SD, $p < 0.05$).

Unpaired two-tailed Student's t-test an unpaired two-tailed Student's t-test was used to statistically analyze the ΔCt values that showed that this decrease was significant with a p-value of less than 0.05. This would indicate a probability less than 5 per cent of the observed difference taking place by chance, the usual level of statistical significance in biological research.

The graphical display of the *PCBB* expression indicated that PC3 and LNCaP groups were distinct, with little overlapping of the expression values. This high level of difference and its statistical significance identify *PCBB* as a differentially expressed gene in the models of metastatic and non-metastatic prostate cancer cells.

4.5 THE EXPRESSION ANALYSIS OF THE *IVD* GENE

The quantitative gene expression of *IVD* revealed no significant difference between *IVD* expression in PC3 and LNCaP cell lines as compared to *PCBB*. The ΔCt normalized values of *IVD* did not significantly differ between the two cell types, and the relative expression values determined using 2^(-ΔCt) revealed that the values were close to one, which suggested an equal level of transcription.

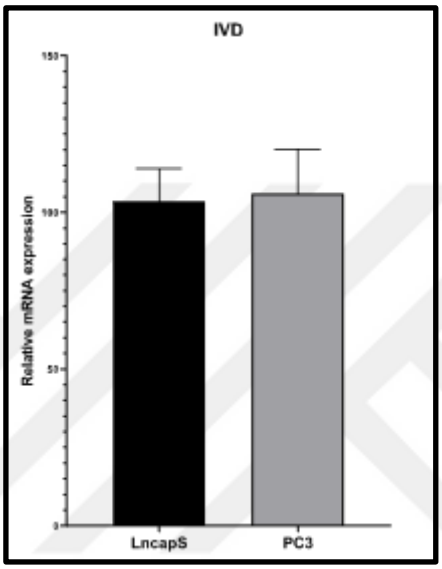

Figure 4.5: Relative mRNA Expression Levels of *IVD* Gene in PC3 and LNCaP Cell Lines (Mean ± SD, $p > 0.05$).

The statistical test did not show that the difference in the *IVD* expression was statistically significant, with a p-value of more than 0.05, and this was indicated by an unpaired two-tailed Student t-test. This result suggests that the extent of the variation of *IVD* expression lies under the experimental variation and does not show a significant biological difference in the conditions applied in the experiment.

This was also supported by the visualization of the *IVD* expression data, in that the standard of the degree of consistency of the mean and range of standard deviation of the overall measure was similar in PC3 and LNCaP samples. The observation that the expression amongst the replicates is similar is evidence that *IVD* expression does not vary throughout the subsequent prostate cancer phenotypes.

4.6 COMPARISON OF THE EXPRESSION PATTERN OF *PCBB* AND *IVD*

The *PCBB* and *IVD* expression patterns were compared, and the pattern of gene regulation is specific. *PCBB* expression was much less in metastatic PC3 cells compared to LNCaP cells, but otherwise, the *IVD* expression was similar in the two models. This deviation implies that transcriptional changes which were observed in this paper cannot be attributed to a blanket down-regulation of metabolic genes, but to specialized and gene-specific changes.

The discrepancies in the statistical outcome (significant in the case of *PCBB* and non-significant in the case of *IVD*) justify the role of particular genomic analysis of the case in the conditions of molecular heterogeneity of populations of prostate cancer cells. These findings are strong with the basis on the normalization consistency, replicate concordance, and the appropriate statistical testing.

4.7 SUMMARY OF RESULTS

The statistically significant decrease in the expression of *PCBB* of metastatic PC3 prostate cancer cells compared to the androgen-sensitive LNCaP cells is evidenced by the qRT-PCR analysis of the study. Contrastingly, *IVD* expression is consistent between cell lines as well as does not show statistically significant variation. These results were obtained in controlled experimental conditions, and normalization and statistical analysis have been validated, giving a valid quantitative basis for discussion and interpretation in the future.

5. DISCUSSIONS AND CONCLUSIONS

5.1 THE INTRODUCTION AND CONTEXTUALIZATION

The present study aimed to study the alterations in expression of the mitochondrial metabolic genes *PCBB* and *IVD* in the prostate cancer cell models that exhibit different biological phenotypes. LNCaP cells and PC3 cells served as an example of early-stage hormone-responsive cancer in the prostate that is androgen-independent and is highly metastatic, which is advanced and aggressive prostate cancer. These results indicated that the *PCBB* in PC3 cells were downregulated significantly compared to the *PCBB* in LNCaP cells without a change in the expression of *IVD*. These findings demonstrate that metabolic regulation of the genes that are associated with specific genes and context in prostate cancer is a possible phenomenon that can be employed in chemoresistance and metastatic development.

Table 5.1: Characteristic Differences between LNCaP and PC3 Cell Lines of Prostate Cancer.

Feature	**LNCaP**	**PC3**
Origin	Lymph node metastasis of prostate adenocarcinoma.	Prostate adenocarcinoma metastatic in the bone.
Androgen Receptor (AR) Status	Functional AR signalling is positive.	Negative, AR-independent
Proliferation Rate	Moderate	High
Metastatic Potential	Low	High

Table 5.1: Characteristic Differences between LNCaP and PC3 Cell Lines of Prostate Cancer (Table Continued).

PSA Secretion	Yes	None
Tumorigenicity in Mice	Forms slow-growing tumors	Very tumorigenic and aggressive tumors.
Chemotherapy Sensitivity	Moderate sensitivity	Resistant to multiple drugs
Normal ROS oxidative phosphorylation, normal ROS.	Re-programmed oxidative metabolism, increased metabolic plasticity.	The metabolism of BCAA (Branched-Chain Amino Acid) is intricate and requires a thorough explanation,Baseline catabolism
The Regulation of Reactive Oxygen Species (ROS).	Normal ROS homeostasis	Increased antioxidant and stressivation.
Epithelial/Mesenchymal Status	Epithelial-like	Mesenchymal-like, invasive
Signaling Pathways	Androgen-dependent proliferation	Androgen-independent, stimulated EMT, re-programming metabolism.
Clinical Relevance	Is an indication of early metastatic prostate cancer that is hormone-sensitive.	Symptomatic, metastatic, castration-resistant prostate cancer.

Table 5.2 contains the cellular and metabolic history of the comprehensiveness of the connection between *PCBB* and *IVD* expression profile and metastatic potential, and chemo resistance.

5.2 COMPARISON AND CONTRAST WITH THE PAST LITERATURE

5.2.1 *PCBB* Gene

The observation that *PCBB* is extremely down-regulated in PC3 cells is consistent with the new paradigm, indicating that the association of metabolic reprogramming of mitochondria with metastatic prostate cancer is established. *PCBB* is an abbreviation that refers to the expression of a beta subunit of propionyl-CoA carboxylase, a protein that participates in the metabolism of branched-chain amino acids and odd-chain fatty acids in the mitochondria. The decreased expression of PC3 cells may represent adaptive-metabolic remodeling whereby the most metastatic cells would be able to remain alive under the conditions of high oxidative stress and energy needs.

The bioinformatics studies performed on the phenotypes of chemoresistance and aggressive prostate cancer have identified the role played by *PCBB* in the signature of mitochondrial genes in chemoresistance and aggressive prostate cancer phenotypes. *PCBB* was mentioned among the candidate genes where the metastatic progression was positively correlated with the differentiation of expression under computational models (Zhang et al., 2023). It is proposed that in prostate cancer, an extensive metabolic reprogramming occurs which involves mitochondrial oxidative metabolism and altered nutrient utilization. This alteration enables metabolic plasticity which supports tumor energy production and redox homeostasis during the progression of the cancer. In our study, these bioinformatic hypothetical thoughts are experimentally tested, since our theoretical scenario is implemented on practical data of transcription in metastatic prostate cancer cells (Wanjari et al., 2023)

The suppressive effect on *PCBB* of PC3 cells is opposite to the effects on other forms of cancers, where high concentrations of propionyl-CoA carboxylase have been linked with the proliferation of the tumor. The up-regulated Branched Chain Amino Acid (BCAA) metabolism promotes anabolic growth in the case of hepatocellular carcinoma. This difference emphasizes the type and situation-specificity of the mitochondrial metabolic control of cancer, which suggests the likelihood that the genomic functionality of metastatic prostate cancer may not be similar to its control in other cancers. This observation is in agreement with the concept of metabolic rewiring, according to which the metastatic cancer

cells not only maximize their survival-promoting pathways to stress as much as they respond to the stress by maximizing their production of energy-generating enzymes.

The downregulation of mechanistically *PCBB* may lead to a reduction in flux through metabolic pathways that may function through propionyl-CoA and may divert the intermediate into alternative forms of energy or biosynthetic activities needed to maintain survival in chemotherapeutic stress. It corresponds to the research carried out by Hekmatshoar, who indicated that alterations in the genes of mitochondria can contribute to adaptive resistance to docetaxel in prostate cancer by regulating the amount of ROS and the extent of apoptosis (Hekmatshoar, 2025). Therefore, silencing of PC3 by *PCBB* may result in chemoresistance of said cells, yet the functional activity must be proven.

5.2.2 *IVD* Gene

On the other hand, LNCaP and PC3 cell lines showed the same expression of *IVD*. *IVD* codes isovaleryl-CoA dehydrogenase, which is an enzyme that belongs to the leucine metabolism process in the mitochondria. It is also not modified during the process of transcription, which also implies that *IVD*, in contrast to *PCBB*, may not be a limitation of the mitochondrial metabolic adaptation to the progression of prostate cancer.

Such observation is partially in line with the study that found that expression of some mitochondrial enzymes is constitutive despite the metabolic stress, which is an indicator of their basic housekeeping roles. *IVD* stability is opposite to *PCBB*, and it confirms that the concept of metabolic rewiring of metastatic prostate cancer is not an up- or down-regulation of enzymes of the mitochondrion but a gene-specific one (Zhou et al., 2024).

5.3 MECHANISTIC IMPLICATIONS, BIOLOGICAL IMPLICATIONS

The dissimilarity between the patterns of divergent expression of the *PCBB* and *IVD* of the metastatic models of prostate cancer shows that the metabolism of the mitochondrion is specifically concentrated to the advantage of the tumor progression and chemoresistance. This may be due to *PCBB* downregulation that offers a check to the initial reliance on the canonical BCAA catabolism that facilitates metabolic plasticity, ROS management and apoptosis prevention. It is presumed that these changes are favorable to the androgen-

independent, high-metastatic cells that are reflected in the augmented proliferation and chemoresistance of PC3 (Wanjari et al., 2023).

In comparison, *IVD* stability shows that some catabolic pathways are kept in order to retain basal mitochondrial activity, which is vital in the survival of the cell. This selective activity promotes the idea that metabolic plasticity of prostate cancer cells consists of re-establishing particular branches of metabolism instead of completely modifying mitochondrial enzymes (Zhou et al., 2024). Therefore, *PCBB* and IVCB gene expression differences result in the metabolism of mitochondria in metastasis prostate cancer, which is responsible for the chemoresistance among the patients.

Besides, the panel of observed *PCBB* downregulation overlaps the established chemoresistance mechanisms:

a. Redox regulation: Systemic chemotherapy destroys cancer cells by inducing oxidative stress. Redox regulation: Red Nr reduction may lead to ROS reduction, which may enable metastatic cells to survive oxidative stress induced by chemotherapy (Wanjari et al., 2023).
b. Redistribution of energy: Metabolic intermediates can be diverted to other pathways with a role in making nucleotides and lipid metabolism that favour cell survival and growth in stressful situations.
c. The modulation of apoptotic threshold: Mitochondrial-mediated apoptosis modulated by a low level of *PCBB* may be an indirect increase in resistance to docetaxel (Hekmatshoar, 2025).

These findings are aligned with the theory on cancer metabolic reprogramming, whereby it has been argued that metastatic and therapy-resistant tumours adaptively choose metabolic processes to sustain survival, invasion, and resistance phenotype.

5.4 THE STRENGTHS AND LIMITATIONS OF THE CURRENT STUDY

5.4.1 Strengths

This research has some methodological and conceptual strengths:

Controlled experimental design: Two well-characterized cell lines (LNCaP and PC3) were used, which made it possible to analyze gene expression in different biological fields without the possibility of confounding differences in the heterogeneity of the genetic background.

Quantitative precision: The purity of RNA (A260/A280 1.821), standardized cDNA synthesis and the strong qRT-PCR protocol with the use of *HPRT*1 as a control provided the reliable and reproducible gene expression data.

Gene-specific analysis: The researchers were keen to differentiate between *PCBB* and *IVD* to gain specific information about mitochondrial adaptation to metabolism, and not about the overall metabolic tendencies.

Integration of the literature critically: Results were related to the existing bioinformatic, transcriptomic and functional data, enhancing the scientific relevance of the identified expression patterns.

5.4.2 Limitations of the Present Study

Although it has strong points, one should admit several limitations:

a. mRNA-only analysis: No measurements of protein expression and activity were performed; hence, functional implications of downregulation of *PCBB* were not examined.
b. 2D cell culture model Monolayer cultures cannot recapitulate the complex tumour microenvironment, such as stromal interactions, hypoxia gradients, and 3D metabolic heterogeneity.
c. Minimal functional studies: The relationship between chemoresistance and literature and cell phenotype is based on inference, but no direct modulation (knockdown or overexpression) of *PCBB*/*IVD* was conducted.
d. Single-gene focus: Gene-specific analysis is a strength; however it fails to appreciate adaptations of the entire metabolic network globally, which might be involved in chemoresistance.

5.5 CONCLUSION

This research shows that *PCBB* is lower in metastatic, androgen-independent PC3 prostate cancer cells than it is in androgen-sensitive cells of the LNCaP cells, and *IVD* does not change. The identified results are the experimental evidence of the bioinformatic predictions of the association between *PCBB* and mitochondrial metabolic reprogramming and chemoresistance, emphasizing gene-specific regulation of metabolic pathways in metastatic prostate cancer.

PCBB only and not *IVD* are differentially expressed, highlighting the specific adaptation of the mitochondrial metabolism in aggressive tumour phenotypes, and providing information on possible mechanisms that underlie therapy resistance. These results highlight the relevance of taking the plasticity of metabolism into account in the design of therapeutic interventions and recommend that the *PCBB* may be used as a biomarker for metastatic evolution or chemoresistant phenotypes (in "waiting" for functional validation).

5.6 FUTURE RESEARCH RECOMMENDATIONS

On the basis of the key findings and the limitations of the current research, the following recommendations can be applied to ensure the validity of the future research regarding the *IVD* and *PCBB* genes in the treatment of prostate cancer.

5.6.1 Protein-level Authentication and Operational Assays

a. Western blot analysis or immunofluorescence analysis to determine the expression of *PCBB* and *IVD* protein in LNCaP and PC3 cells.
b. Perform functional studies: Knockdown of *PCBB* in LNCaP and overexpression in PC3 cells to ascertain the cause-and-effect relationship on the proliferation process, regulation of ROS, and chemoresistance.
c. Measure apoptosis and response to a metabolic flux alteration after manipulation of *PCBB* expression to directly correlate gene expression to functional consequences.

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