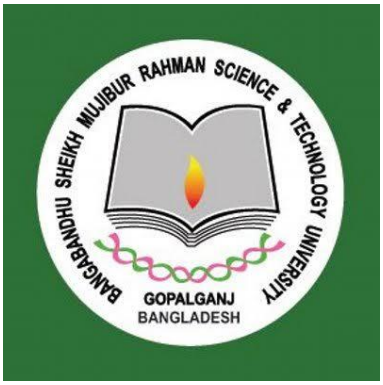
Identification of Key Genes as Potential Drug Targets for Prostate Cancer



A Dissertation Submitted to the Department of Statistics of Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj, Bangladesh in Partial Fulfillment of the Requirements for the Degree of Bachelor of Science.

Submitted By

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July 2023

Bangabandhu Sheikh Mujibur Rahman Science and Technology University, Gopalganj-8100, Bangladesh

CERTIFICATE OF APPROVAL

It's my great pleasure to certify that the project report permitted "Identification of Key Genes as Potential Drug Targets for Prostate Cancer" is an original work done by Md Amanat Ullah Arman. He has completed the project report work under my supervision. As far as I know, the project has not been previously submitted to any university for any kind of degree.

I also certify that I have examined the project and found it satisfactory for submission to the Department of Statistics, Bangabandhu Sheikh Mujibur Rahman Science and Technology University for Bachelor of Science (B.Sc.) in Statistics.

……..

Dr. Md Tofazzal Hossain
Supervisor
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DEDICATED

To

My Parents and Honorable Teachers

DECLARATION

I certify that, to the best of my knowledge, my project does not infringe upon anyone's copyright nor violate any proprietary rights and that any ideas, techniques, quotations, or any other material from the work of other people included in my project, published or otherwise, are fully acknowledged in accordance with the standard referencing practices. Furthermore, to the extent that I have included copyrighted material that surpasses the bounds of fair dealing within the meaning of the Canada Copyright Act, I certify that I have obtained a written permission from the copyright owner(s) to include such material(s) in my project and have included copies of such copyright clearances to my appendix.

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Acknowledgement

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Finally, I must express my very profound gratitude to my parents, all teachers, and all my friends for providing me with unfailing support and continuous encouragement throughout my years of study and through the process of researching and writing this project. This accomplishment would not have been possible without them.

List of Abbreviations

PCa – Prostate Cancer

GLOBOCAN- Global Cancer Observatory

PSA- Prostate Specific Antigen

BPH- Benign Prostatic Hyperplasia

FNA- Fine-Needle Aspiration

NGS- Next Generation Sequencing

DE - Differentially Expressed

DEGs - Differentially Expressed Genes

cDEGs - Common Differentially Expressed Genes

NCBI - National Center for Biotechnology Information

GEO - Gene Expression Omnibus

TCGA - The Cancer Genome Atlas

LIMMA - Linear Models for Microarray Data

PPI - Protein-Protein Interaction

ENCODE - Encyclopedia of DNA Elements

MCODE - Molecular Complex Detection

KGs - Key Genes

KPs - Key Proteins

DR - Drug Repurposing

3D- Three-Dimensional

2D- Two-Dimensional

PDB- Protein Data Bank

MCC – Maximal Clique Centrality

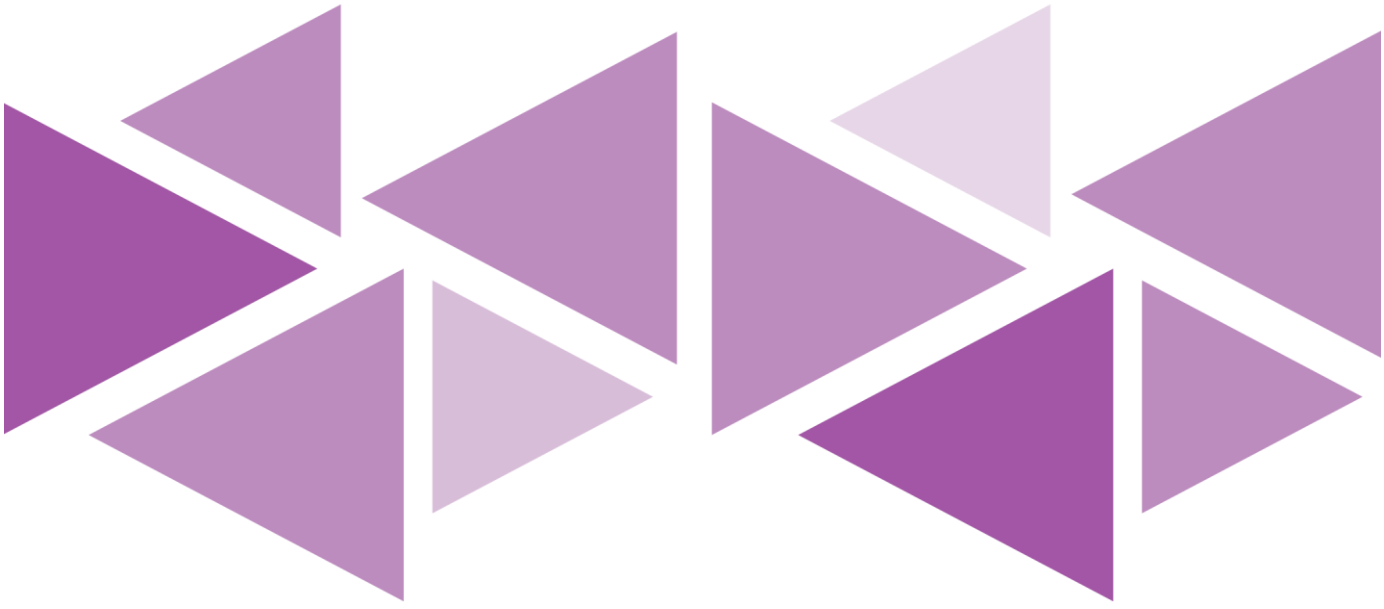
Abstract

Bioinformatics analysis has been playing a vital role in identifying potential genomic biomarkers more accurately from an enormous number of candidates by reducing time and cost compared to the wet-lab-based experimental procedures for disease diagnosis, prognosis, and therapies. Prostate cancer (PCa) is one of the most malignant diseases seen in men worldwide. This study aimed at identifying potential key genes (KGs), and candidate drugs for PCa diagnosis and targeting therapies. Three publicly available microarray datasets of PCa were analyzed for identifying differentially expressed genes (DEGs) by Bioconductor R package called limma. We identified 458 common DEGs (cDEGs) and these genes were mapped to STRING database to construct the protein-protein interaction (PPI) network. We extracted the top 15 hub genes (TOP2A, RRM2, NCAPG, BUB1B, CENPU, CENPF, AURKA, TPX2, MKI67, BIRC5, CDCA5, NUSAP1, EZH2, ECT2, and TK1) by the PPI network analysis. The survival analysis of the hub genes disclosed that the lower expressions of the five hub genes BIRC5, CDCA5, CENPF, NUSAP1, and TK1 were associated with better survival of PCa patients. These five genes might be the candidate biomarkers for the diagnosis and treatment of PCa. Then, we considered 5 key proteins (genomic biomarkers) (BIRC5, CDCA5, CENPF, NUSAP1, and TK1) as the drug target proteins (receptors), performed their docking analysis with the 211 meta-drug agents, and found Adapalene, Ergotamine, Irinotecan Hydrochloride, Drospirenone, Abiraterone Acetate, Zafirlukast, Ibrutinib, Abiraterone, Imatinib, and Telmisartan as the top ranked 10 candidate drugs with respect to our proposed target proteins for the treatment against PCa patients. Therefore, the proposed drugs might play vital role for the treatment against PCa patients.

Key words: prostate cancer; hub genes; candidate genes; molecular docking; candidate drug

Chapter 1

General Introduction



Chapter 1: General Introduction

1.1 Introduction to Project

Prostate cancer (PCa) is a malignancy cancer in urinary system of men with a high incidence. The prostate is a small gland in the pelvis found only in men. About the size of a walnut, it is located between the penis and the bladder. It surrounds the urethra, a tube that carries urine from the bladder to the penis. The main function of the prostate is to help in the production of semen. It produces a thick white fluid that is mixed with the sperm produced by the testicles, to create semen. It is rank second most common male cancer in the world. PCa is the most prevalent cancer (20%) in males and is accountable for a fifth (6.8%) cancer-related deaths in males globally. According to the World Cancer Survey Statistics (GLOBOCAN), the number of newly diagnosed cases of PCa was ~1.41 million in 2020, with ~375 thousand new deaths (Sung *et al.*, 2021; Ferlay *et al.*, 2019). Especially in recent years, this cancer rate in Asian men is increasing at an alarming rate. And, Bangladeshi men are also becoming victims of this fatal disease nowadays. In 2020, a statistical data of GLOBOCAN calculated 2,441 new prostate cancer cases, resulting in 1,289 deaths in Bangladeshi males. By 2040, the global PCa burden is expected to increase to 2.43 million new cases and 740 thousand new deaths due to population growth and ageing (Sung *et al.*, 2021; Ferlay *et al.*, 2019).

Smoking, obesity, race/ethnicity, diet, age, chemicals and radiation exposure, sexually transmitted diseases, etc., are among the most common risk factors for prostate cancer(Shen and Abate-Shen, 2010). However, the basic change at the molecular level is the manifested confirmation of PCa.

PCa is one of the most frequent cancers in males and has the highest prevalence of male malignant tumors among the 112 nations in the global cancer statistics in 2020.

Although prostate specific antigen (PSA) level is the most frequently used screening tool for prostate cancer detection, it doesn’t stand as an absolute method to predict disease malignancy. Furthermore, the use of genetic profiling may provide additional benefits for early PCa detection (Herget *et al.*, 2016; Albertsen, 2008b). Prostate cancer may be rectified at an early stage of cancer by surgery or radiation therapy, but patients with advanced or metastatic disease may have no curative therapeutic options (Albertsen,

2008a; So *et al.*, 2005). Therefore, it is urgent to screen out diagnostic and prognostic biomarkers, precisely distinguishing the various stages of PCa, and determining the exact therapy for PCa is critical.

Microarray technology and bioinformatics analysis have been also widely applied, in order to explain the differences of gene expression which can help us better understand mechanisms about prostate tumorigenesis and progression (Huang *et al.*, 2017). In the present study, we downloaded three gene expression profiles (GSE104749, GSE46602 and GSE55945) from the Gene Expression Omnibus (GEO) database, which contain benign prostatic hyperplasia (BPH) and prostate cancer (PCa) fine-needle aspiration (FNA) biopsy tissue samples.

However, *de novo* (new) drug discovery is a tremendously challenging, time-consuming, and expensive task due to several steps involved in this process, from the target-based drug selection to clinical validation. Drug repurposing (DR) is a promising approach to overcome many of those obstacles in discovering and developing new drugs by exploring the new therapeutic applications of approved drugs that are established for different diseases (Rudrapal *et al.*, 2020). It is considered as a supporting process to the conventional drug discovery. To explore more suitable repurposable drugs for a new disease, it requires identifying appropriate target proteins associated with the new disease.

In the current study, we used DR approach in finding potential drug targets. We analyzed publicly available next generation sequencing (NGS) data collected from PCa tissue and adjacent normal tissue. After finding the differentially expressed (DE) genes, we mapped 458 common differentially expressed genes (cDEGs) to STRING (Szklarczyk *et al.*, 2019) database to construct a protein-protein interaction (PPI) network. From the PPI network, the top 15 hub genes were selected for further analysis. The expressions of these hub genes were validated by the cancer genome atlas (TCGA) data. The survival analysis of the hub genes using the TCGA data showed that the five genes BIRC5, CENPF, TK1, CDCA5, and NUSAP1 were associated with the prognosis of PCa. Overall, the genes BIRC5, CENPF, TK1, CDCA5, and NUSAP1 might be the potential candidates for PCa prognosis and treatment. Finally, we analyzed the molecular docking of key genes with

meta-drug agents, and suggested therapeutic targets with lead small compounds against PCa.

1.2 Introduction to Bioinformatics

Bioinformatics is the sophisticated branch of molecular biology (Osareh and Shadgar, 2013). It is involved the biology, computer science and statistical tools. There are four main branches of bioinformatics such as genomics, transcriptomics, proteomics, and metabolomics. Where, genomics deals only DNA. A genome can be thought of as the complete set of DNA sequences that codes for the hereditary material that is passed on from generation to generation (Robert D Fleischmann *et al.*, 1995). These DNA sequences include all the genes (the functional and physical unit heredity passed from parent to offspring) and, transcriptomics deals RNA which is the next part of genomics. When it cannot specifically describe any new disease in the short time in genomics then used transcriptomics (Johansen *et al.*, 2011). Proteomics deals with the information about proteins and it defines proteins, modification proteins, interactions between proteins(Anderson and Anderson, 1998). And Metabolomics is the study of chemical processes involving metabolites, the small-molecule intermediates and products of metabolism that including the amino acids, nucleotides, lipids and sugars (Jordan *et al.*, 2009). The details of Bioinformatics with its branches are given in Figure 1.1.

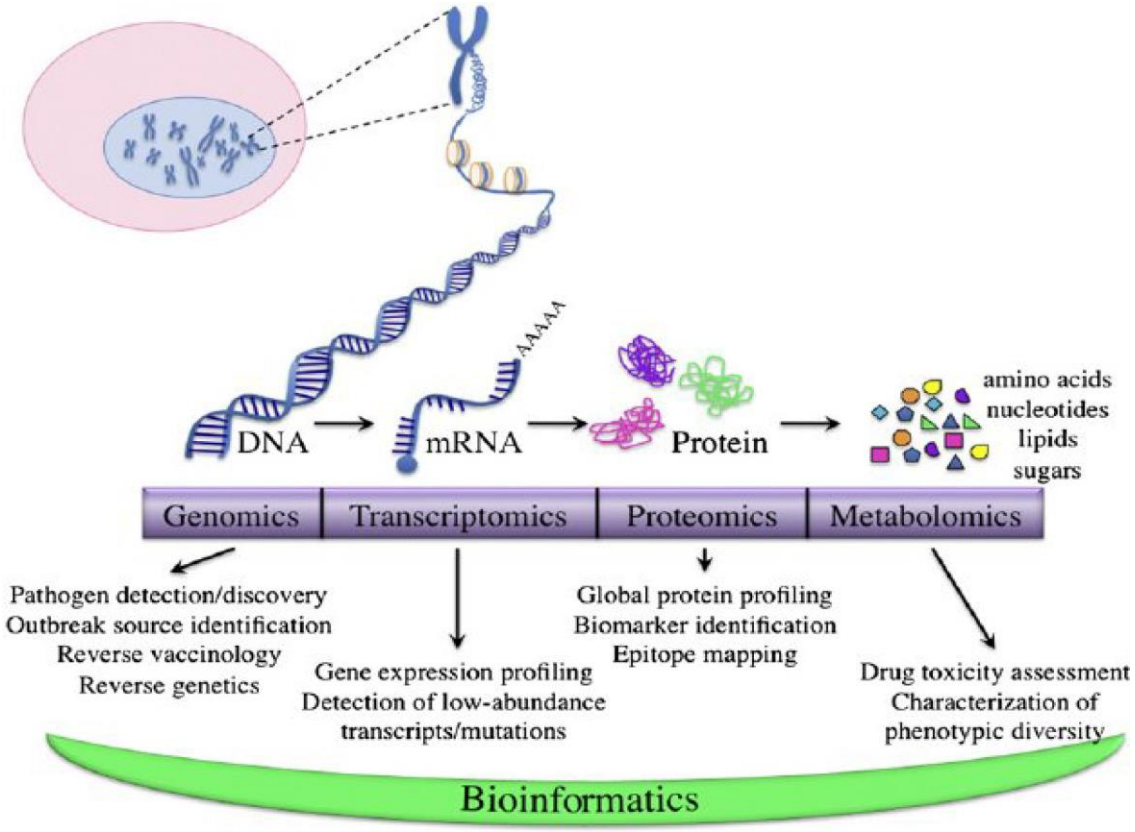


Figure 1.1: Main Branches of Bioinformatics.

1.3 Central Dogma of Molecular Biology

Central dogma of molecular biology is a classification of the flow of genetic information within a biological system. It is described as "DNA makes RNA and RNA makes protein" (Hook *et al.*, 2010). In short: DNA → RNA → Protein, or DNA to RNA to Protein.

It was first stated by Francis Crick in 1958 (CRICK, 1958). According to him, "the central dogma of molecular biology deals with the detailed residue by residue transfer of sequential information. It states that such information cannot be transferred back from protein to either protein or nucleic acid.'' The process is replication, transcription and translation are given in Figure1.2.

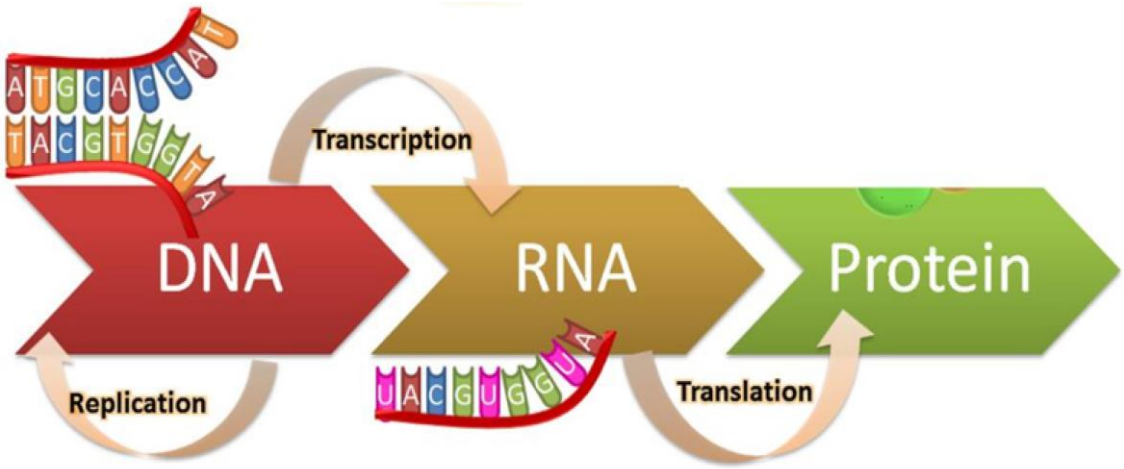


Figure 1.2: Central Dogma of Molecular Biology.

Transcription contain information in a section of DNA is transferred to a newly assembled piece of messenger RNA (mRNA) (Black, 2003). It is facilitated by RNA polymerase and transcription factors. Translation is the process of finding to a ribosome from a mature mRNA, where it is translated (Konikkat, 2016). In prokaryotic cell, which has no nuclear component, the process of transcription and translation may be linked together. The final step in the central dogma, to transmit the genetic information between parents and progeny, the DNA must be replicated faithfully. It is carried out by a complex group of proteins that unwind the super helix, unwind the double helix and using DNA polymerase and its associated proteins.

1.3.1 DNA Replication

DNA replication is the process by which a double-stranded DNA molecule is copied to produce two identical DNA molecules. Replication is an essential process because, whenever a cell divides, the two new daughter cells must contain the same genetic information, or DNA, as the parent cell.

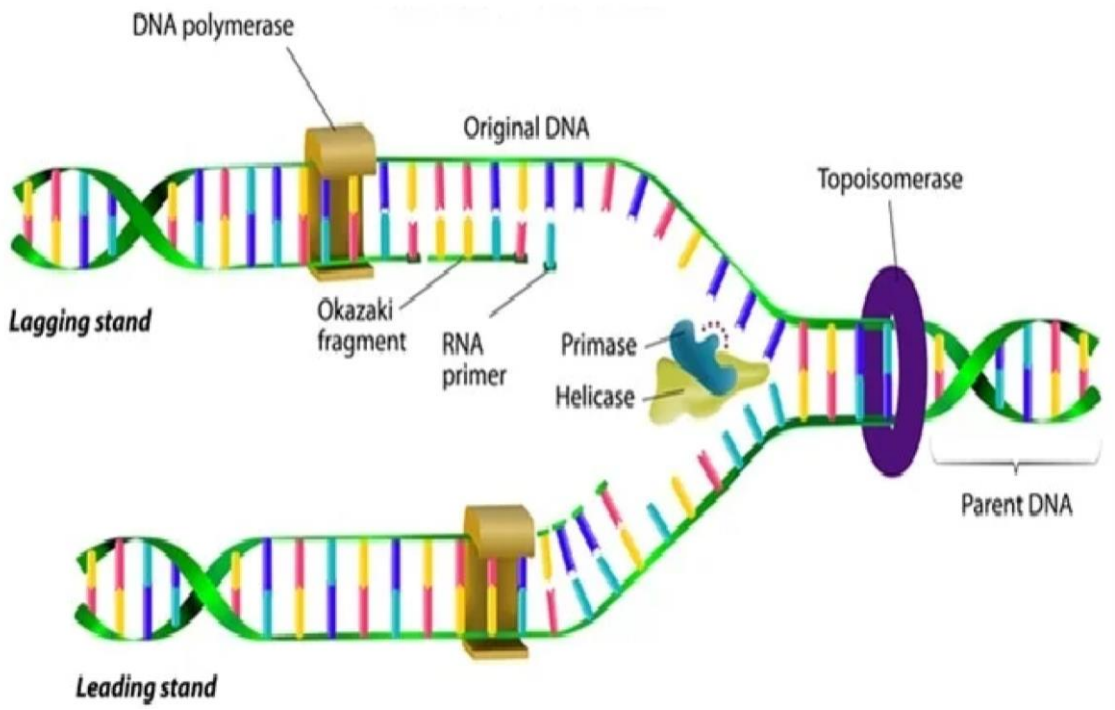


Figure 1.3: DNA Replication.

1.3.2 Transcription

Transcription is the process by which DNA is copied (transcribed) to mRNA, which carries the information needed for protein synthesis. Transcription takes place in two broad steps. First, pre-messenger RNA is formed, with the involvement of RNA polymerase enzymes. The process relies on Watson-Crick base pairing, and the resultant single strand of RNA is the reverse-complement of the original DNA sequence. The pre-messenger RNA is then "edited" to produce the desired mRNA molecule in a process called RNA splicing.

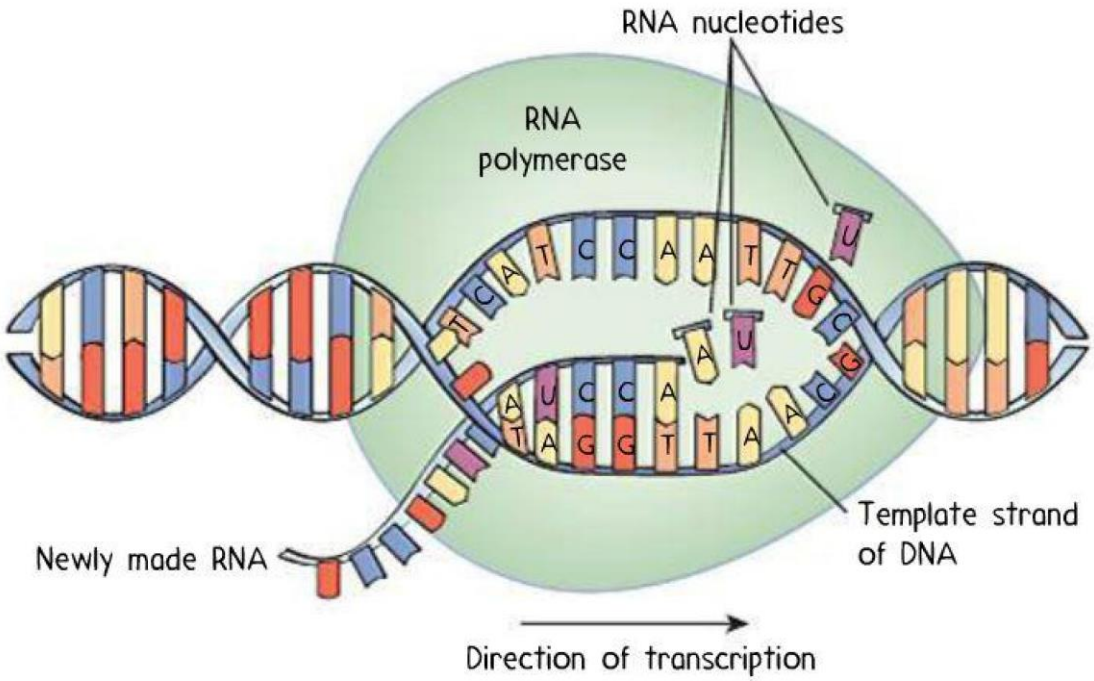


Figure 1.4: The Process of Transcription.

1.3.3 Translation

Translation is the process by which the genetic code contained within a messenger RNA (mRNA) molecule is decoded to produce a specific sequence of amino acids in a polypeptide chain. It occurs in the cytoplasm following DNA transcription and, like transcription, has three stages: initiation, elongation and termination. In other way, Translation is the process of translating the sequence of a messenger RNA (mRNA) molecule to a sequence of amino acids during protein synthesis.

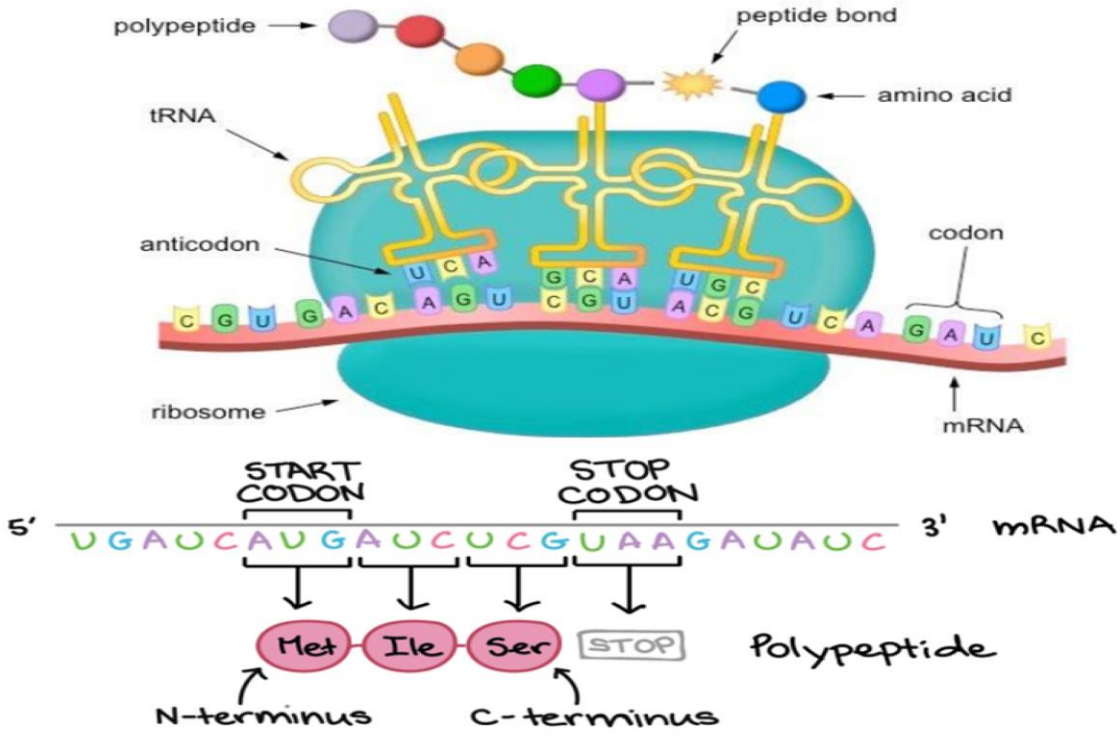


Figure 1.5: The Process of Translation.

1.4 Research Interest in Microarray Gene Expression Data Analysis

It is observed that new diseases are coming day by day. To early detection of the disease status and discovering the drug, the most influential genes identification is very important. Moreover, till now there are very few FDA based genes biomarker for diagnosing cancer. Therefore, every new disease, the research interest of microarray data analysis is to identify the transcriptomic biomarker as well as the biomarker pathways and also its biological validation also its interpretation for every new disease. Although there are some tools and techniques are available for the above analysis as a result these are underdeveloped. To reach the targeted goal, researchers often face some problems for microarray data analysis.

1.5 Transcriptomics

The study of the complete set of RNAs (transcriptome) encoded by genome of a specific cell or organism at a specific time under a specific set of conditions (Lowe *et al.*, 2017). It is sometimes used to refer all RNAs, or mRNA, depending on the experiment (Énzsöly *et al.*, 2014).The details description of transcription age given by gene expression, regulation and gene profiling.

1.5.1 Gene Expression and Regulation

A cell stores its genetic information in DNA molecule, which is contained genes. Depending on the function of the cell, it used to different genes to make proteins by copying the code of the gene into messenger RNA (mRNA) that procedure called transcription. Gene regulation could happen at the transcription level, translation level and post translation level (Fleishman and Anderson, 1980; Li and Bingham, 1991; GANOZA *et al.*, 1989; Harata and Mizuno, 1988; Monahan and Hall, 1974; Abraham *et al.*, 1993). Since it was the first level of the gene regulation and was believed that most of the gene regulation happened at that level, transcription regulation was the focus of the gene regulation studies. Due to the technical limitation, sometimes scientists could not obtain enough information to provide them a more thoroughly and detailed picture of the gene regulation. The details description of DNA replication are given in Figure 1.6.

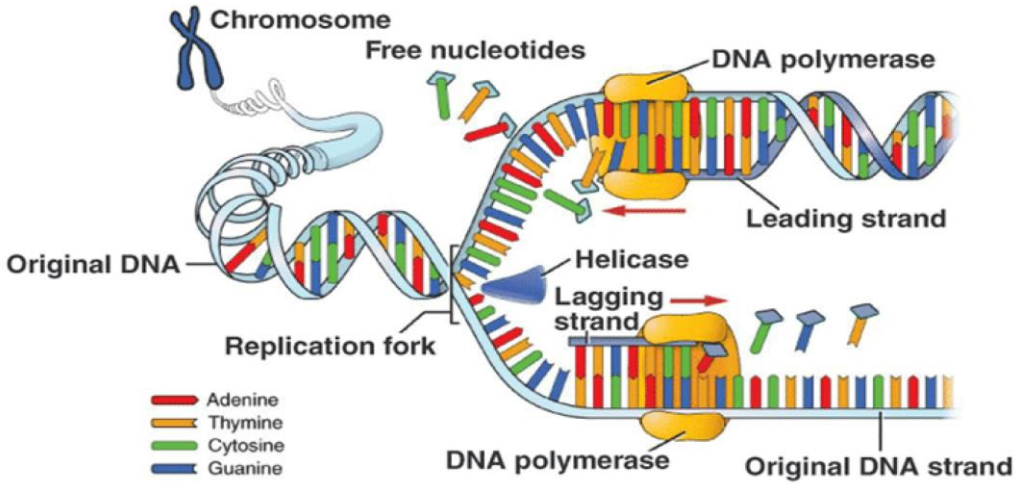


Figure 1.6: Gene Expression and Regulation.

1.5.2 Gene Profiling

Microarray gene expression profiling is used to simultaneously compare gene transcription in cancer cells versus normal tissues in thousands of genes that allows for the identification of genes. Among these genes some are over expressed and unexpressed in cancers. Currently gene expression profiling is used as a primarily research tool. The flowchart of gene expression profiling (Dufva, 2009) is showed in Figure 1.7.

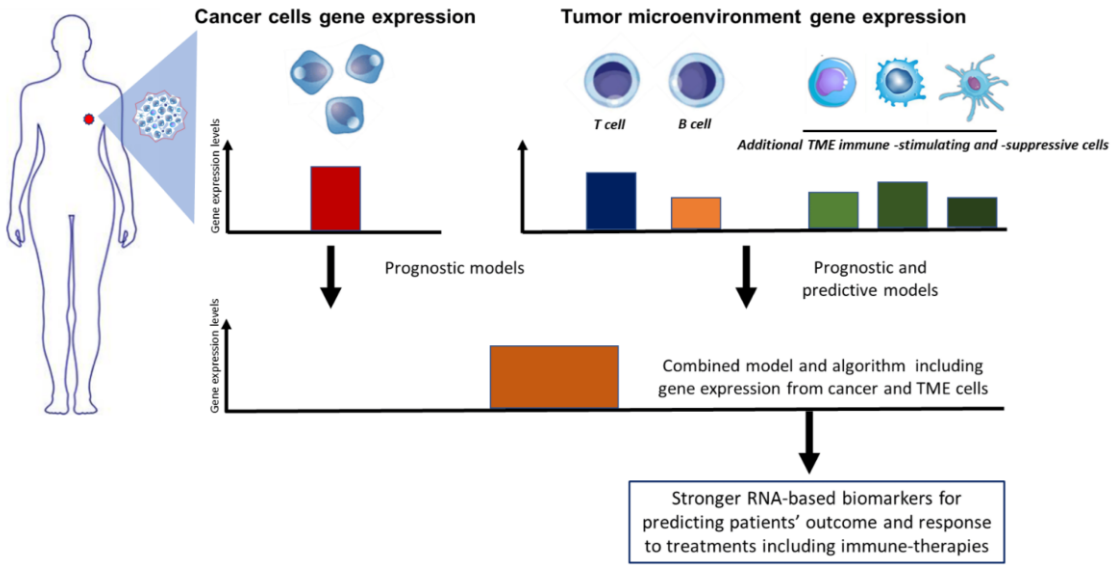


Figure 1.7: Gene Expression Profiling.

The expression profile may provide diagnostics information addressing the aggressiveness of a tumor, including cancer disease diagnosis and its response to therapies. It may also information about potential therapeutic targets, as well as sensitivity and specificity to different chemotherapies.

Analysis of microarray gene expression data commonly yields a list of differently expressed genes. List of this genes are helpful in providing researchers capability research ways and shedding lights on the roles those genes may be played in the phenomenon that is under investigation.

1.6 Gene Expression and Microarray

Microarrays are a two-dimensional arrangement of biological samples, with samples placed in spots numbering in the thousands and organized into columns and rows. They allow for efficient analysis of genetic material.

Gene expression is the process by which the genetic instructions are used to combination of gene products. These products are usually proteins, which go on to perform essential functions as enzymes hormones and receptors.

Gene expression microarrays exposure of all the transcriptional activity in a biological sample. Identify dissimilarity of most conventional molecular label of the gene by using statistical tools, which are generally allowed the study of single gene or a small set of genes, microarrays facilitate the invention of totally expected and unexpected functional roles of genes (Slonim and Yanai, 2009). The power of these tools has been applied to a range of applications, including discovering cancer types, developing new symptomatic tools, and identifying underlying test and fitting the different model of the specific disease.

1.6.1 Different Types of Microarrays

Microarrays are divided into three types based on the preparation mode of the array;

a) The Spotted Array on Glass: Spotted arrays are arrays made on poly-lysine coated glass microscope slides. This provides binding of high-density DNA by using slotted pins. It allows fluorescent labeling of the sample.

b) Self-Assembled Arrays: These are fiber optic arrays made by the deposition of DNA synthesized on small polystyrene beads. The beads are deposited on the etched ends of the array. Different DNA can be synthesized on different beads and applying a mixture of beads to the fiber optic cable will make a randomly assembled array.

c) In-situ Synthesized Arrays: These are made by chemical synthesis on a solid substrate. In the chemical synthesis, photo labile protecting groups are combined with photolithography to perform the action. These arrays are used in expression analysis, genotyping and sequencing.

On the basis of the use of different types of probes, Microarrays are classified into twelve types

DNA Microarrays: DNA microarray is also known as gene chip, DNA chip or biochip. It either measures DNA or uses DNA as a part of its detection system. There are four types of DNA microarrays: cDNA microarrays, oligo DNA microarrays, BAC microarrays and SNP microarrays.

a) MMChips: MMChips allows the integrative analysis of cross-platform and between laboratory data. It studies interactions between DNA and protein. ChIP-chip (Chromatin immunoprecipitation (ChIP) followed by array hybridization) and ChIP-seq (ChIP followed by massively parallel sequencing) are the two techniques used.

b) Protein Microarrays: It acts as a platform for characterization of hundreds of thousands of proteins in a highly parallel way. Protein microarrays is of three types, and these are analytical protein microarrays, functional protein microarrays and inverse-phase protein microarrays.

c) Peptide Microarrays: These types of arrays are used for the detailed analyses or optimization of protein-protein interactions. It helps in antibody recognition by screening proteomes.

d) Tissue Microarrays: Tissue microarray paraffin blocks that are formed by separating cylindrical tissue cores from various donors and embedding it into a single microarray. It is mainly used in pathology.

e) Cellular Microarrays: They are also called transfection microarrays or living-cell- microarrays and are used for screening large-scale chemical and genomic libraries and systematically investigating the local cellular microenvironment.

f) Chemical Compound Microarrays: This is used for drug screening and drug discovery. This microarray has the capacity to identify and evaluate small

molecules and so it is more useful than the other technologies used in the pharmaceutical industry.

g) Antibody Microarrays: They are also referred to as antibody array or antibody chip. These are protein-specific microarrays that contain a collection of capture antibodies placed inside a microscope slide. They are used for detecting antigens.

h) Carbohydrate Arrays: They are also called glycol arrays. Carbohydrate arrays are used in screening proteomes that are carbohydrate binding. They can also be utilized in calculating protein binding affinities and atomization of solid-support synthesis for glycans.

i) Phenotype Microarrays: Phenotype microarrays or PMs are mainly used in drug development. They quantitatively measure thousands of cellular phenotypes all at once. It is also used in functional genomics and toxicological testing.

J) Reverse Phase Protein Microarrays: They are microarrays of lysates or serum. Mostly used in clinical trials, especially in the field of cancer, they also have pharmaceutical uses. In some cases, they can also be used in the study of biomarkers.

k) Interferometric Reflectance Imaging Sensor or IRIS: IRIS is a biosensor that is used to analyze protein-protein protein-DNA and DNA-DNA interactions. It does not make use of fluorescent labels. It is made of Si or SiO2 substrates prepared by robotic spotting.

1.6.2 Elements of Microarray Technology

A microarray technology includes Affymetrix, Array, Probes and Spotter. The short description of Affymetrix, Array, Probes and Spotter is given by

Affymetrix: Affymetrix makes quartz chips for analysis of microarray called Gene Chip arrays (Lowe *et al.*, 2017). Affymetrix Gene Chip is a popular microarray

platform for genome wide expression profiling and has been widely used in functional genomics in the classification of cancers.

Array: This was the robust based on the patrimonial material of known arrangements were organized efficiently along opened. The process of arrangements was called spotting. Now a day the array is made up of glass or nylon which bears many wells to hold the complementary gene expressions. Each of the point on the microarray represents an independent experimental stress test is used to measure the presence and absence of specific gene expression in the sample cell.

Probes: The single beached cDNAs that are known as the "probes'' which were spotted on the exhibits. The target polynucleotide groupings in the organic specimen arrangements were hybridized with the complimentary tests. The attachment of the probe to the array is very essential to maintain point integrity and prevention of the probe from being washed away during array processing. Also, it is critical as a fitfully followed test can cause commotion to leak in subsequently decreasing the nature of resultant figure.

Spotter: A few methods have been developed to understand the behavior of genes. Microarray technology is an important one of them. It is used to monitor large amount of genes expression level in parallel. The spotting is carried out utilizing either contact or non-contact strategies, whereas contact spotters have the spotting spout like an ink pen where connected weight discharges the tests on the shows. Non-contact spotters' utilization ink-plane engineering or the piezoelectric narrow impacts for spotting justifications. Non-contact spotters are speedier then contact spotters. However, contact spotters have more exactness as contrasted with non- reaching ones (Cheung, 2012).

1.7 Workflow of the Project

The flowchart of microarray data analysis workflow is given in Figure 1.8.

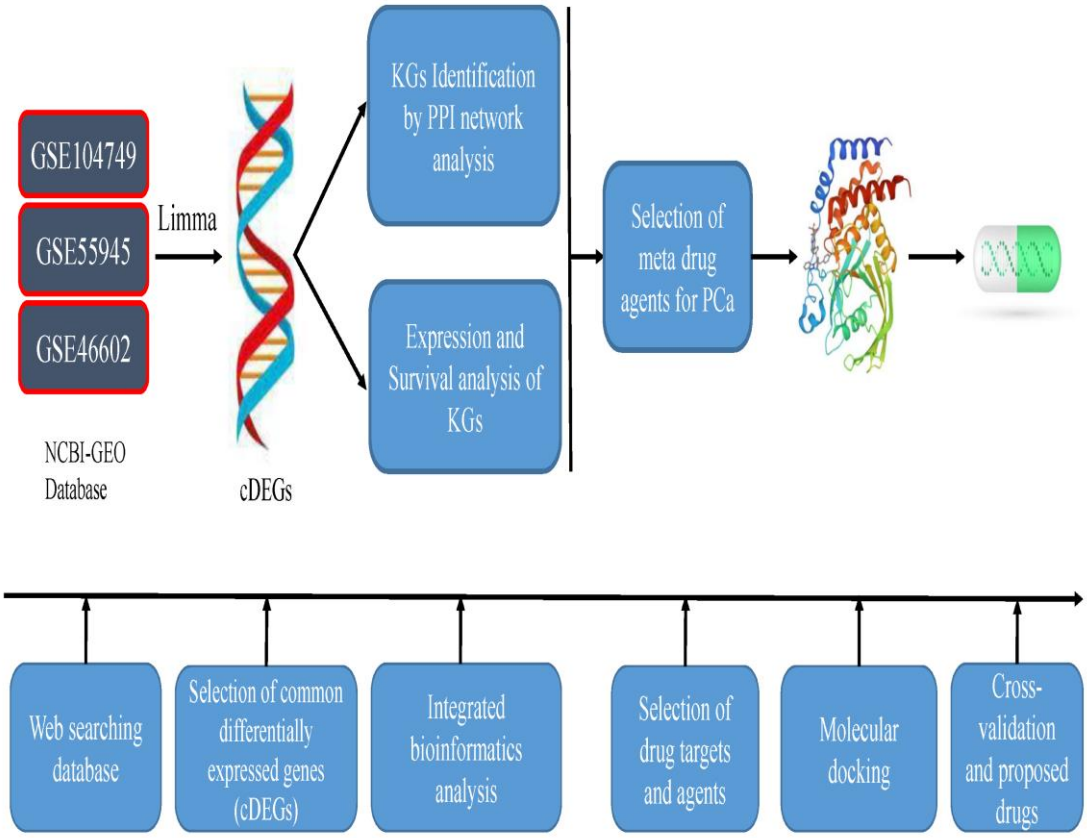


Figure 1.8: Workflow of the Project.

1.8 Problems Identification for the Project

Although Prostate cancer (PCa) is a malignancy cancer of men with a high incidence, besides it is quite challenging to detect biomarkers and appropriate drug.

1.9 Objectives of the Project

The main objectives of the project were

(i) Computational identification of genomic biomarkers (drug targets) for PCa.

(ii) Exploring proposed genomic biomarkers-guided candidate drugs for the treatment against PCa.

1.10 Limitations of the Project

The study has been done based on the secondary data downloaded from public repository. So, the limitations of secondary data remain in the study. Furthermore, all of these studies have been done in the dry laboratory. That means we have not validated our results by wet lab experiments. Thus, using the integration of wet and dry laboratories this study would be an avenue for further research.

1.11 Layout

Chapter 1: This chapter includes the basic concept of Bioinformatics, problems of microarray analysts, objectives, and limitations of the project.

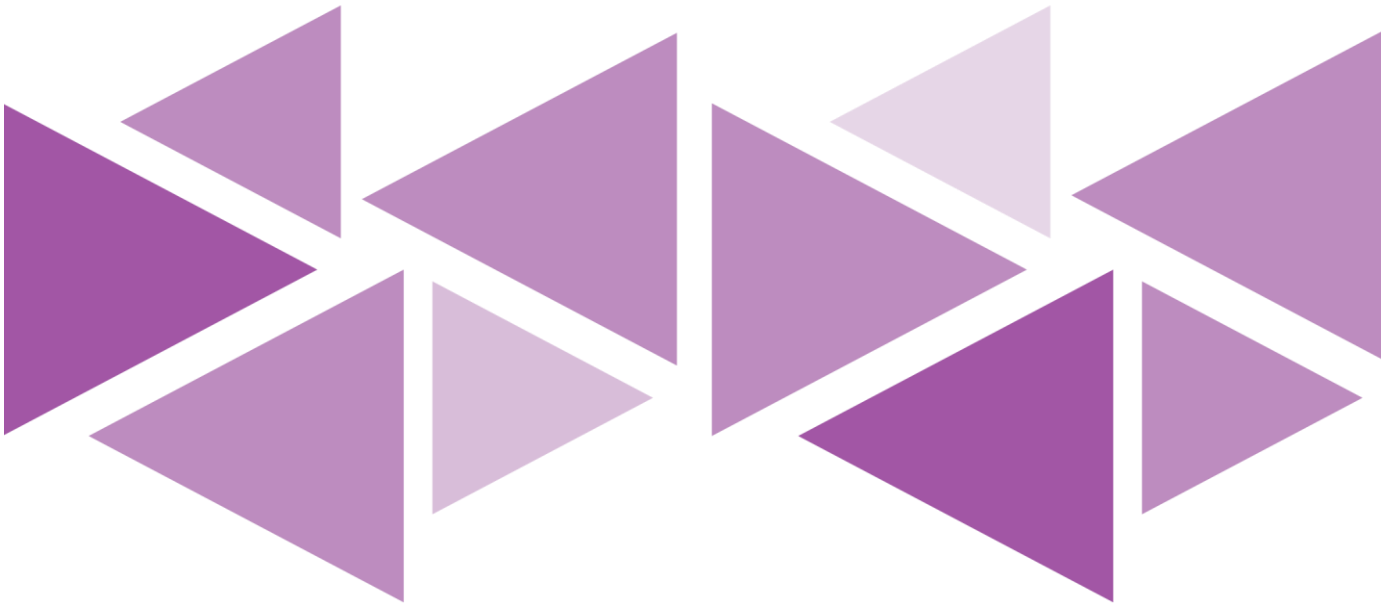
Chapter 2: This chapter includes method and material for the identification of DE genes from microarray data, PPI networking, expression and survival analysis, and drug repurposing by molecular docking study.

Chapter 3: This chapter includes the results of DE genes from microarray data, PPI networking, expression and survival analysis, and drug repurposing by molecular docking study.

Chapter 4: This chapter includes a discussion, conclusion, and areas of further research of the project.

Chapter 2

Method and Material



Chapter 2: Method and Material

2.1 Introduction

Methodology refers to the overarching strategy and rationale of your research project. It involves studying the methods used in the research field and the theories or principles behind them, to develop an approach that matches the research objectives. Data source, quality, methodology, and strategy are the critical portion for getting precise discoveries and in conclusion comment on this finding for each inquiries about. So, we choose to gather the secondary data for our inquiry about this project. This chapter is certain to the portrayal of all vital steps for the secondary data collection and all other related issues to study.

2.2 Selection of Project Title

There is a system in our department to make some groups by dividing the total student with the teacher for completion of project work after being selected group I met our honorable supervisor and after a discussion with my supervisor and we select the project title as **"Identification of Key Genes as Potential Drug Targets for Prostate Cancer"**.

2.3 Data Sources and Descriptions

We used both original data and meta-data to reach the goal of this study as described below.

2.4 Collection of Microarray Exploring Profiles for Genomic Biomarkers and Drug Target Receptors

We collected microarray profiles for PCa from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) website (http://www.ncbi.nlm.nih.gov/geo/, (accessed on 9 April 2023). PCa patients microarray

datasets of GSE46602 (Mortensen *et al.*, 2015), GSE55945 (Arredouani *et al.*, 2009), and GSE104749 (Shan *et al.*, 2017) were all based upon the GPL570 Platforms ((HG-U133_Plus_2) Affymetrix Human Genome U133 Plus 2.0 Array), which included 34 PCa tissues and 14 normal prostate tissues, 13 PCa tissues and 8 normal prostate tissues, and 4 PCa tissues and 4 normal prostate tissues, respectively.

2.5 Collection of Meta-Drug Agents for Exploring Candidate Drugs

We collected host transcriptome-guided 211 meta-drug agents by the literature review of PCa patients (Table A1) for exploring candidate drugs. Thus, we considered 211 drug agents to explore candidate drugs by molecular docking with the identified proteins.

2.6 Finding Differentially Expressed Genes

2.6.1 Bioconductor

Current statistical inference problems in biomedical and genomic data analysis routinely involve the simultaneous test of thousands, or even millions, of null hypotheses. These testing problems share the following general characteristics: inference for high-dimensional multivariate distributions, with complex and unknown dependence structures among variables; a broad range of parameters of interest, for example, regression coefficients and correlations; many null hypotheses, in the thousands or even millions; and complex dependence structures among test statistics.

The Bioconductor project started in 2001 and is an open-source, open-development software project to provide tools for the analysis and comprehension of high throughput genomic data. It is based primarily on the R programming language, and most of the Bioconductor components are distributed as R packages. It provides widespread access to a broad range of powerful statistical and graphical methods for the analysis of genomic data. Bioconductor's software can be used for microarray analysis (data import, quality assessment, normalization, differential expression analysis, clustering, classification, and many more applications); annotation (using microarray probe, gene, pathway, gene ontology, homology, and other annotations); high throughput assays (importing, transforming, editing, analyzing and visualizing various types of assays); and

transcription factors analysis (finding candidate binding sites for known transcription factors via sequence matching) (www.Bioconductor.org).

2.6.2 Linear Model

The model that models the expression of each gene as a linear function of explanatory variables (Groups, Treatments, Combinations of groups and treatments, etc.)

$$\mathbf{Y = X\beta + \varepsilon}$$

Where,

y - Vector of observed data

X - Design matrix (with row for each observation and column for each parameter)

β - Vector of parameters to estimate

ε - Random errors

2.6.3 LIMMA or Linear Models for Microarray Data

This method represents the design of any microarray experiment in terms of a linear model fitted for each gene separately. To test for differential expression, the gene-specific variance estimates are improved in a Bayesian way by using the information from all genes. The method is implemented in a Bioconductor package called limma. We will give here an overview of the method; a more detailed description can be found in Smyth (2004).

It is designed to analyze complex microarray experiments involving comparisons between many RNA targets simultaneously. By fitting a linear model to the expression data for each gene, this package allows analyses of contrasts of interest. Empirical Bayes and other shrinkage methods are used to borrow information across genes, making the analyses stable even for experiments with small number of arrays (Smyth, 2004, 2005). Limma uses linear models to analyze designed microarray experiments (Yang and Speed, 2002)(Smyth, 2004). This approach allows very general experiments to be analyzed nearly as easily as a simple replicated experiment.

Mathematically,

We assume a linear model $\mathbf{E[y_j] = X\alpha_j}$,

Where,

$\mathbf{y}_j$ contains the expression data for gene *j*,

$\mathbf{X}$ is the design matrix, and

$\boldsymbol{\alpha}_j$ is a vector of coefficients.

Here y_j^T is the j^{th} row of the expression matrix and contains either log-ratios or log intensities. The contrasts of interest are given by $\beta_j = C^T\alpha_j$ where C is the contrast matrix. The coefficients component of the fitted model contains estimated values for the α_j. After applying the contrast step, the coefficients component now contains estimated values for the β_j. With common reference microarray data, linear modeling is much the same as ordinary ANOVA or multiple regression except that a model is fitted for every gene.

The basic statistic used for significant analysis is the moderated t-statistic, which is computed for each probe and for each contrast. This has the same interpretation as an ordinary t-statistic except that the standard error is estimated by pooling variance estimates across genes, that is, shrunk toward a common value, using a simple Bayesian model. This has the effect of borrowing information from the ensemble of genes to aid with inference about each individual gene (Smyth, 2004).

Moderated t-statistics (for specified t-tests among 2-groups) lead to p-values in the same way that ordinary t-statistics do except that the degrees of freedom are increased, reflecting the greater reliability associated with the smoothed standard errors. These *p*-values are adjusted for multiple testing. The most popular form of adjustment is “FDR”, which is Benjamini and Hochberg’s method to control the false discovery rate (Benjamini and Hochberg, 1995).

The *B* statistic (lods or *B*) is the log odds that the gene is differentially expressed (Smyth, 2004). The *B* statistic is automatically adjusted for multiple testing by assuming that 1% of all genes, or some other percentage specified, are expected to be differentially expressed. The *p*-values and *B*-statistics will normally rank genes in the same order.

The empirical Bayes step computes one more useful statistic. The moderated *F*-statistic (*F*) combines the *t*-statistics for all the contrasts into an overall test of significance for that gene. The *F*-statistic tests whether all contrasts are non-zero for that gene against a general alternative. The denominator degrees of freedom is the same as that of the moderated *t*. It is similar to the ordinary *F*-statistic from analysis of variance except that the denominator mean squares are shrunken, as described above.

2.6.4 Identification of cDEGs for PCa Patients

The identification of common differentially expressed genes (cDEGs) was a key step of this study. To identify cDEGs, we identified DEGs for each of GSE46602, GSE55945,and GSE104749 datasets separately, the R package linear models for microarray (LIMMA) (Smyth, 2004) was used to identify DEGs between two groups (normal vs. cancer). |Log2Fold Change | > 1.0 and adjusted p-value < 0.05 were considered as the cut-off for defining significant DEGs. The LIMMA approach calculates the p-value by using the modified t-statistics to test the significance of differential gene expressions between two conditions, and p-value is then adjusted by using the Benjamini–Hochberg procedure (Benjamini and Hochberg, 1995)**.** Finally, we selected 458 common DEGs by using three DEGs sets derived from three publicly available microarray datasets.

2.7 Protein-Protein Network Analysis of cDEGs for Identification of KGs

The PPI network of cDEGs was constructed through the STRING online database (https://string-db.org/, (accessed on 10 April 2023)) (Szklarczyk *et al.*, 2011). For improving the quality of PPI network, we used the Cytoscape software (Shannon *et al.*, 2003). The Cytoscape plugin cytoHubba was used to select the Hub Genes (HubGs) or Hub Proteins (HubPs) from PPI network (Shannon *et al.*, 2003)(Chin *et al.*, 2014). The PPI network provides several nodes and edges, which indicate proteins and their interactions, respectively. A node with the largest number of significant interactions/connections/edges with other nodes is considered as the top-ranked HubGs. We constructed a sub-network with the top 15 hub genes using the CytoHubba. The HubGs were selected by using the topological analyses Maximal Clique Centrality (MCC) (Chin *et al.*, 2014) of the PPI network. The hub genes were then extracted from the sub-network.

2.8 Expression and Survival Analyses of the Hub Genes in Prostate Cancer

We examined the expression of hub genes in PCa tissue samples compared to normal tissue samples using GEPIA (Tang *et al.*, 2019) database. GEPIA is a new web based tool that uses gene expressions from TCGA database to compare the expression profiles of genes between normal and cancer samples. In GEPIA, there were 492 PCa tumor tissue samples and 52 normal tissue samples from the TCGA database. The default cutoffs |Log2Fold Change | > 1.0 and p-value < 0.01 were considered as statistically significant. The overall survival analysis of the hub genes in PCa was also performed by the GEPIA tool. There were survival data of 100 patients with PCa in the GEPIA database. Log rank $P < 0.05$ was considered to be statistically significant.

2.9 Drug Repurposing by Molecular Docking Study

We performed a molecular docking analysis of suggested receptor proteins with drug agents to proposein-silico validated efficient candidate drugs for the treatment of PCa. As previously mentioned in the data sources (Table A1), we considered our proposed genes based key proteins (KPs) as drug target proteins and 211 meta-drug agents. Both receptor proteins and meta-drug agents require 3-dimensional (3D) structures for molecular docking studies. All of the targeted proteins' 3D structures were downloaded from the protein data bank (PDB) (Berman *et al.*, 2000) and SWISS MODEL (Waterhouse *et al.*, 2018). All meta-drug agents' 3D structures were downloaded from the PubChem database (Kim *et al.*, 2019). Using discovery studio visualizer 2019 (Biovia *et al.*, 2000), the 3D structures of the target proteins were displayed, and the target chains that were not part of the genes were deleted. Every protein was defined as a receptor, and the proteins' active sites were found from the receptor cavities using the discovery studio tool. The protonation state of protein was assigned using the PDB2PQR and HCC servers (Dolinsky *et al.*, 2007)(Gordon *et al.*, 2005). All the absent hydrogen atoms were properly added as well. The pKa for the receptor amino acids were examined under the physical conditions of pH=7, salinity =0.15, external dielectric = 80, and internal dielectric = 10. Then, using PyRx (Dallakyan and Olson, 2015; Rehman *et al.*, 2021)

tools , the receptor was prepared for molecular docking study by eliminating water molecules and ligand heteroatoms and by addition of polar hydrogens (Morris *et al.*, 2009). The ligands were prepared for molecular docking study by using PyMol (Yuan *et al.*, 2017; Delano, 2002; Seeliger and De Groot, 2010) tools to set the torsion tree and rotatable and nonrotatable bonds in the ligand. PyRx was used to calculate binding affinities between target proteins and drug agents (Trott and Olson, 2009). The PyRx scoring functions was as given below.

$$E = \sum_i^{ligand} \sum_j^{protein} e_{pair}(d_{ij}) \quad (1)$$

Here, *d* is the surface distance calculated with Eq. (2).

$$d_{ij} = r_{ij} - R_j - R_j \quad (2)$$

Where r is the interatomic distance, and R_i , and R_j are the radii of the pair's atoms. Every atom pair interacts via a steric interaction described by the 1st component of Eq. (3). In addition, depending on the atom type, hydrophobic and non-directional H-bonding interactions may exist, as indicated by the last two components of Eq. (3).

$$\sum_i^{ligand} \sum_j^{protein} e_{pair}(d_{ij}) = \sum_i^{ligand} \sum_j^{protein} \omega_1 \times gauss_1(d_{ij}) + \omega_2 \times gauss_2(d_{ij}) + \omega_3 \times repulsion(d_{ij}) + \sum_i^{ligand} \sum_j^{protein} \omega_4 \times hydrophobic(d_{ij}) + \sum_i^{ligand} \sum_j^{protein} \omega_5 \times Hbond(d_{ij}) \quad (3)$$

Where ω_1; ω_2; ω_3; ω_4; and ω_5 are weight values equal to 0.0356; 0.00516; 0.840; 0.0351; and 0.587; respectively. The combination of an attractive Gaussian function with a repulsive parabolic function reproduces the general shape of a typical Lennard-Jones interaction, provided the Gaussian term is negative and the parabolic positive. If both

atoms in the pair are hydrophobic, the linear function in Eq. (7) is included. Also, if the pair consists of an H-bond donor and an H-bond acceptor, Eq. (8) is added.

$$\text{gauss}_1 = e^{-(\frac{d}{0.5})^2} \tag{4}$$

$$\text{gauss}_2 = e^{-(\frac{d-3}{2})^2} \tag{5}$$

$$repulsion(d) = \begin{cases} d^2, & if\ d < 0; \\ 0, & if\ d \geq 0 \end{cases} \tag{6}$$

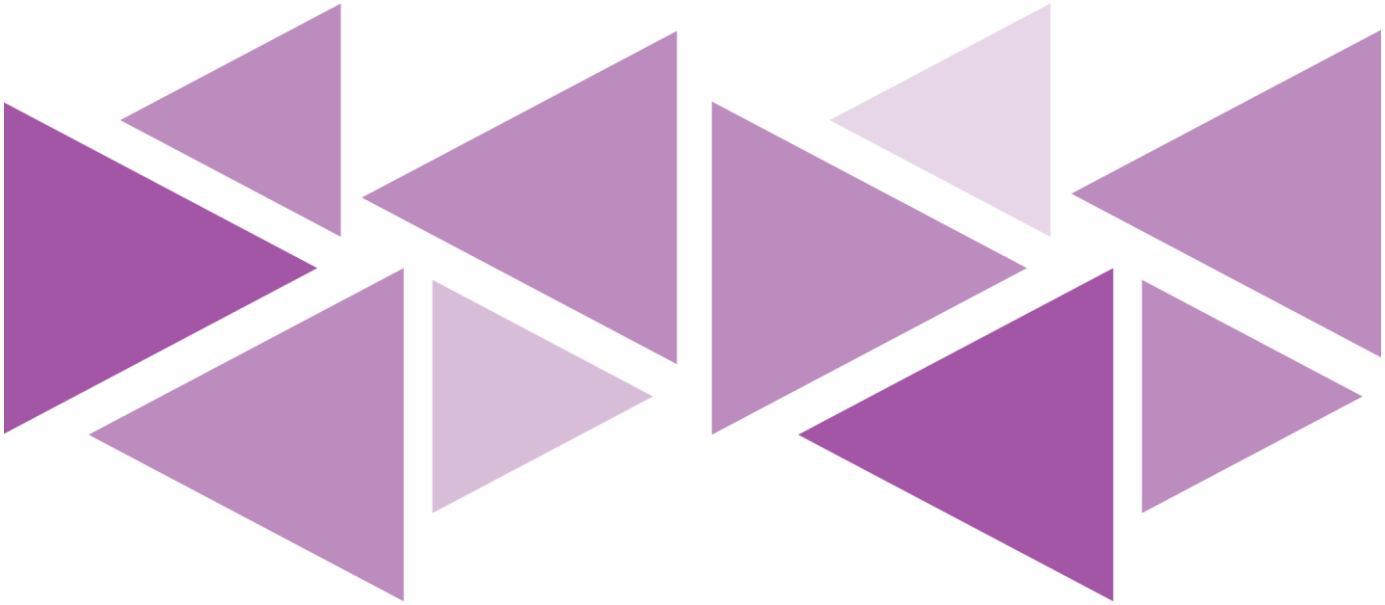
$$hydrophobic(d) = \begin{cases} 1.0, & if\ d < 0.5; \\ 1.5 - d, & if\ 0.5 \leq d \leq 1.5; \\ 0, & if\ d > 1.5 \end{cases} \tag{7}$$

$$Hbond(d) = \begin{cases} 1.0, & if\ d < -0.7; \\ \frac{d}{-0.7}, & if\ -0.7 \leq d \leq 0; \\ 0, & if\ d > 0 \end{cases} \tag{8}$$

The exhaustiveness parameter was set to 10. PyMol (Delano and Bromberg, 2004) and discovery studio visualizer 2019 (Biovia *et al.*, 2000) were used to analyze the docked complexes for surface complexes, types, and distances of non-covalent bonds. Let A_{ij} denote the binding affinity between the i-th target protein (i = 1, 2, . . . , m) and the j-th drug agent (j = 1, 2, . . . , n). To select the top-ranked lead compounds as the candidate drugs, we ordered the drug target proteins and agents according to the descending order of row sums $\sum_{j=1}^{n}$ Aij; i = 1,2, . . . ,m and column sums $\sum_{i=1}^{m}$ Aij; j = 1,2, . . . ,n, respectively. The discovery studio visualizer 2019 and PyMol software (Biovia *et al.*, 2000; Seeliger and De Groot, 2010; Delano and Bromberg, 2004; Delano, 2002; Janson and Paiardini, 2021; Morris *et al.*, 2009; Trott and Olson, 2009; DeLano, 2020) were used to examine the hydrogen bonds and hydrophobic interactions between CA compound and the hVDAC protein, as well as -helix part of hVDAC protein. Besides, the two-dimensional (2D) and 3D structures of the complexes were analyzed using discovery studio visualizer 2019.

Chapter 3

Result



Chapter 3: Result

3.1 Introduction

The process of data casting and computerization was addressed in the previous chapter. Before undertaking analysis, it is often necessary to understand the qualities or nature of the data. The graphical depiction might be highly beneficial for some subjects.

3.2 Identification of cDEGs for PCa Patients

The datasets GSE104749, GSE55945, and GSE46602 were analyzed to identify DEGs between PCa infections and control samples, and the DEGs in each dataset were presented using the volcano plots (Figure 3a–c), where red and blue dots represented the up-regulated and down-regulated genes, respectively. In GSE104749, a total of 4934 DEGs with 2075 up-regulated and 2859 down-regulated genes; in GSE55945, a total of 9445 DEGs with 3787 up-regulated and 5658 down-regulated genes; and in GSE46602, a total of 2403 DEGs with 794 up-regulated and 1609 down-regulated genes were identified by the R package limma with $|\log FC| > 1.0$ and p-value < 0.05. Then, we found 458 cDEGs for PCa patients (Figure 3d).

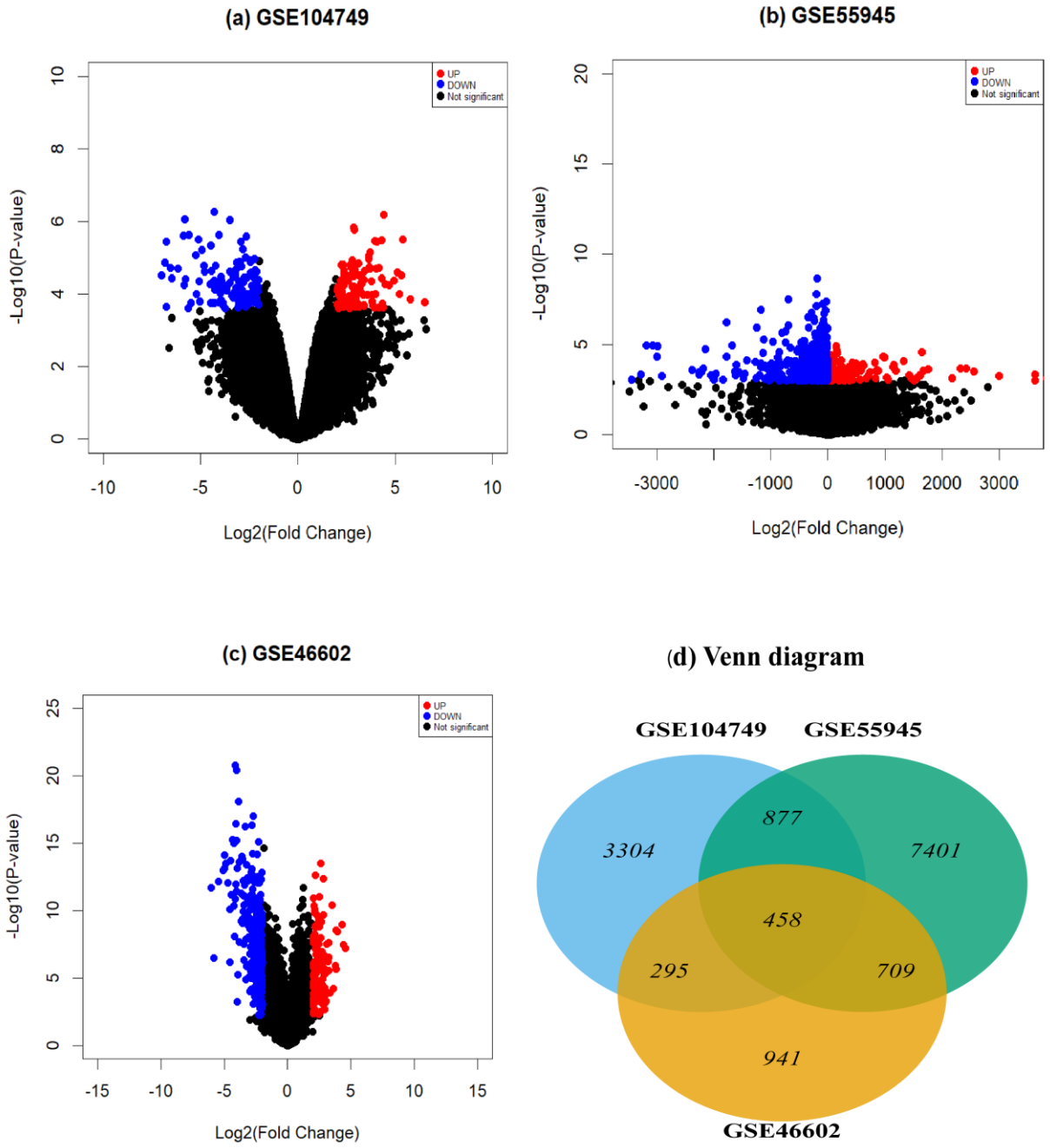
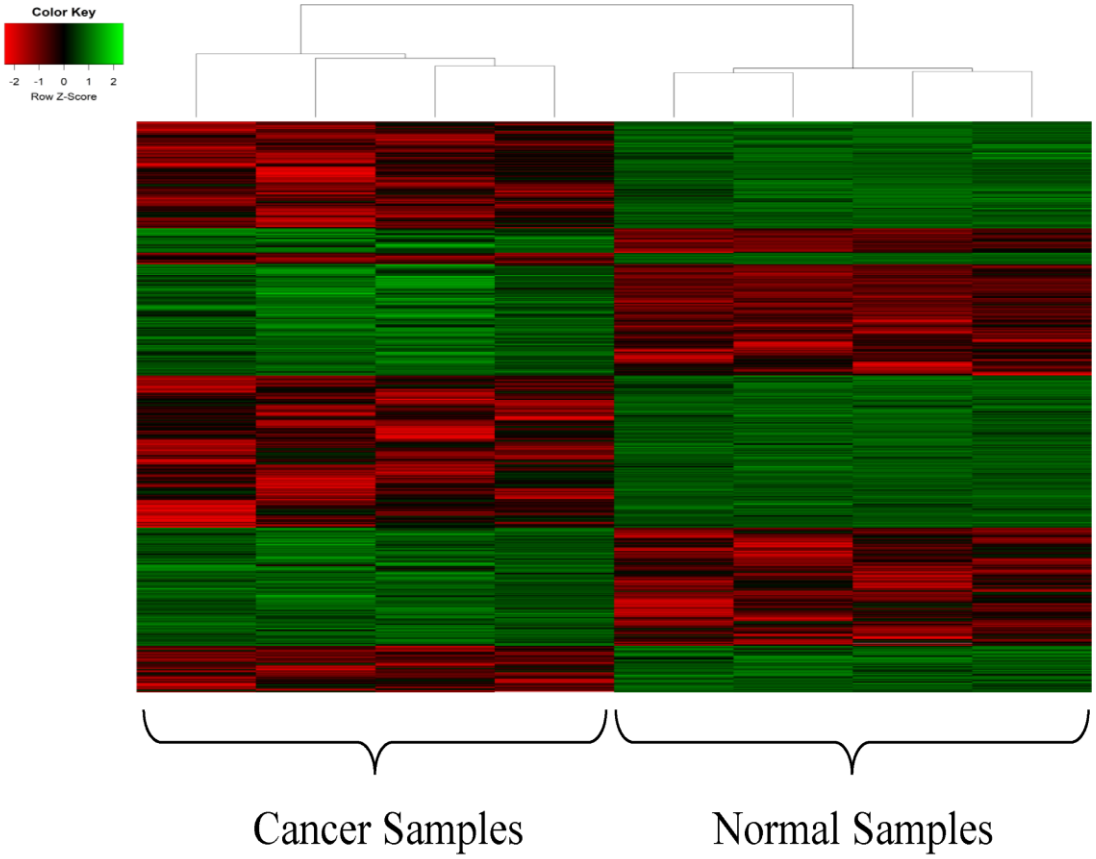
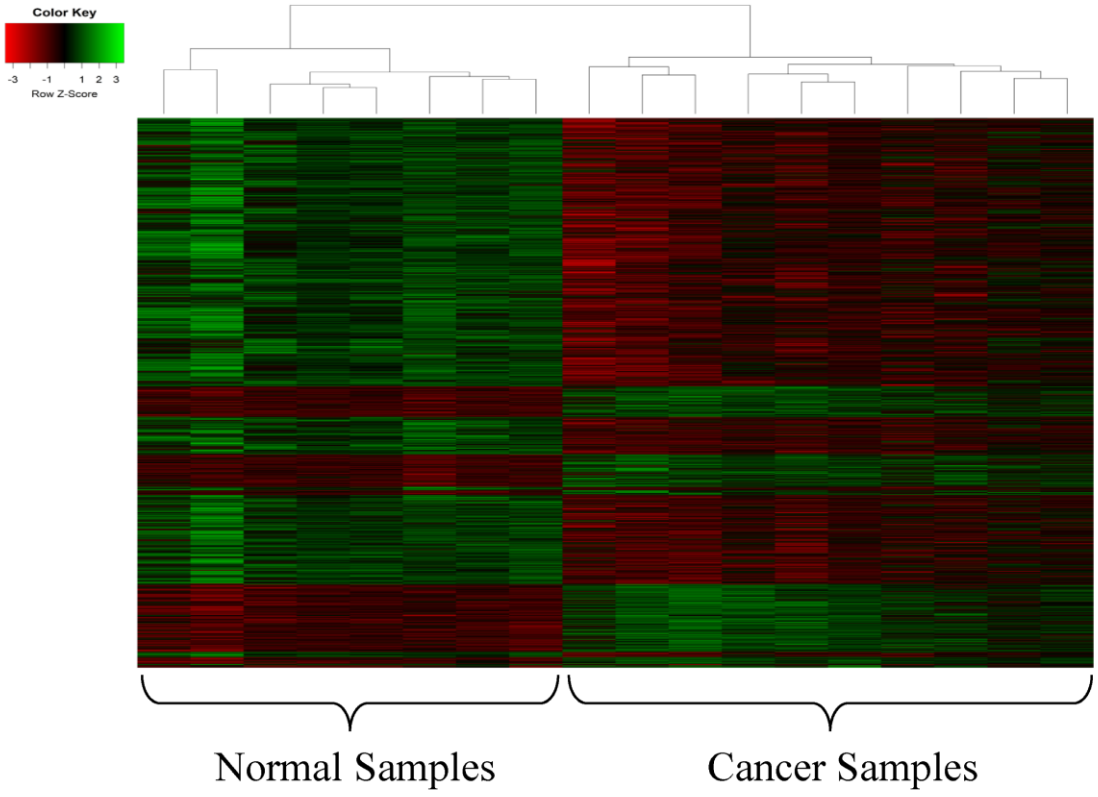


Figure 3.1: Screening of the overlapping DEGs among GSE104749, GSE55945, and GSE46602 datasets. The volcano plots of DEGs in (a) GSE104749, (b) GSE55945 and (c) GSE46602; blue dots and red dots represented the significantly down-regulated and up-regulated DEGs, respectively. (d) Common up-regulated and down-regulated differentially expressed genes from PCa visualized through a Venn diagram. Four-hundred and fifty eight genes were founded common- up-regulated and down-regulated in PCa patients.

(a) GSE104749



(b) GSE55945



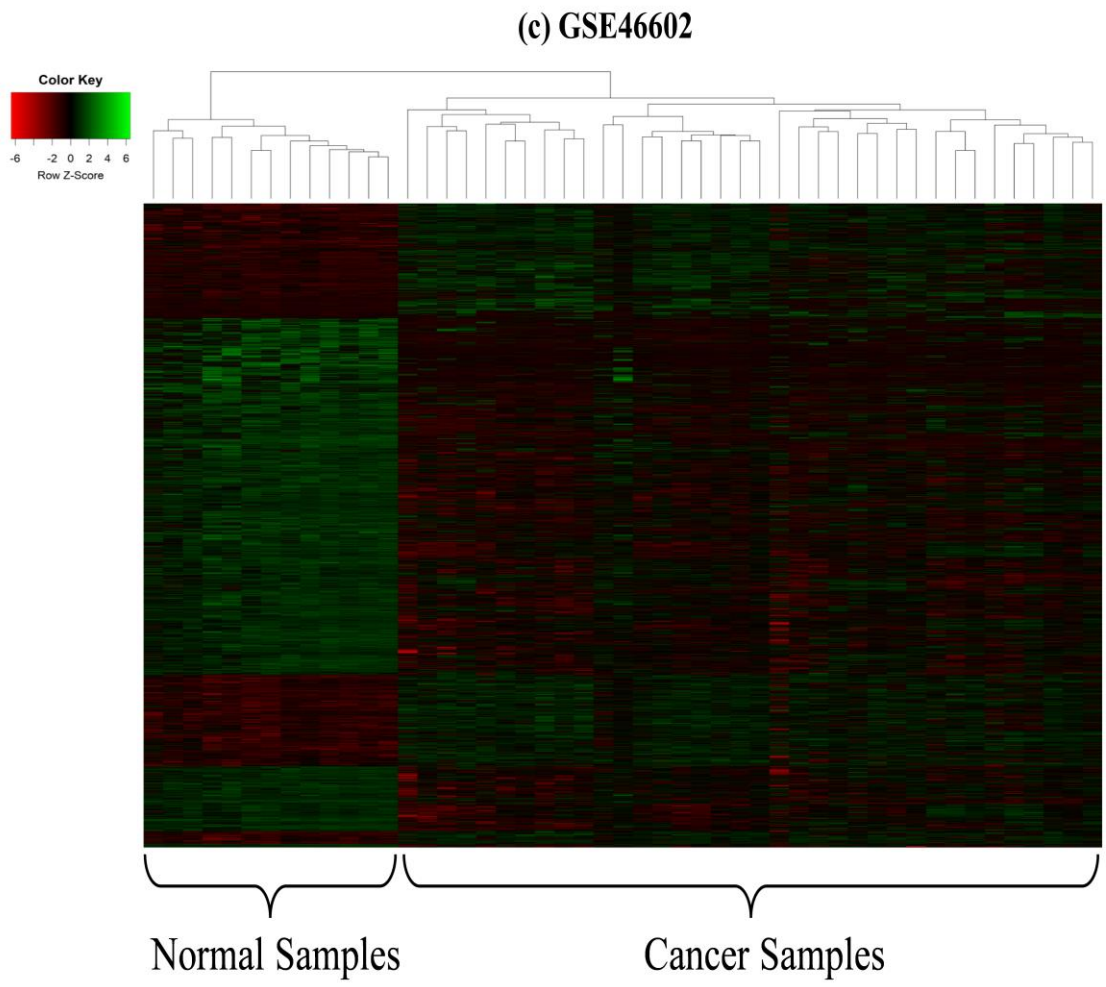


Figure 3.2: Heatmaps of the DEGs. The heatmaps show the expression profile of the DEGs in PCa compared to the adjacent normal tissues. The color scale indicates the Log2FoldChange of the expression value for each gene in cancer vs. normal tissues. Red colors indicate down-regulation and green colors indicate up-regulation.

3.3 Protein-Protein Network Analysis of cDEGs for Identification of KGs

The PPI network of cDEGs was constructed using STRING database, which contained 238 nodes and 539 edges. From the PPI network, we constructed a sub-network with the top 15 hub genes (HubGs) {TOP2A, RRM2, NCAPG, BUB1B, CENPU, CENPF, AURKA, TPX2, MKI67, BIRC5, CDCA5, NUSAP1, EZH2, ECT2, and TK1}, applying MCC, topological measures in the PPI network. The sub-network was shown in Figure 3.3. So, we considered these fifteen key genes (KGs) for further analysis.

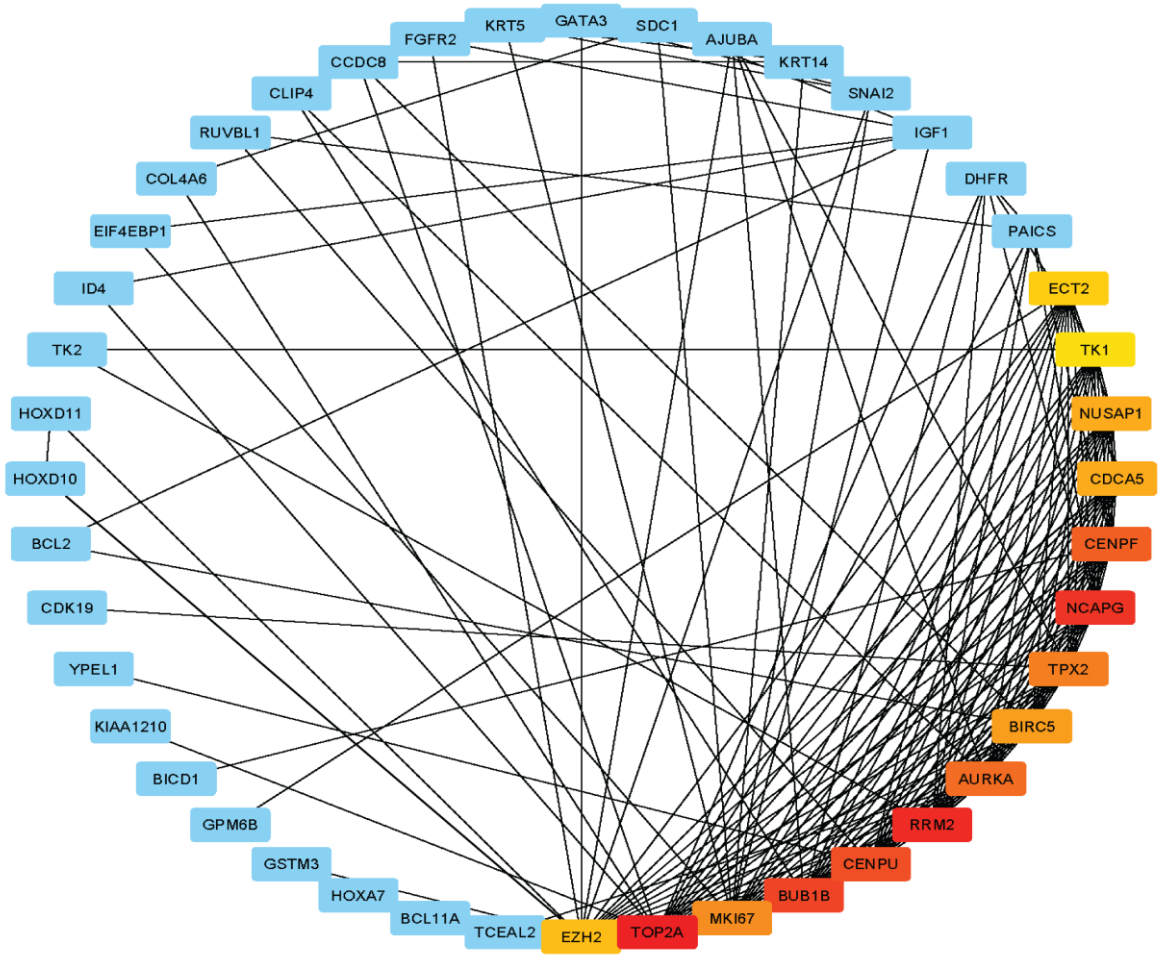


Figure 3.3: A sub-network with the top 15 hub genes.And their interacted genes constructed with the CytoHubba plugin of Cytoscape from the PPI network obtained from STRING database after mapping the 458 differentially expressed genes. The deepness of the red color indicates higher degree nodes.

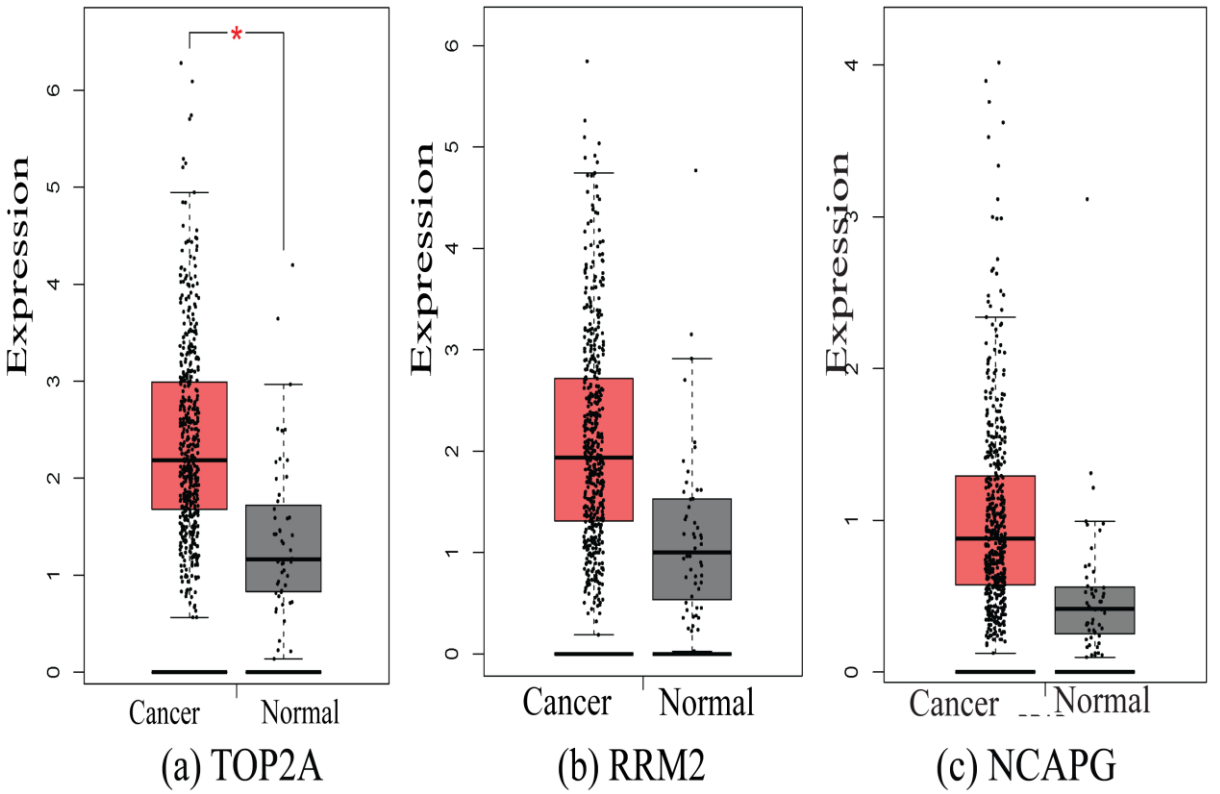
Table 1: Top 15 Hub Genes Ranked by MCC Method.

Rank	Gene Name	Score
1	TOP2A	6706022669.00
2	RRM2	6706022666.00
3	NCAPG	6706022664.00
4	BUB1B	6706022546.00
5	CENPU	6706022523.00
6	CENPF	6706022521.00
7	AURKA	6706022427.00
8	TPX2	6706022425.00
9	MKI67	6706022412.00
10	BIRC5	6706022403.00
11	CDCA5	6706022400.00
11	NUSAP1	6706022400.00
13	EZH2	6227020834.00
14	ECT2	6227020801.00
15	TK1	479001722.00

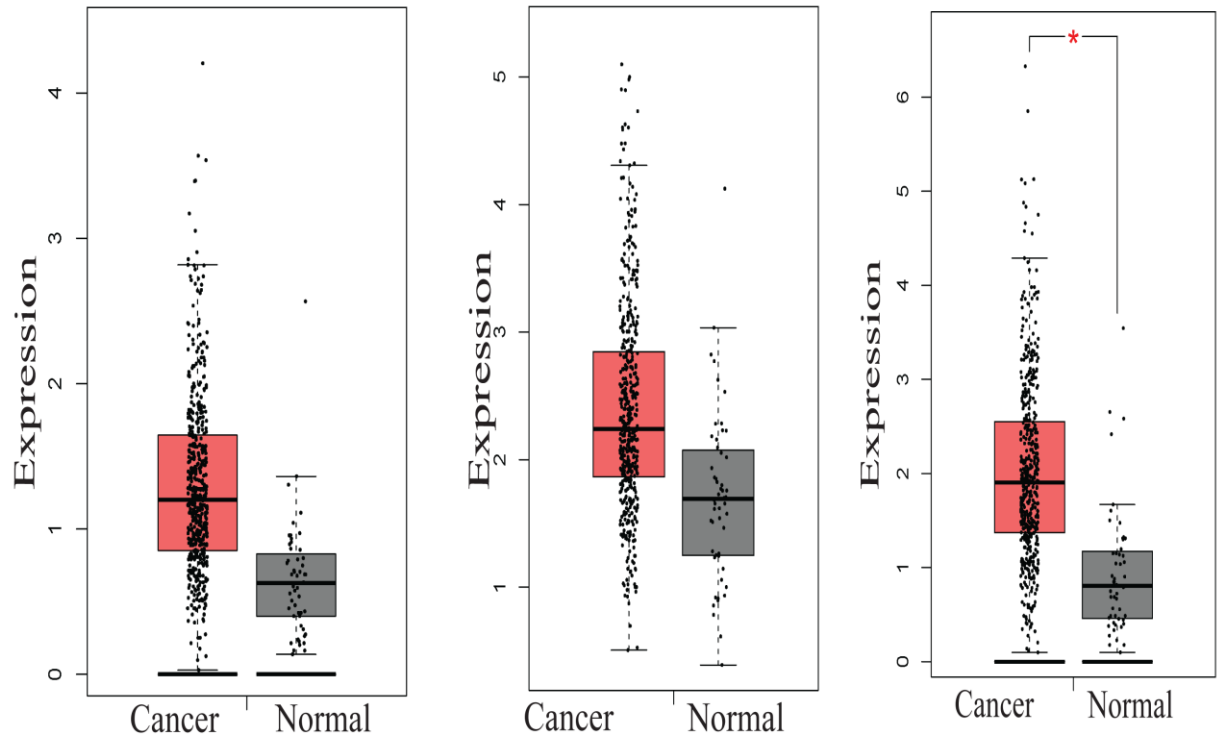
3.4 Expression and Survival Analyses of the Hub Genes in Prostate Cancer

The expressions of the 15 hub genes in the normal tissue samples and prostate tissue samples were examined by the GEPIA database. There were 492 prostate tumor samples and 52 normal samples in GEPIA obtained from TCGA database. We found that all 15 genes were upregulated in the PCa tissue compared to the normal tissue (Figure. 3.4(a)–3.4(o)). Thus, our findings were validated by the TCGA data.

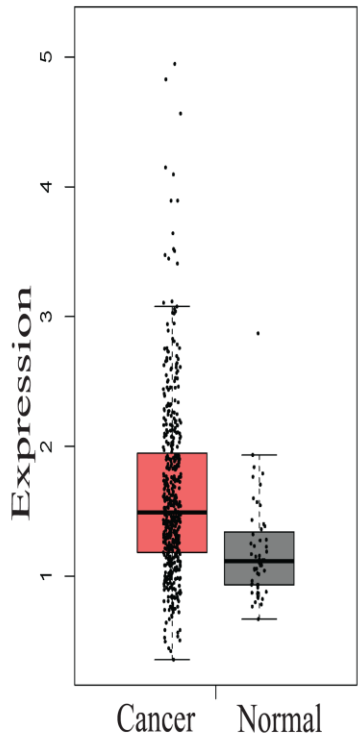
The correlation analysis between the expression of the hub genes in PCa and the overall survival of the PCa patients was performed by GEPIA tool using TCGA data. The survival data of 100 patients with PCa were given in the GEPIA database. The patients were classified into higher and lower groups based on the median values of the gene expression. As shown in Figure 3.5(a)-3.5(e), the lower expression of the genes BIRC5, CDCA5, CENPF, NUSAP1, and TK1 was associated with better survival of the PCa patients. And we found that BIRC5, CDCA5, CENPF, NUSAP1, and TK1 were statistically significant.



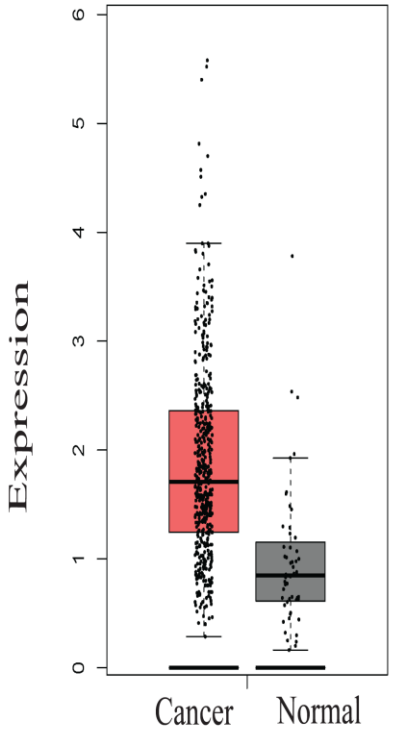
(a) TOP2A (b) RRM2 (c) NCAPG



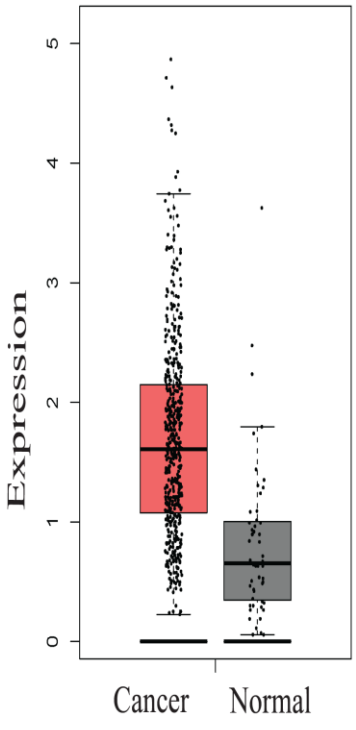
(d) BUB1B (e) CENPU (f) CENPF



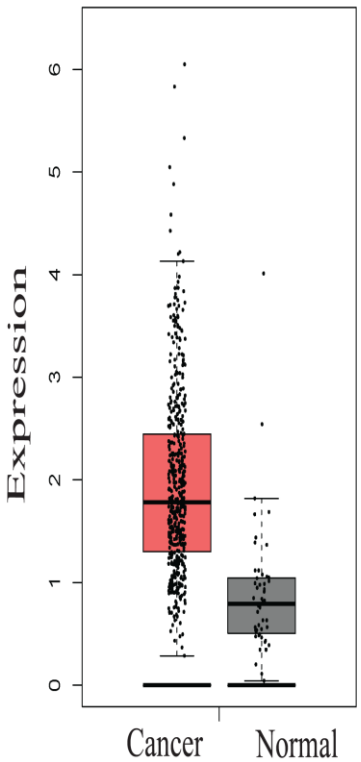
(g) AURKA



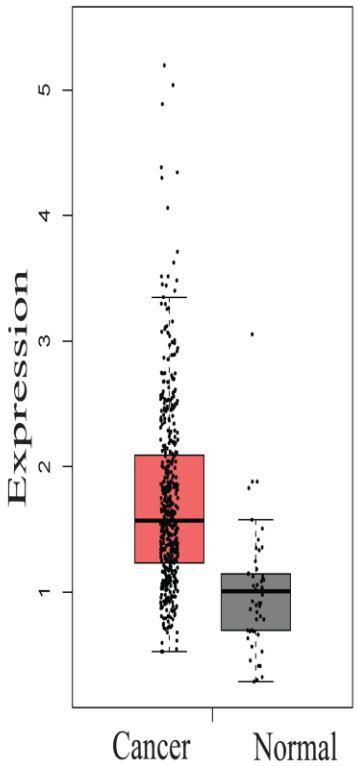
(h) TPX2



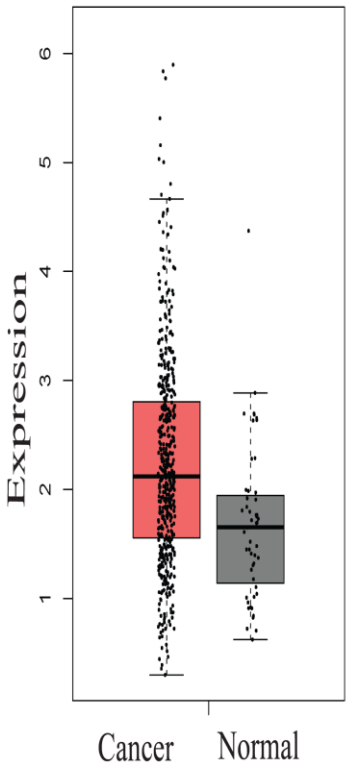
(i) MKI67



(j) BIRC5



(k) CDCA5



(l) NUSAP1

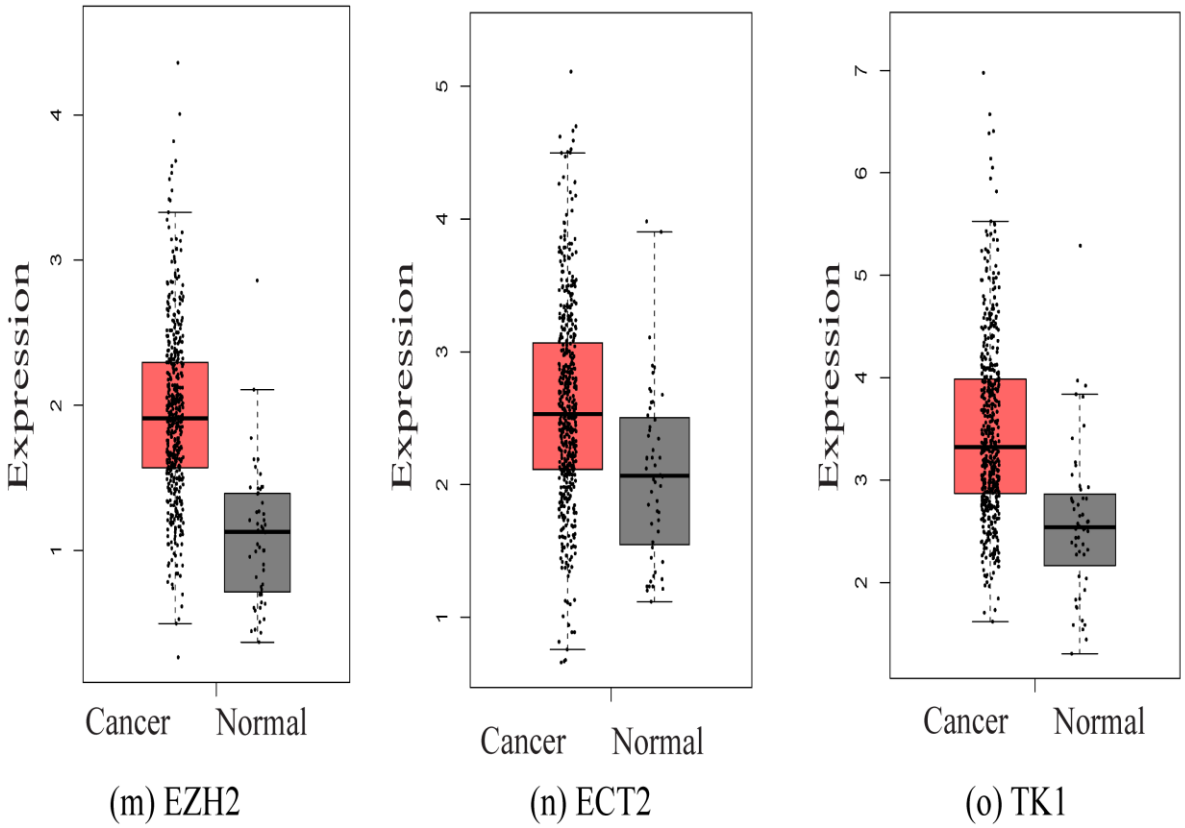


Figure 3.4: Validation of the expression of 15 hub genes in PCa. The expression of (a) TOP2A, (b) RRM2, (c) NCAPG, (d) BUB1B, (e) CENPU, (f) CENPF, (g) AURKA, (h) TPX2, (i) MKI67, (j) BIRC5, (k) CDCA5, (l) NUSAP1, (m) EZH2, (n) ECT2, and (o) TK1 were upregulated in prostate tumor tissues compared to normal tissues from the TCGA data through GEPIA. The cutoffs |Log2FoldChange|>1 and *p*-value<0.01 were considered as statistically significant.

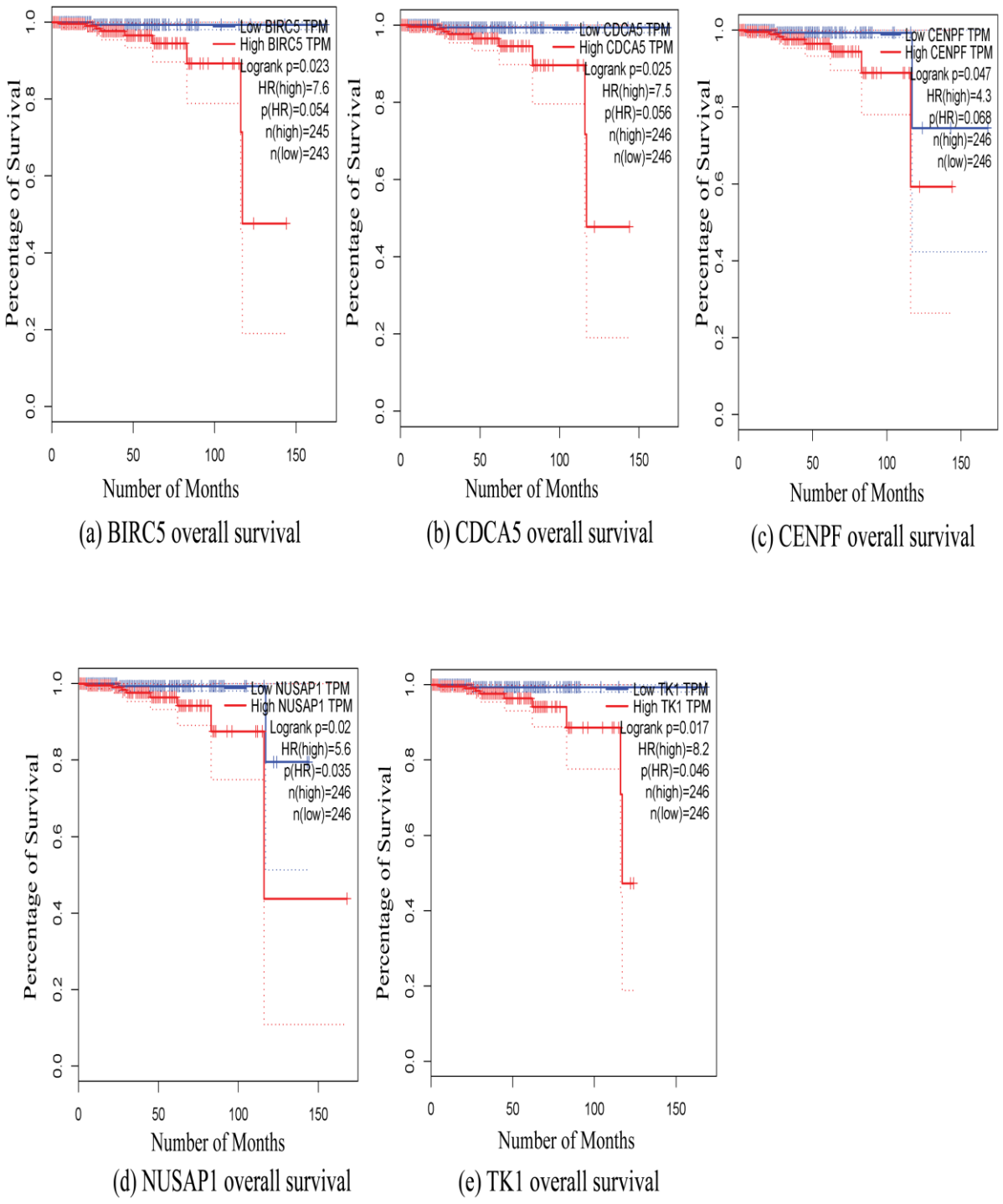


Figure 3.5: Overall survival analysis of the candidate hub genes.The association between the expression levels of (a) BIRC5, (b) CDCA5 (c) CENPF, (d) NUSAP1, and (e) TK1 and the overall survival of the patients with PCa was obtained from the TCGA data through GEPIA. The threshold log rank $P<0.05$ was considered as statistically significant.

3.5 Drug Repurposing by Molecular Docking Study

To explore candidate drugs by molecular docking simulation, we considered m= 5 drug target proteins (receptors) and n =211 meta-drug agents as mentioned in the data source. we downloaded 3D structure of our BIRC5 and TK1 from protein data bank (PDB) (Berman *et al.*, 2000) with source codes 1e31, and 2wvj, respectively. On the other hand, the 3D structures of CENPF, CDCA5,and NUSAP1 proteins were downloaded from SWISS-MODEL (Waterhouse *et al.*, 2018) with sources ID of P49454, Q96FF9,and Q9BXS6, respectively. The 3D structures of 211 meta-drug agents (Table A1) were downloaded from PubChem database (Kim *et al.*, 2019) as mentioned previously. Then, the molecular docking was carried out between total 5 proteins and 211 meta-drug agents to calculate the binding affinity scores (kcal/mol) for each pair of proteins and drugs. Next, we ordered the proteins in descending order of row sums of the binding affinity matrix and drug agents according to the column sums to select few drug agents as the candidate drugs for PCa (Figure 3.1 and Table A2) (Hossain *et al.*, 2023; Reza *et al.*, 2022; Sarker *et al.*, 2023). Thus, we selected top-ranked ten drug agents (Adapalene, Ergotamine, Irinotecan Hydrochloride, Drospirenone, Abiraterone Acetate, Zafirlukast, Ibrutinib, Abiraterone, Imatinib, and Telmisartan) as candidate drugs with the binding affinity scores –6.3 kcal/mol or less against the 5 proteins (Figure 3.2). The docked complexes of the top three virtual hits from PyRx docking were further considered for protein-ligand interaction profiling. As shown in Figure 3.3a TK1_ Adapalene complex showed interacting amino acids Arg82, Ala85, Leu89, Ala111, Ala115, Val106, Glu107, and Ala111 respectively. In Figure 3.7b, Ergotamine_BIRC5 complex showed Lys15, Asp16, Glu40, Ile74, Lys78, Val89, Lys91, Gln92, and Phe93 respectively. And, in Figure 3.7c, Irinotecan Hydrochloride_TK1 complex showed Arg18, Gln20, Glu35, Glu144, Ser145, Val147, Val147, Leu149, Glu159, and Glu159 respectively.

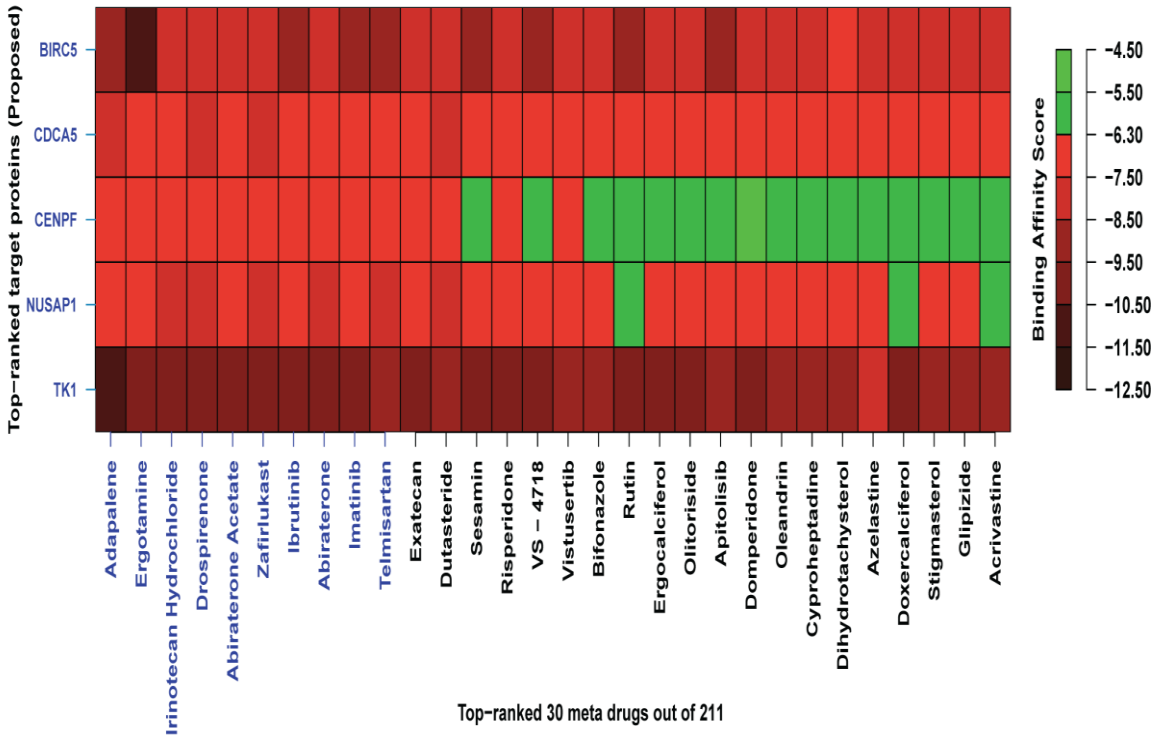


Figure 3.6: Image of binding affinity scores based on the top-ordered 30 meta-drug agents out of 211 against the ordered 5 receptors.Where red colors indicate the strong binding affinities between target proteins and drug agents, and green colors indicate their weak bindings.

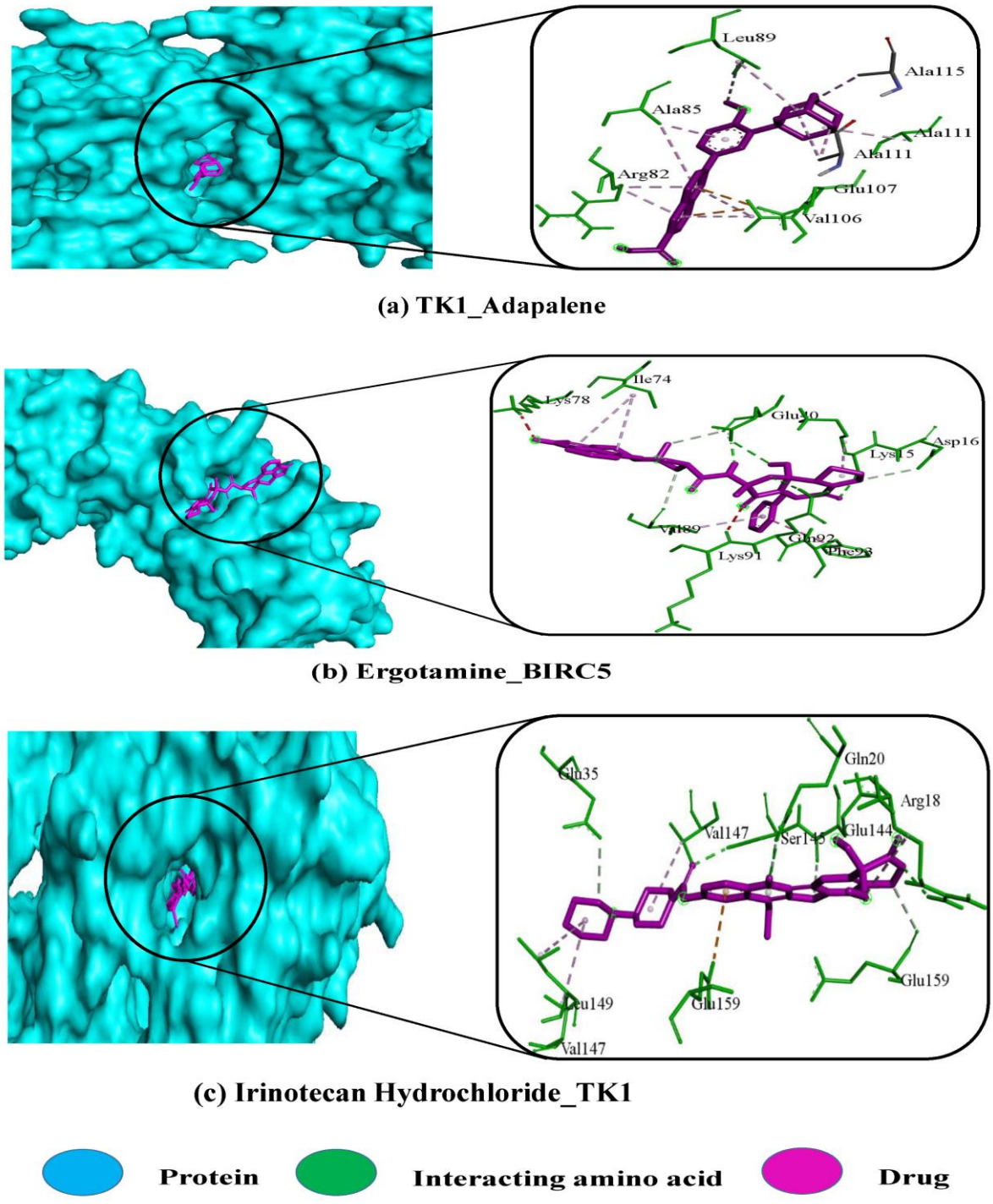
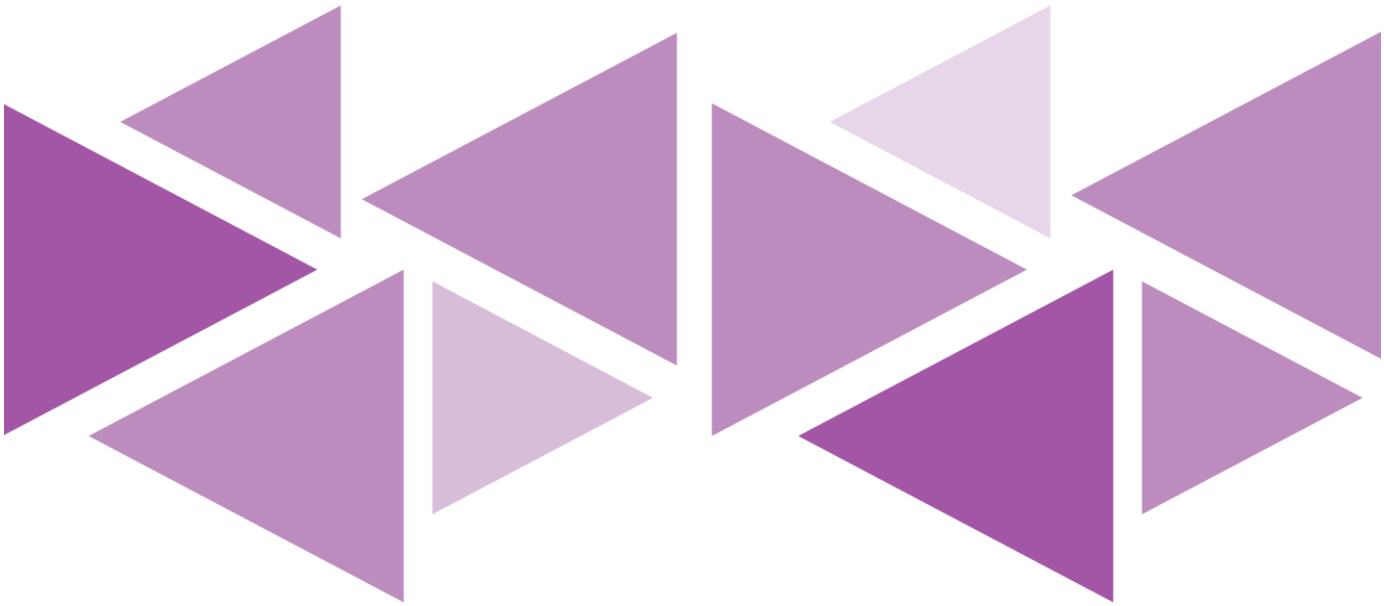


Figure 3.7: Top three potential targets and top three lead drugs based on docking results. Lead three drugs Adapalene, Ergotamine, and Irinotecan Hydrochloride were selected by investigating the binding affinity score. The 3D structure of the key protein with candidate drugs is shown in left side. The 2D Schematic diagram of key protein with candidate drugs interaction and the neighbor residues (within 410–10 m of the drug) are given in right side.

Chapter 4

Discussion and Conclusion



Chapter 4: Discussion and Conclusion

In reality, research is a methodical quest for information. It's a low-key endeavor to uncover the truth. It's a thorough investigation or examination that aims to broaden and confirm current knowledge. With the use of study, a researcher strives to build his impression. The goal of our research is to alter the delivery method. The point of the study is to gain a clear understanding of the proposed molecular biomarkers and repurposing candidate drugs presented in this study have merit for diagnosis and therapies of PCa disease. We employed a modest sample size and a purposeful approach due to time and money restrictions. Finally, this research will benefit societal awareness, men, and researchers who want to do further research.

4.1 Discussion

PCa is a malignancy cancer in urinary system of men, it is the most prevalent cancer in males and is accountable for a fifth cancer-related deaths in males globally. Although a lot of advancements have been made in PCa diagnosis and treatment, the survival rate is still poor due to lack of early diagnosis and proper treatments at advanced level of PCa. Thus, more research is required to discover potential biomarkers and candidate drugs for improving the survival rate and reducing the mortality rate of PCa patients.

To investigate the genetic influence of PCa infections, we identified 458 cDEGs. Among them, we detected fifteen hub genes TOP2A, RRM2, NCAPG, BUB1B, CENPU, CENPF, AURKA, TPX2, MKI67, BIRC5, CDCA5, NUSAP1, EZH2, ECT2, and TK1 from the PPI network of cDEGs. The survival analysis showed that the lower expression of the genes BIRC5, CDCA5, CENPF, NUSAP1, and TK1 were associated with better survival of the PCa patients (Figure 3.5). Five of these genes were reported as important biomarkers of PCa by previous studies. For example, BIRC5 might play a critical role in metastasis of PCa (Fan *et al.*, 2018). BIRC5, which play an important role in the occurrence and progression of cancer (Altieri, 2008). Wang et al reported that BIRC5 was involved in the tumorigenesis of colorectal cancer (Wang *et al.*, 2014). BIRC5 was reported to be associated with microtubule-kinetochore attachment, interacting with cell adhesion (Vitale *et al.*, 2011). BIRC5 is an immune-related gene that inhibits apoptosis

and promotes cell proliferation. It is highly expressed in most tumors and leads to poor prognosis in cancer patients (Xu *et al.*, 2021). Survivin (BIRC5) gene expression was increased in primary prostate cancers and metastases, but did not differ in recurrent vs non-recurrent prostate cancers (Hennigs *et al.*, 2020). CENPF has been shown to be a synergistic master regulator of prostate cancer malignancy and a prognostic indicator of poor survival and metastasis (Aytes *et al.*, 2014). CENPF upregulation is linked to aggressive tumor features in a variety of malignancies including prostate cancer (Göbel *et al.*, 2018). CENPF, which are master regulators of prostate cancer malignancy (Mitrofanova *et al.*, 2015). CDCA5 was upregulated and affected the prognosis in patients with PCa. Decreased expression of CDCA5 inhibited PCa cell proliferation by inhibiting the ERK signaling pathway. Consequently, CDCA5 may be a potential therapeutic target for PCa (Ji *et al.*, 2021). Upregulated CDCA5 expression in PCa tissues may play a crucial role in the occurrence of PCa (Gu *et al.*, 2022). The cell division cycle associated protein (CDCA) family, which plays a critical role in cell division and proliferation, is upregulated in the PCa cell lines (Zhang *et al.*, 2020). In prostate cancer, NUSAP1 increases invasion, cell migration, and metastasis (Gulzar *et al.*, 2013). NSAP1 is found in prognostic gene sets associated with high grade compared to low grade prostate cancer (Penney *et al.*, 2011). TK1 is a prognostic biomarker for PCa (Zhang *et al.*, 2022). Recent findings suggest that intracellular TK1 is correlated with cancer cell invasion and progression, along with its signature role in cancer cell proliferation (Alshabi *et al.*, 2019). The blood concentration of TK1 at baseline was associated with future PCa-specific mortality (Lundgren *et al.*, 2022). Serum concentrations of TK1 and PSA can both predict long-term risk in PCa (Tribukait *et al.*, 2023). Thus, these five genes BIRC5, CDCA5, CENPF, NUSAP1, and TK1 might serve as potential biomarkers for PCa.

To explore effective candidate drugs for the treatment against PCa disease, we proposed 5 key proteins (BIRC5, CDCA5, CENPF, NUSAP1, and TK1) as the drug target receptors and performed their docking simulation with the 211 meta-drug agents (Table A2). Then, we selected top ranked ten drugs (Adapalene, Ergotamine, Irinotecan Hydrochloride, Drospirenone, Abiraterone Acetate, Zafirlukast, Ibrutinib, Abiraterone, Imatinib, and Telmisartan) as the most probable repurposable candidate drugs for PCa infections based on their strong binding affinity scores (kcal/mol) with all the target proteins (Figure 3.6). Among the identified candidate drugs, Adapalene inhibited

prostate cancer cell proliferation, elicited apoptosis, and arrested the cell cycle in the S-phase. Adapalene also slowed the rate of tumor growth and bone destruction *in vitro*. Adapalene may be a potential treatment against prostate cancer (Nong *et al.*, 2022). Adapalene exerts strong in vitro and in vivo antiproliferative effects on different PC cell lines (i.e., DU-145, PC-3, RM-1), by inducing DNA damage, S-phase cell cycle arrest, and apoptosis (Nong *et al.*, 2022; Lu *et al.*, 1999). We have not found many supporting articles of Ergotamine for treatment against PCa, we found these two articles for Ergotamine (Watanabe *et al.*, 2017; Bai *et al.*, 2020) because Ergotamine gives a good binding affinity, so it may be applied as a therapeutic drug for the prostate cancer. Irinotecan hydrochloride, IRI-STNM performed significantly better than the native IRI and non-targeted nanomicelles which was led by higher cellular uptake of IRI-STNM, indicating the role of serotonin in targeting of drug loaded nanomicelles (Tunki *et al.*, 2020). Drospirenone exhibits antiandrogenic activity in castrated, testosterone-substituted male rats as shown by dose-dependent inhibition of accessory sex organ growth (prostate, seminal vesicles) (Muhn *et al.*, 1995). Abiraterone acetate is an irreversible inhibitor of CYP17A1, a 17–20 lyase and 17-α hydroxylase of the cytochrome P450 family, which converts pregnanes into steroid hormones, including androgen precursors. It can, therefore, block androgen production in the testis and adrenal glands, and also in prostate tumors, thus preventing prostate cancer growth (Scott, 2017; Nevedomskaya *et al.*, 2018). Long-term use of Zafirlukast decreases cancer risk (Wolf *et al.*, 2022). Zafirlukast inhibited the growth of multiple cancer cell lines (Gelzinis 2023). Ibrutinib is a potent BTK inhibitor which targets B-cell signaling pathways, has an established safety profile, and has been shown to inhibit in vivo prostate tumor growth pre-clinically (Nawaf *et al.*, 2023). Ibrutinib may be applied as a therapeutic drug for the prostate cancer (Zhu *et al.*, 2020). The drug (Ibrutinib, Ibr) encapsulated with different nanocomposites was used for effective prostate cancer treatment (Murugesan *et al.*, 2021). The efficacy of Morusflavone, in comparison with Abiraterone, in interacting stably with CYP17A1, which is a therapeutic target for prostate cancer (Abdi *et al.*, 2021a). For patients with metastatic castration-resistant PCa (mCRPC) several survival-prolonging therapeutic options, like androgen receptor (AR)-directed substances (Abiraterone) are available (Hebenstreit *et al.*, 2020). Imatinib studies in prostate cancer have now demonstrated that p-PDGFR inhibition can control local tumors but facilitate metastatic progression (Rosenberg and Mathew, 2013). This first study evaluated the efficacy and safety of Imatinib in patients with early androgen

sensitive prostate cancer following local therapy (Rao *et al.*, 2005). Prostate Cancer Cultured DU145 cells were treated pharmacologically with Telmisartan, Telmisartan induces prostate cancer DU145 cells apoptosis through the up-regulation of PPAR-delta protein expression (Wu *et al.*, 2016). Telmisartan is a potent target for prevention and treatment in PC (Funao *et al.*, 2008). The Telmisartan-containing complex inhibited the proliferation of prostate cancer cells at lower concentrations than the free drug (Novel antidote to prostate cancer, 2013).

The literature review also supported our proposed drugs for the treatment against PCa infections. Therefore, the proposed ten candidate drugs might play the vital role for the treatment against PCa patients with comorbidities as our proposed target proteins are also associated with several comorbidities. Further wet lab experimental validation is needed for both the proposed target proteins and candidate drugs to confirm the role of the candidate drugs for the treatment of PCa.

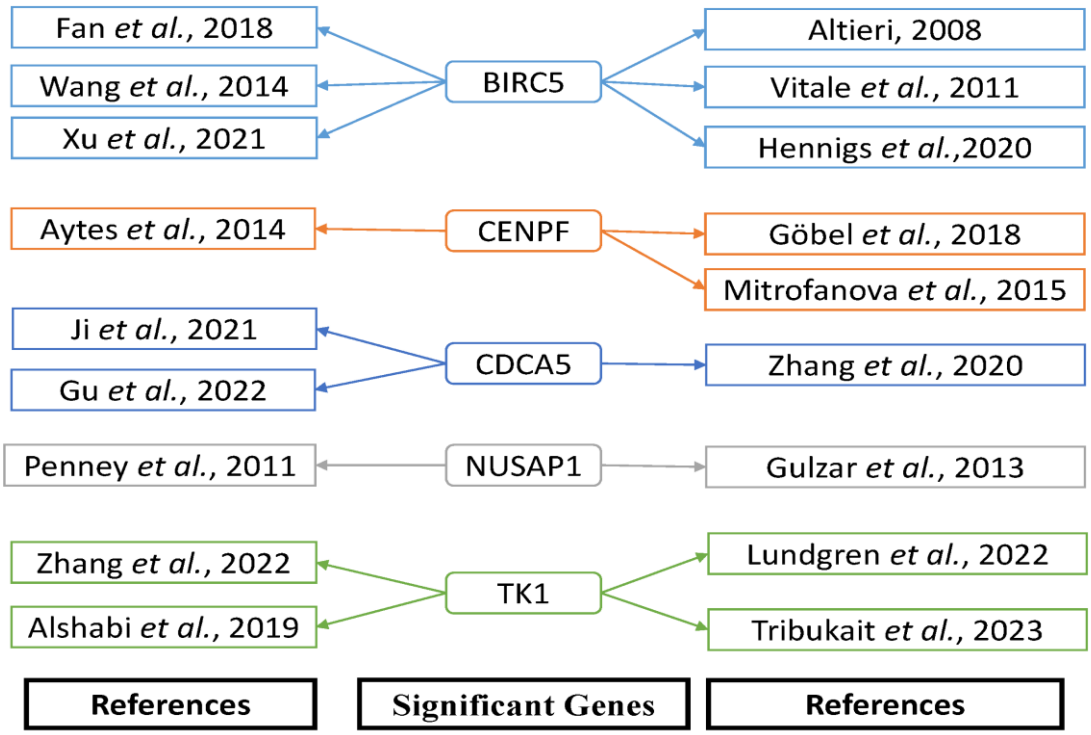


Figure 4.1: Five proposed hub genes. Here, the first and third columns indicated different articles, and the second column indicated the top ranked hub genes. Different color connected networks indicated the different significant genes.

4.2 Conclusion

We identified a total of 458 cDEGs. Through the PPI network analysis, we screened 15 hub genes. The TCGA data validated the expressions of the hub genes. Among the 15 hub genes, five genes, BIRC5, CDCA5, CENPF, NUSAP1, and TK1, were significantly associated with the PCa patients found by survival analysis. Therefore, these five genes, BIRC5, CDCA5, CENPF, NUSAP1, and TK1, might be considered as potential biomarkers for PCa diagnosis and treatment. In this project, we also attempted to suggest effective supporting drugs for the treatment against PCa patients. For this purpose, we identified 5 host receptor proteins (BIRC5 , CDCA5 , CENPF, NUSAP1, and TK1) guided top ranked 10 repurposable drugs (Adapalene, Ergotamine, Irinotecan Hydrochloride, Drospirenone, Abiraterone Acetate, Zafirlukast, Ibrutinib, Abiraterone, Imatinib, and Telmisartan) for the treatment against PCa patients by molecular docking simulation. The literature review also supported our proposed drugs for the treatment against PCa patients. Thus, the proposed molecular biomarkers and repurposing candidate drugs presented in this study have merit for diagnosis and therapies of PCa disease.

4.3 Areas of Further Research

In this project, we use the R package LIMMA, however other methods can be applied. The gene ontology (GO) term and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analyses can also be used. We only use Molecular Docking for drug discovery, However, Molecular Dynamic Simulations also can be used for better validation.

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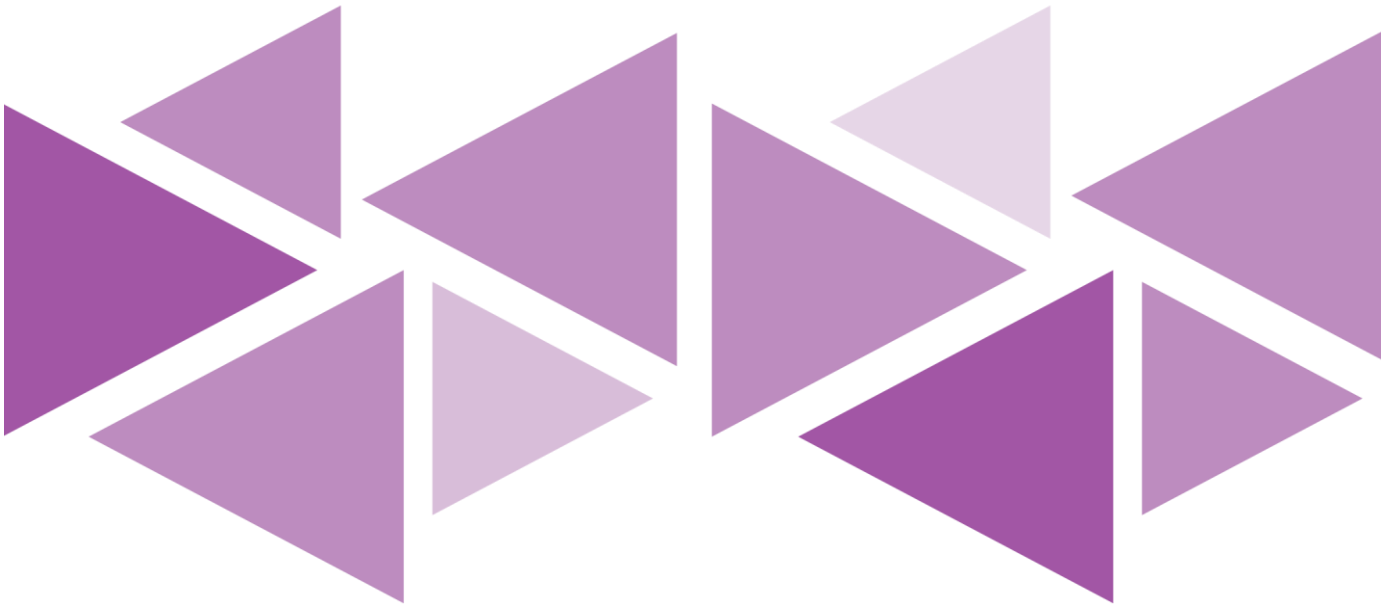

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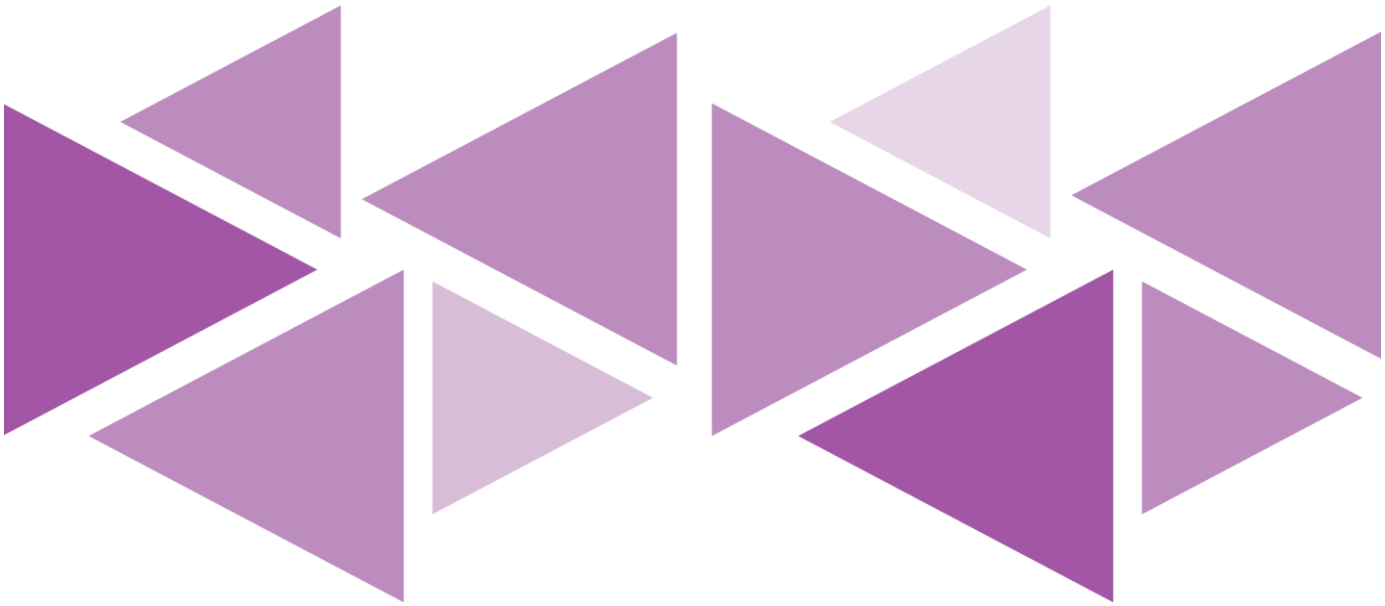
APPENDIX



APPENDIX

Table A1: 211 meta-drug agents list.

Drug name	Reference
Adapalene, Ergotamine, Irinotecan Hydrochloride, Drospirenone, Abiraterone Acetate, Zafirlukast, Ibrutinib, Abiraterone, Imatinib, Telmisartan, Exatecan, Dutasteride, Sesamin, Risperidone, Vs-4718, Vistusertib, Bifonazole, Rutin, Ergocalciferol, Olitoriside, Apitolisib, Domperidone, Oleandrin, Cyproheptadine, Dihydrotachysterol, Azelastine, Doxercalciferol, Stigmasterol, Glipizide, Acrivastine, Ouabain, Lapatinib, Beta-Sitosterol, Nelfinavir, Epirubicin, Methysergide, Testolactone, Cholecalciferol, Desonide, Celecoxib, Daridorexant, Luteolin, Testosterone, Idelalisib, Doxorubicin, Eriodictyol, Fluorometholone, Isofucosterol, Alfacalcidol, Berotralstat, Ethynodiol_Diacetate, Clocortolone, Calcitriol, Bisacodyl, Morindone, Methotrexate, Mifepristone, Azatadine, Prasterone, Dexamethasone, Naftopidil, Tolazamide, Zaleplon, Alprazolam, Bms-214662, Anthragallol, Strophanthidin, Mianserin, Soranjidiol, Triamcinolone, Azd5363, Ormeloxifene, Ertapenem, Indomethacin, Taxifolin, Finasteride, Dht (Dihydrotestosterone), Prednisone, Simvastatin, Zopiclone, Afuresertib, Catechin, Butorphanol, Diflunisal, Quercetin, Romidepsin, Budesonide, Calycosin, Kaempferol, Lucidin, Nortriptyline, Buparlisib, Niclosamide, Isorhamnetin, Thalidomide, Morphine, Tretinoin, Formononetin, Hydrocortisone, Bimiralisib, Medroxyprogesterone Acetate, Protriptyline, Tetracycline, Atorvastatin, Vs-5584, Bicalutamide, Enzalutamide, Ipatasertib, Nalbuphine, Sulindac, Flutemetamol (18F), Amitriptyline, Damnacanthal, Picropodophyllin, Flavin Mononucleotide, Gentian Violet Cation, Syringaresinol, Demeclocycline, Tapinarof, Levofloxacin, Norgestimate, Trimetrexate, Hydroxyzine, Imiquimod, Esculin,	(Abdi *et al.*, 2021b; Bahmad *et al.*, 2022; Bernal *et al.*, 2023; Bibby *et al.*, 2021; Green and Sambrook, 2022; Lourenço and Vale, 2023a; Lu *et al.*, 2022; Malik *et al.*, 2022; Nevedomskaya *et al.*, 2018; Rahman, 2015; Selvaraj *et al.*, 2021; Srivastava *et al.*, 2021; Tan *et al.*, 2015; Funao *et al.*, 2008; Rao *et al.*, 2005; Murugesan *et al.*, 2021; Muhn *et al.*, 1995; Zhu *et al.*, 2020; Nawaf *et al.*, 2023; Scott, 2017; Wolf *et al.*, 2022; Wu *et al.*, 2016; Tunki *et al.*, 2020; Hebenstreit *et al.*, 2020; Nong *et al.*, 2022; Lu *et al.*, 1999; Rosenberg and Mathew, 2013; Fan *et al.*, 2018; Abdi *et al.*,

(to be Continued)

Table A1: 211 meta-drug agents list.

Drug name	Reference
Saxagliptin, Erlotinib, Clofoctol, Tofranil, Zidovudine, Clemastine, Rosuvastatin, Bimatoprost, Omacetaxine Mepesuccinate, Tiaprofenic Acid, Cinoxacin, Patent Blue, Tetryzoline, Curcumin, Lesinurad, Vernakalant, Flutamide, Clobazam, Trimipramine, Propranolol, Hexachlorophene, Biib021, Ethylhexyl Methoxycrylene, Guanadrel, Methylphenobarbital, Plumbagin, Cladribine, Matrine, Trifluridine, Homoharringtonine, Atropine, Tioconazole, Zenarestat, Chloramphenicol, Latanoprost, Diclofenac, Formoterol, Gemcitabine, Clofarabine, Melatonin, Melphalan Flufenamide, Chlorothiazide, Ciclopirox, Menadione, Perindopril, Oseltamivir, Nitroxoline, Pindolol, Vorinostat, Azacitidine, Cedazuridine, Ethotoin, Ferroquine, Albendazole, Iobenguane, Tropicamide, Lidocaine, Talbutal, Acetazolamide, Gemeprost, Pentobarbital, Aspirin, Didanosine, Profenamine, Memantine, Levomilnacipran, Triclosan, Salicylic Acid, Mexiletine, Norepinephrine, Midodrine, Zoledronic Acid, Caffeine, Pilocarpine, Phenylephrine, Chlorzoxazone, Carboplatin, Chloroquine, Cimetidine, Thiopental, Valproic Acid, Fluorouracil, Ethchlorvynol, Busulfan, Histamine, Sodium Acetate	2021a; Yuan *et al.*, 2017; Wu *et al.*, 2021; Turanli *et al.*, 2018; Lourenço and Vale, 2023b; Chen *et al.*, 2016; Robert D. Fleischmann *et al.*, 1995)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Adapalene	-8.8	-7.8	-7	-7.3	-11.4
Ergotamine	-10.7	-7.3	-6.8	-6.9	-10.5
Irinotecan Hydrochloride	-8.3	-7.5	-6.5	-8.1	-10.5
Drospirenone	-8	-7.7	-6.6	-8	-9.7
Abiraterone Acetate	-8.4	-7.3	-6.4	-7.4	-10.4
Zafirlukast	-8	-7.8	-6.5	-8	-9.6
Ibrutinib	-8.7	-6.9	-6.5	-7.4	-10.3
Abiraterone	-7.8	-7.5	-6.7	-7.6	-10.1
Imatinib	-8.8	-7.5	-6.4	-7.1	-9.9
Telmisartan	-8.7	-7.5	-6.6	-8	-8.6
Exatecan	-7.8	-7.3	-7.1	-7.4	-9.6
Dutasteride	-7.8	-7.8	-6.4	-7.8	-9.2
Sesamin	-8.7	-7.2	-6.3	-6.7	-9.9
Risperidone	-8.4	-6.6	-6.4	-7.3	-9.9
Vs-4718	-8.7	-7	-6.3	-6.4	-10
Vistusertib	-7.8	-6.9	-6.9	-7.2	-9.4
Bifonazole	-8.2	-7.4	-6.1	-6.8	-9.1
Rutin	-9.1	-6.9	-5.7	-6.2	-9.7
Ergocalciferol	-8.2	-7.1	-5.9	-6.6	-9.7
Olitoriside	-8	-6.7	-6.1	-6.6	-10.1

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Apitolisib	-8.7	-7	-6.2	-6.4	-9.1
Domperidone	-8.4	-6.6	-5.5	-6.6	-10.2
Oleandrin	-8	-6.9	-6.2	-7	-8.7
Cyproheptadine	-7.9	-6.4	-6.1	-7	-9.3
Dihydrotachysterol	-7.5	-6.5	-6	-7	-9.5
Azelastine	-8.1	-6.9	-6.2	-6.8	-8.4
Doxercalciferol	-7.8	-6.6	-5.8	-6.2	-10
Stigmasterol	-7.8	-6.9	-5.9	-6.6	-9
Glipizide	-8.2	-6.4	-5.8	-6.7	-9
Acrivastine	-7.7	-7	-5.7	-6.2	-9.2
Ouabain	-8.5	-5.8	-5.8	-6.5	-9.1
Lapatinib	-8.2	-5.5	-5.9	-6.8	-9.2
Beta-Sitosterol	-7.8	-6.6	-5.2	-6.5	-9.5
Nelfinavir	-7.8	-6.6	-5.7	-6.1	-9.4
Epirubicin	-7.6	-6.4	-6	-6.6	-8.9
Methysergide	-8	-6.6	-5.6	-6.4	-8.9
Testolactone	-7.4	-6.7	-5.6	-7.3	-8.5
Cholecalciferol	-7.3	-6.9	-5.6	-6.1	-9.5
Desonide	-7.7	-6.6	-6.1	-6.7	-8.3
Celecoxib	-8.5	-6.8	-5.5	-6.6	-7.9

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Daridorexant	-7.8	-6.6	-5.8	-6.5	-8.6
Luteolin	-7.6	-6.2	-6.3	-6.1	-9
Testosterone	-7.3	-6.3	-5.9	-6.8	-8.9
Idelalisib	-7.8	-7.1	-5.6	-6.5	-8.1
Doxorubicin	-7.3	-6.1	-5.7	-6.7	-9.3
Eriodictyol	-7.8	-6	-6.4	-6.1	-8.7
Fluorometholone	-7.8	-6.1	-6	-6.7	-8.4
Isofucosterol	-6.5	-6.9	-5.9	-6.6	-9.1
Alfacalcidol	-7.8	-6.7	-5.6	-5.9	-8.9
Berotralstat	-7.3	-7.2	-5.2	-6.8	-8.4
Ethynodiol Diacetate	-7.5	-6.7	-5.5	-6.8	-8.3
Clocortolone	-7.4	-6.3	-6	-6.3	-8.7
Calcitriol	-7.8	-6.5	-5.5	-5.7	-9.1
Bisacodyl	-8	-6.5	-5.6	-6.2	-8.2
Morindone	-7.7	-6	-5.4	-6.6	-8.8
Methotrexate	-7.8	-5.6	-6	-5.8	-9.2
Mifepristone	-7.2	-6.8	-5.9	-6.2	-8.3
Azatadine	-7	-6.7	-5.8	-6.6	-8.2
Prasterone	-7.4	-6.1	-5.3	-6.5	-9
Dexamethasone	-7.3	-6.3	-5.9	-6.2	-8.5

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Naftopidil	-7.3	-6.3	-6	-6.1	-8.5
Tolazamide	-8	-6.5	-5.8	-5.7	-8.2
Zaleplon	-6.5	-6.8	-5.9	-5.9	-9
Alprazolam	-7.9	-6.1	-5.7	-6	-8.3
Bms-214662	-6.8	-6.6	-5.6	-6.8	-8.1
Anthragallol	-7.4	-6.1	-5.6	-6.3	-8.4
Strophanthidin	-7.5	-6	-5.6	-6.3	-8.4
Mianserin	-7.7	-6.5	-5.5	-6.3	-7.8
Soranjidiol	-7.4	-5.9	-5.7	-6.2	-8.6
Triamcinolone	-7.7	-5.9	-5.7	-6.1	-8.4
Azd5363	-7.1	-6.2	-5.5	-5.9	-9
Ormeloxifene	-6.4	-5.9	-5.4	-6.5	-9.5
Ertapenem	-7.3	-5.5	-5.9	-6.4	-8.5
Indomethacin	-7.8	-6	-5.3	-5.9	-8.6
Taxifolin	-7.8	-6	-5.4	-5.9	-8.5
Finasteride	-7.3	-6.4	-5.6	-6.7	-7.6
Dht (Dihydrotestosterone)	-7.4	-6.2	-5.5	-6.8	-7.5
Prednisone	-7.2	-5.8	-5.6	-6.6	-8.2
Simvastatin	-6.7	-6.1	-5.4	-6.2	-9
Zopiclone	-7.3	-6.8	-5.1	-5.9	-8.3

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Afuresertib	-7.2	-6.5	-5.3	-6.3	-8
Catechin	-7.5	-5.8	-5.6	-6.1	-8.3
Butorphanol	-6.8	-6	-5.8	-6.1	-8.5
Diflunisal	-7.1	-5.8	-5.8	-6.5	-8
Quercetin	-7.5	-5.9	-5.5	-5.8	-8.5
Romidepsin	-6.7	-6.3	-5.6	-6.5	-8
Budesonide	-7.1	-6.1	-5.8	-6.4	-7.6
Calycosin	-7.1	-5.9	-5.7	-5.9	-8.4
Kaempferol	-7.6	-5.9	-5.4	-5.7	-8.4
Lucidin	-7.4	-5.9	-5.2	-6.3	-8.2
Nortriptyline	-6.9	-5.7	-5.7	-6.3	-8.4
Buparlisib	-6.3	-6.5	-5.4	-6	-8.6
Niclosamide	-7.5	-5.9	-5.4	-5.6	-8.4
Isorhamnetin	-7.5	-5.7	-5.3	-5.8	-8.4
Thalidomide	-6.9	-6	-5.6	-6	-8.2
Morphine	-6.6	-6.1	-5.6	-6	-8.4
Tretinoin	-7.3	-6.1	-5.5	-6	-7.8
Formononetin	-7.6	-5.5	-5.6	-5.6	-8.2
Hydrocortisone	-7.5	-6.1	-5.2	-6.1	-7.6
Bimiralisib	-7.1	-5.5	-5.3	-5.9	-8.6

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Medroxyprogesterone Acetate	-6.8	-5.6	-5.2	-7	-7.6
Protriptyline	-6.6	-5.5	-5.3	-6.1	-8.6
Tetracycline	-6.7	-6.2	-5.9	-5.8	-7.5
Atorvastatin	-7.1	-6	-5.4	-5.3	-8.2
Vs-5584	-7.5	-6	-5.5	-5.5	-7.5
Bicalutamide	-6.6	-6.7	-5.2	-5.3	-8.1
Enzalutamide	-6.6	-5.7	-5.6	-6	-8
Ipatasertib	-6.6	-6.2	-5.2	-5.6	-8.2
Nalbuphine	-6.6	-5.4	-5.6	-5.9	-8.3
Sulindac	-6.5	-5.9	-5.5	-6.2	-7.7
Flutemetamol (18F)	-7	-5.8	-5.4	-5.3	-8.2
Amitriptyline	-6.6	-5.6	-5.3	-6	-8.2
Damnacanthal	-6.8	-5.8	-5.4	-5.6	-8
Picropodophyllin	-6.4	-6.1	-4.5	-6.1	-8.5
Flavin Mononucleotide	-6.5	-6.2	-5.1	-5.7	-8.1
Gentian Violet Cation	-6.5	-5.8	-5.4	-6.2	-7.6
Syringaresinol	-6.8	-5.7	-5.2	-5.8	-8
Demeclocycline	-7	-5.5	-5.2	-6.1	-7.7
Tapinarof	-7.1	-5.6	-5.2	-5.6	-7.9
Levofloxacin	-7.1	-6	-5.2	-5.5	-7.5

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Norgestimate	-6.5	-6	-5.2	-5.8	-7.5
Trimetrexate	-6.3	-5.3	-5.3	-5.9	-8.2
Hydroxyzine	-7.4	-5.7	-5.2	-5.4	-7.2
Imiquimod	-6.9	-5.3	-5	-5.7	-8
Esculin	-6.9	-5.6	-5.5	-5.5	-7.3
Saxagliptin	-6.7	-5.2	-5.2	-5.7	-7.9
Erlotinib	-6.9	-5.8	-4.9	-5.4	-7.7
Clofoctol	-6.5	-5.5	-5.1	-6.1	-7.4
Tofranil	-6.4	-5.5	-4.9	-5.7	-8.1
Zidovudine	-7.1	-5.8	-5.1	-5.2	-7.4
Clemastine	-6.6	-5.9	-4.6	-5.2	-8.2
Rosuvastatin	-5.9	-5.9	-4.8	-5.7	-8.2
Bimatoprost	-7	-5	-5.1	-5.8	-7.4
Omacetaxine Mepesuccinate	-6.8	-5.5	-5.1	-5.2	-7.7
Tiaprofenic Acid	-6.9	-5.4	-4.8	-5.4	-7.6
Cinoxacin	-7.1	-5.3	-5	-5.2	-7.5
Patent Blue	-6.5	-5.9	-4.7	-5.8	-7.1
Tetryzoline	-6.8	-5.3	-5.1	-5.8	-7
Curcumin	-7.1	-5.2	-4.6	-5.2	-7.8
Lesinurad	-6.5	-5.8	-5	-5.9	-6.6

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Vernakalant	-6.9	-5.7	-5	-5.2	-6.9
Flutamide	-6.9	-5.6	-4.8	-5.1	-7.2
Clobazam	-6	-5.7	-5	-6	-6.8
Trimipramine	-5.9	-5.5	-5	-5.7	-7.3
Propranolol	-6.6	-5.4	-5	-5	-7.2
Hexachlorophene	-6.2	-5.3	-5	-5.7	-6.9
Biib021	-6.1	-5.2	-5.4	-5.3	-7
Ethylhexyl Methoxycrylene	-5.5	-6	-4.6	-5.5	-7.3
Guanadrel	-6.8	-5.1	-4.7	-4.7	-7.5
Methylphenobarbital	-6.2	-5.7	-4.8	-5	-7.1
Plumbagin	-6.2	-4.9	-5.2	-5.1	-7.2
Cladribine	-6.4	-5.5	-4.5	-5.2	-6.9
Matrine	-6.5	-4.9	-5.1	-5.2	-6.8
Trifluridine	-6.7	-5.1	-4.6	-4.9	-7.2
Homoharringtonine	-5.7	-5.5	-4.9	-5	-7.3
Atropine	-6.4	-5.4	-4.4	-5.6	-6.5
Tioconazole	-6.4	-5.1	-4.5	-4.8	-7.5
Zenarestat	-6.3	-5.3	-4.1	-5.5	-7
Chloramphenicol	-7.2	-5.1	-4.4	-4.9	-6.5
Latanoprost	-6.8	-5.1	-4.1	-4.1	-8

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Diclofenac	-6.2	-5.2	-4.6	-5.1	-6.9
Formoterol	-6.5	-4.9	-3.9	-4.8	-7.8
Gemcitabine	-6.7	-4.8	-4.6	-5.1	-6.7
Clofarabine	-6.4	-4.8	-4.6	-4.9	-7.2
Melatonin	-6.3	-5	-4.7	-5	-6.9
Melphalan Flufenamide	-6.6	-5.4	-4	-4.4	-7.4
Chlorothiazide	-6.1	-5.4	-4.4	-4.6	-7.2
Ciclopirox	-7.1	-4.6	-4.4	-5	-6.5
Menadione	-5.9	-4.6	-4.7	-5.3	-7
Perindopril	-6.3	-5.2	-4.1	-5.1	-6.7
Oseltamivir	-6.8	-5.1	-4.3	-4.7	-6.3
Nitroxoline	-6	-4.5	-4.7	-5.2	-6.5
Pindolol	-6.4	-4.9	-4.6	-4.6	-6.4
Vorinostat	-5.7	-5.1	-4.7	-4.7	-6.6
Azacitidine	-6.2	-4.7	-4.6	-4.3	-6.7
Cedazuridine	-6.1	-4.7	-4.6	-4.4	-6.7
Ethotoin	-5.4	-4.9	-4.4	-4.9	-6.9
Ferroquine	-7.1	-4.6	-3.7	-4.8	-6.2
Albendazole	-5.6	-4.3	-5.1	-5	-6.3
Iobenguane	-6.3	-4.5	-4.7	-4.6	-6.2

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Tropicamide	-5.5	-5.1	-4.4	-4.6	-6.7
Lidocaine	-5.9	-4.4	-4.1	-5	-6.6
Talbutal	-5.2	-5.1	-4.2	-4.7	-6.8
Acetazolamide	-5.7	-4.8	-4.5	-4.5	-6.3
Gemeprost	-5.7	-4.8	-3.9	-5	-6.4
Pentobarbital	-5	-5.1	-4.2	-4.6	-6.8
Aspirin	-5.9	-4.8	-4.2	-4.6	-6.1
Didanosine	-5.2	-4.5	-4.3	-4.8	-6.8
Profenamine	-5.7	-4.5	-4.3	-4.6	-6.3
Memantine	-5.3	-4.6	-4.5	-4.6	-6.4
Levomilnacipran	-5.3	-5	-4.2	-4.5	-6.3
Triclosan	-5.5	-4.5	-4.2	-4.5	-6.6
Salicylic Acid	-5.9	-4.3	-4.6	-4.8	-5.6
Mexiletine	-5.5	-4.4	-4.2	-4.6	-6.2
Norepinephrine	-5.7	-4.2	-4.7	-4.4	-5.9
Midodrine	-5.9	-4.6	-4.1	-4.2	-6
Zoledronic Acid	-5.9	-4.3	-4.2	-4.1	-6
Caffeine	-5.6	-4.3	-4	-4.6	-5.9
Pilocarpine	-5.9	-4.3	-4	-3.9	-6.1
Phenylephrine	-5.5	-4.2	-4.2	-4.4	-5.7

(to be continued)

Table A2: Docking score of target proteins with meta-drug agent against PCa.

Drug Name	Docking score of target proteins				
	BIRC5	CDCA5	CENPF	NUSAP1	TK1
Chlorzoxazone	-5.1	-4.4	-3.9	-4.2	-5.9
Carboplatin	-5	-4.1	-4.3	-3.7	-5.8
Chloroquine	-5.2	-3.4	-4.3	-4.9	-5
Cimetidine	-5.4	-3.6	-3.9	-3.7	-6
Thiopental	-4.7	-4.1	-4.1	-3.8	-5.2
Valproic Acid	-5.3	-3.8	-3.5	-3.5	-5.4
Fluorouracil	-4.9	-3.7	-3.6	-3.6	-5.3
Ethchlorvynol	-4.9	-3.8	-3.6	-3.6	-4.8
Busulfan	-4.4	-3.8	-3.2	-3.5	-4.7
Histamine	-3.9	-3.4	-3.1	-3.1	-4.5
Sodium Acetate	-3.1	-2.5	-2.4	-2.6	-4