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In-Silico Analysis and Molecular Docking of Phytochemicals Targeting Genes as Therapeutic Sites for Prostate Cancer

Gayatree Pradhan[1], Debasmita Roy[2], Dharitri Priyadarsini[3], Rajat Kumar Nayak[4]

[1]Research Associate, SBIO SCIENCE Pvt Ltd, Bhubaneswar, Odisha.
[2]M.Sc. Microbiology, MITS school of biotechnology, Bhubaneswar, Odisha.
[3,4]Senior Research Associate, SBIO SCIENCE Pvt Ltd, Bhubaneswar, Odisha.

ABSTRACT

Prostate carcinoma is known to be a hypoxic and lipogenic solid tumor, exhibiting a remarkable oncogenic modulated metabolic programming. Increasing intake of glucose and aerobic glycolysis, called the Warburg effect, are main metabolic changes in hypoxic tumors. Protein, nucleic acid, and lipid biosynthesis are the other metabolic processes associated with cancer metabolic rewiring.

Research on new treatments and prevention strategies for prostate cancer has increased due to the disease's rising occurrence globally. Broccoli and other plants of the Brassica genus contain sulforaphane, a phytochemical with anticancer qualities. Several investigations have demonstrated that sulforaphane stops prostate cancers from growing and spreading. This study assesses the most current research on sulforaphane's ability to stop prostate cancer from progressing in vitro, in vivo, and in clinical settings along with Genistein and Silibinin. The suggested methods of action of sulforaphane on prostatic cells are described in depth. We also go over the difficulties, restrictions, and potential applications of sulforaphane as a therapeutic agent in the management of prostate cancer. Molecular docking was held between the compounds and the genes associated with prostate cancer: PDGFRA, PDGFRB, GSTP1, GPX2, GPX3 and MGST2.

Using several techniques, this brief in silico analysis revealed notable diversity in the prediction of SFN's physicochemical and pharmacokinetic properties as well as its toxicological potential. Even though SFN showed a strong potential to prevent metastases in several tumor models, further extensive in silico and toxicity studies are required to fully investigate its toxicological profile.

KEYWORDS: Sulforaphane, Genistein, Silibinin, Prostate, Drug likeness, Phytochemicals, PDGFRA, PDGFRB, GSTP1, GPX2, GPX3 and MGST2

INTRODUCTION:

Normal cells progressively acquire a series of biological characteristics, referred to as the hallmarks of cancer, throughout the multistep process of human tumor development. Among these characteristics, oncometabolic reprogramming, which supports tumor formation, is characterized by the increased activity of glycolysis, the phosphogluconate pathway, the production of new mitochondria,

glutaminolysis, and lipid metabolism. This metabolic reprogramming is essential for tumor cells to thrive in the nutrient- and oxygen-deficient environments that characterize tumor microenvironments during carcinogenesis **[1]**.

Highly dynamic organelle called mitochondria are involved in nearly all cellular functions, including the formation of reactive oxygen species (ROS) in both healthy and diseased conditions. This mostly happens during Oxidative Phosphorylation (OX-PHOS) and the Electron Transport Chain (ETC). Complex-I (NADH-ubiquinone oxidoreductase), Complex-II (succinate-ubiquinone oxidoreductase), Complex-III (ubiquinol-cytochrome-c reductase), and Complex-IV (cytochrome-c oxidase) are the four enzyme complexes that are essential for ETC and OX-PHOS to function properly. Thirteen polypeptides involved in the ETC are encoded by the mitochondrial genome, and mutations in mitochondrial DNA (mtDNA) are known to play a major role in the development and spread of cancer **[2]**.

Prostate cancer ranks among the most frequently diagnosed solid tumors and is the sixth leading cause of cancer-related mortality in men globally. This multifactorial disease is influenced by a combination of environmental, ethnic, hormonal, and lifestyle factors. Gleason score is a pathological indicator of the differentiation of tissue of the prostate tumor which is the best predictors of devastating prostate cancer. Prostate cancer is considered as a lipogenic tumor. Prostate cancer cells were reprogrammed their lipid metabolism as a way to proliferate under hypoxic conditions **[3]**.

The treatment options for prostate cancer currently include the use of steroidal and nonsteroidal antiandrogens, radiation therapy, chemotherapy, surgery, or a combination of these methods. These approaches often lead to prolonged side effects that can impair sexual and urinary functions, which is why complementary and alternative therapies, including herbal remedies, are sought after. Timely and precise diagnosis of prostate cancer is crucial for establishing effective treatment strategies **[4]**.

Glucosinolates are abundant in cruciferous vegetables, including broccoli and broccoli sprouts. The phytochemical sulforaphane (SFN) is created when the glucosinolate glucoraphanin in broccoli sprouts combines with the human enzyme myrosinase during chewing or chopping. In both hereditary and carcinogen-induced models of prostate cancer, broccoli sprouts and SFN exhibit chemo-preventive and cancer-suppressive qualities **[5]**.

Bioinformatics tools and databases serve as essential resources for investigating the intricate relationships between oxidative stress (OS)-related genes and proteins in cancer. Platforms like CBioPortal provide comprehensive genomic and transcriptomic information across various cancer types, facilitating large-scale analyses that reveal the molecular mechanisms driving cancer. Additionally, other bioinformatics tools support differential gene expression analyses, allowing for the identification of genes associated with OS that are regulated differently in cancerous tissues compared to normal ones. By synthesizing data from multiple sources, recent research has identified genetic alterations that influence vulnerability to oxidative damage and the progression of cancer, shedding light on critical processes and molecular functions affected by OS in diverse tumors. These initiatives contribute to mapping the molecular landscape of cancer, thereby assisting in the identification of new biomarkers and therapeutic targets for cancers associated with oxidative stress **[6,7]**.

Important computational methods encompass virtual screening and molecular docking, pharmacophore modelling, quantitative structure-activity relationship (QSAR) models, integration of genomic and proteomic data, as well as the application of artificial intelligence, deep learning, and machine learning in clinical trials, along with drug repurposing strategies **[8]**.

MATERIALS AND METHODS:

Data Retrieval: Finding genes linked to processes related to oxidative stress was the first step. The Gene Ontology Resource database was accessed (https://geneontology.org/), which offers a structured framework to describe the activities and functions of gene products. Furthermore, we incorporated genes from the Molecular Signatures Database (MSigDB) that are part of the Gene Ontology Biological Processes response to oxidative stress pathway. Only genes from the Homo sapiens species were chosen **[9,10]**.

Selection of Bioactive Compounds against Prostate Cancer: According to earlier publications, a small number of molecules were chosen as targets. The PubChem database included the compounds' SMILE and 3D structural formats along with their CID numbers: Silibinin (31,553), Genistein (5,280,961), and Sulforaphane (5350) **[11,12,13]**.

Preparation of Protein: Using the protein preparation wizard, the crystal structure of PDGFRA, PDGFRB, GSTP1, GPX2, GPX3 and MGST2 were downloaded. The proteins were processed by adding charges, removing water and setting the heat states **[14]**.

Drug-likeness analysis: The ADMET-lab platform was utilized to ascertain the chosen compounds' drug-likeness characteristics. The platform was used to submit the compounds in SMILE format that were taken from PubChem. A number of expert criteria that are utilized in drug design and are thought to be essential for every drug candidate were employed to screen the compounds for drug-likeness features. Lipinski's rule, Ghose's rule, Operea's rule, Veber's rule, and Varma's rule are the most often utilized rules taken into consideration in this study **[15,16]**.

Molecular docking studies: The methodology outlined by Fatoki et al. was followed in the molecular docking studies. The ligand files were converted from .mol to .pdb format using PyMol program. AutoDock Tools was used to prepare the proteins and ligands for docking using default parameters, and the pdbqt format was used to save the result file. The AutoDock Vina was used to conduct the molecular docking studies. After the docking process, Discovery Studio software was used to analyze and visualize the interactions that led to the ligands' binding to the target protein. Using an effective optimization technique and a scoring function that calculates the binding affinity of ligands to their targets, AutoDock Vina is an open-source molecular docking program that increases the precision and speed of docking simulations. This technique is commonly used to help identify and optimize possible drug candidates in structural biology and computational drug discovery **[17]**.

RESULTS AND DISCUSSION:

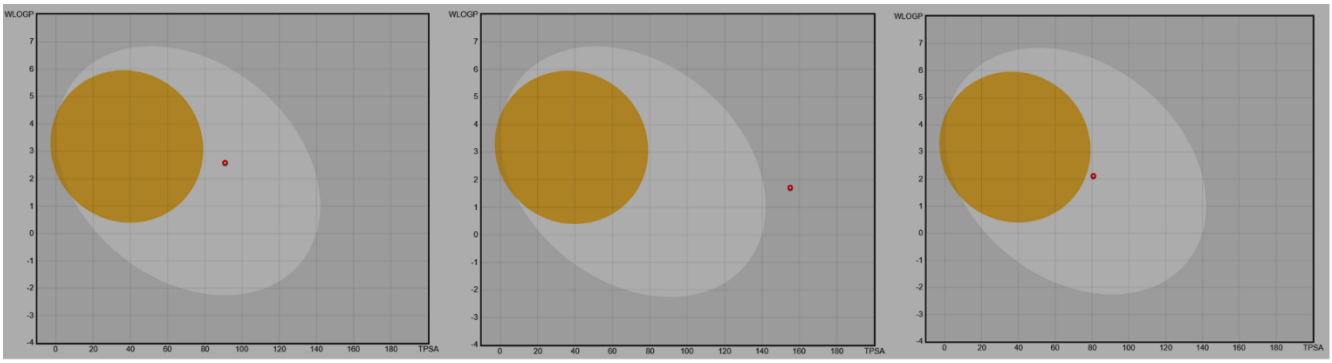
Selection of the bioactive compounds and their epigenetic targets: The selection was mainly based on a preliminary literature search by the individual authors to identify phytochemicals reported to alter epigenetic modifications in prostate cancer. For the receptors, the Swiss-target prediction database, drug bank, binding database and open target platform. Only six possible targets were identified (PDGFRA, PDGFRB, GSTP1, GPX2, GPX3 and MGST2) for three bioactive compounds (Silibinin, Genistein and Sulforaphane).

Drug-likeness analysis: Drug-likeness showed that the compounds complied with these guidelines between 54 and 100 percent

of the time, passing through all of the drug-likeness filters utilized in this investigation. With a greater topological polar surface area (TPSA), sulforaphane exhibits fewer rings, stiff and rotatable bonds, and less number of rotatable bonds. Silibinin had a low number of rotatable bonds, a high molecular weight,

and a high hydrogen-bond acceptance. Genistein displayed a limited quantity of rotatable bonds, hydrogen donors, and rings.

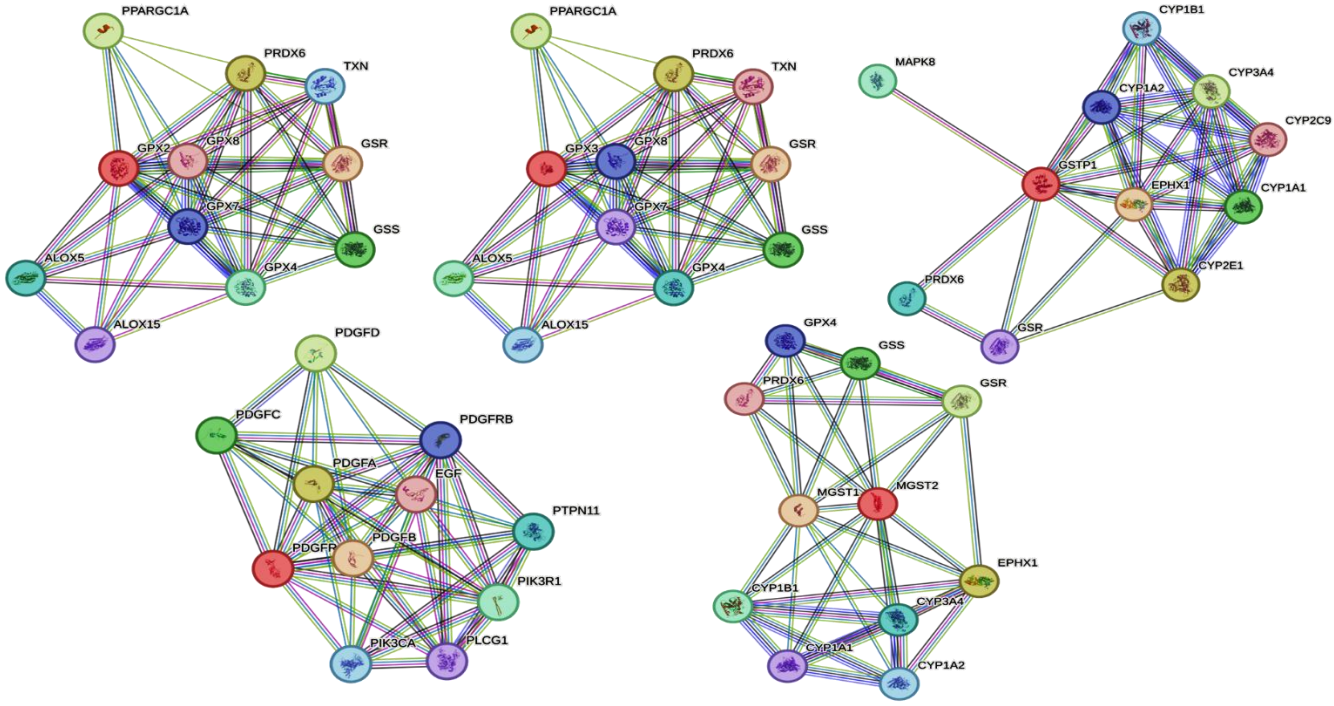
White region in BOILED-Egg diagram represents high probability of passive absorption by the gastrointestinal tract; yellow region (yolk) represents high probability of brain penetration. Yolk and the white region are not mutually exclusive and the Redpoint represent No P-glycoprotein substrate.



[Figure-1: Pharmacokinetic profile. BOILED-Egg diagram of synthesized compounds]

Molecular docking studies: Prostate cancer epigenetic targets were identified on the open target platform by crossmatching with several targets of the selected medications. The best cross-matched targets were chosen based on a stronger association with prostate cancer. The best affinity was discovered when silibinin was docked with GPX2, followed by GPX3, after the receptors and selected compounds were evaluated for binding affinity. Sulforaphane bound more strongly to PDGFRA, PDGFRB, while genistein bound less strongly with GSTP1 followed by MGST2.

Every full node of a STRING represents a gene, and the edges connecting nodes show how the related genes' protein products interact. The forms of evidence supporting the association are represented by different edge colours. The primary activity or location of genes and gene clusters is indicated by their annotation. Genes are coloured in accordance with the selection signatures that have been detected and either described here or in earlier publications.



[Figure-2: STRING interaction diagram of the analyzed genes]

Erectile dysfunction, pain, exhaustion, anaemia, nausea, and hair loss are side effects of the current conventional treatment choices for prostate cancer. Phytochemicals are naturally occurring bioactive substances with pharmacological characteristics such as anti-mutagenic, anti-proliferative, and anti-carcinogenic effects. Phytochemicals exert anticancer effects via altering the membrane potential of cancer cells, which triggers apoptosis, and by obstructing signaling pathways that promote carcinogenesis. Some dietary phytoconstituents have shown great promise in the therapy, according to in vitro studies. Prostate Cancer prevention and treatment have been associated with high consumption of specific cruciferous vegetables, such as mustard and broccoli, which contain phytochemicals like DIM and sulforaphane. Studies demonstrating these natural compounds' low toxicity, excellent tolerance, and bioavailability have increased research interest in them **[3,5,8]**.

Prior research on the chosen drugs' inhibitory effect on their epigenetic targets mostly documented the targets' downregulation via transcription factors. However, our study revealed that the target proteins may be directly inhibited by binding to their active sites, suggesting that the targets may be regulated at the protein level in addition to transcription regulation. Our results thus shed further light on their modes of action. In conclusion, this study's results have supported earlier findings and shed more information on the possible ways in which these substances may work to prevent prostate cancer **[1,10,15]**.

CONCLUSION:

In conclusion, Sulforaphane (SFN) may function as a possible chemo preventive drug, modify epigenetic processes by blocking PDGFRA, PDGFRB activity, and reactivate epigenetically silenced Tumor Suppressor Genes by changing the methylation state of these genes' promoter regions. SFNs are useful PDGFRA, PDGFRB inhibitors that can be utilized to stop cancer. Furthermore, it is yet unknown if SFN can be used as an epigenetic modulator in people or animal models.

Clinical trials and experimental validation are essential for next stages to verify their safety and therapeutic potential. By incorporating these discoveries into personalized medicine strategies, Prostate Cancer treatment could be transformed and patients could receive more individualized and efficient treatment alternatives. In order to improve patient outcomes and quality of life, future research should concentrate on applying these discoveries to clinical practice.

Future research designs should make it a standard practice to combine sulforaphane with other treatments, like radiation therapy and chemotherapy, as this may increase its effectiveness. Sulforaphane is a promising chemo preventive phytocompound that can stop the progression of prostate cancer, according to the findings in this review.

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