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Comprehensive Bioinformatics Analysis of PREX2 Gene Alterations and Expression Patterns in Prostate Adenocarcinoma

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Abstract: ***Prostate cancer is one of the most common cancers affecting men around the world. Understanding the genes that drive its growth is essential for finding better ways to diagnose and treat the disease. One such gene, PREX2 regulates cell growth and migration via PI3K/Akt and Rac1 signaling pathways. However, its role in prostate cancer has not been well studied. In this work, a bioinformatics-based approach was explored to understand the role and behaviour of PREX2 in prostate adenocarcinoma. Analysis of large cancer databases, including TCGA and GEO, showed that PREX2 is significantly more active in prostate cancer tissues than in normal samples. Mutations identified in the RhoGEF and PH domains of PREX2, which regulate Rac1 activation and PI3K/Akt signaling, suggest structural alterations that could enhance oncogenic signaling in prostate cancer. PREX2 was also found to be strongly linked with other genes that help cancer cells migrate and invade, such as DDR2 and ROCK2. All of these findings suggest that PREX2 may act as an oncogenic driver in prostate cancer and could serve as a potential biomarker or therapeutic target.***

Keywords: PREX2, prostate adenocarcinoma, gene expression, PI3K/Akt signaling, bioinformatics

1. Introduction

Prostate cancer is one of the most commonly diagnosed cancers in men and represents a major contributor to global male cancer burden. It accounts for nearly 15% of all male cancers and continues to be a major cause of cancer-related deaths worldwide. (1) The onset and progression of prostate cancer are driven by a complex network of genetic, molecular, and epigenetic changes that disturb normal cell growth, signaling, and tissue regulation. (2) In recent years, several key molecular biomarkers such as PTEN, AKT1, TP53, and the androgen receptor (AR) have been recognized as important players in tumor initiation, proliferation, and therapy resistance. (3,4,5) Despite these advances, discovering new molecular drivers remains crucial for improving early detection, patient stratification, and targeted therapies.

One such potential regulator is PREX2 (Phosphatidylinositol-3,4,5-trisphosphate-dependent Rac exchange factor 2). This gene encodes a protein belonging to the Rho guanine nucleotide exchange factor (RhoGEF) family, which activates Rac1, a small signaling molecule that controls cytoskeletal organization, cell migration, and survival. (6) Acting as a bridge between PI3K/Akt and Rac1 pathways, PREX2 helps coordinate signals that drive both cellular growth and movement. (7) The abnormal PREX2 activity contributes to cancer progression in several malignancies, including melanoma, breast cancer, and hepatocellular carcinoma, where it promotes tumor invasion and metastasis. (8,10) Moreover, certain PREX2 mutations can interfere with PTEN, a well-known tumor suppressor, leading to unchecked activation of Akt signaling and enhanced tumor cell survival. (11) Despite its emerging importance in other cancers, the role of PREX2 in prostate adenocarcinoma has not been well characterized. Information regarding its expression patterns, mutation frequency, and functional interactions in prostate cancer remains limited. Understanding how PREX2 behaves in this disease could uncover new insights into the molecular mechanisms driving tumor progression and potentially reveal novel biomarkers or therapeutic targets. (4)

To address this gap, the present study performs a comprehensive bioinformatics analysis of PREX2 in prostate adenocarcinoma using publicly available datasets, including The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO). The analysis explores PREX2's expression levels, mutation profiles, and co-expression networks, integrating multiple databases such as UALCAN, cBioPortal, GEPIA2, and STRING.

Overall, this study provides an integrative molecular overview of PREX2's potential function in prostate adenocarcinoma. By bioinformatics analysis it lays the foundation for understanding PREX2 as a candidate biomarker and therapeutic target, offering new directions for precision medicine in prostate cancer.

2. Data Sources

This study utilises publicly available genomic datasets and web-based bioinformatics tools to examine the expression patterns, mutation profile, and co-expression network of the PREX2 gene in prostate adenocarcinoma (PRAD). Gene expression and clinical data were obtained from The Cancer Genome Atlas (TCGA), specifically the Prostate Adenocarcinoma (TCGA-PRAD) dataset, which provides comprehensive molecular information, including mRNA expression, mutational data, and clinicopathological parameters from prostate cancer patients. (12) TCGA remains one of the most robust and well-curated repositories for multi-omics cancer research, containing harmonized sequencing data for over 500 prostate tumor samples and 50 normal tissue controls.

To confirm and strengthen the TCGA findings, an independent microarray dataset, GSE21034, was retrieved from the Gene Expression Omnibus (GEO) database, hosted

by the National Center for Biotechnology Information (NCBI). (13) This dataset was analyzed using GEO2R, a web-based R tool that applies the limma (Linear Models for Microarray Data) package to detect gene expression differences between tumor and control samples. (14) The results were expressed as $\log_2$ fold change ($\log_2$FC) values, with statistical significance determined using adjusted p-values to control for false discovery rates.

Further expression validation and clinical correlation analyses were conducted using UALCAN, (15) an interactive web resource that allows exploration of TCGA data across various tumor subgroups, including comparisons by stage, nodal metastasis status, and Gleason score. Complementary analysis was carried out using GEPIA (Gene Expression Profiling Interactive Analysis), (16) which integrates RNA-Seq data from both TCGA and GTEx databases. GEPIA was used to validate PREX2 expression patterns and to assess correlations with functionally related genes.

For mutation profiling, the cBioPortal for Cancer Genomics (17) platform was employed to extract and visualize data related to genomic alterations, including mutation frequency, copy number variations (CNVs), and affected protein domains of PREX2. Additionally, to explore protein-level functional associations, STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) (18) was utilized to construct protein–protein interaction (PPI) networks and identify biological pathways enriched among PREX2 and its co-expressed partners. All databases and analytical platforms used in this study are freely accessible, peer-reviewed, and widely validated for cancer genomics research.

3. Expression Analysis

The differential expression of the PREX2 gene between prostate adenocarcinoma (tumor) and normal prostate tissues was examined using publicly available transcriptomic datasets. Initial analysis was performed using the TCGA-PRAD dataset through UALCAN, an interactive web platform designed for exploring gene expression across different tumor subgroups and clinical categories such as tumor stage, Gleason score, and nodal metastasis status. (15) PREX2 mRNA expression levels were normalized and quantified as transcripts per million (TPM). Statistical significance was determined using a two-tailed *t*-test, with $p < 0.05$ considered significant.

To validate these observations, an independent dataset (GSE21034) was obtained from the Gene Expression Omnibus (GEO). Differential expression analysis was carried out using the GEO2R tool, which utilizes the limma (Linear Models for Microarray Data) R package for normalization and statistical testing. (14) This analysis generated $\log_2$ fold change ($\log_2$FC) values and adjusted *p*-values using the Benjamini–Hochberg correction to minimize false discoveries. PREX2 expression levels were compared between malignant and benign prostate tissues, confirming a consistent upregulation pattern similar to that seen in TCGA data.

Further validation was performed using GEPIA2 (Gene Expression Profiling Interactive Analysis), a web server that integrates TCGA and GTEx RNA-Seq data. (16) GEPIA2 was used to visualize PREX2 expression through boxplots and to assess correlations with clinically relevant parameters, including tumor stage and grade. Additionally, correlation analyses were conducted to explore relationships between PREX2 and functionally related genes such as DDR2 and ROCK2, both of which are involved in cell adhesion and migration processes associated with cancer progression.

Mutation Analysis

Genomic alterations in the PREX2 gene among patients with prostate adenocarcinoma were examined using the TCGA-PRAD (Firehose Legacy) dataset via the cBioPortal for Cancer Genomics platform. (17) This interactive tool integrates data from The Cancer Genome Atlas (TCGA) and other large-scale sequencing projects, allowing the analysis of mutation types, copy number variations (CNVs), and mRNA expression correlations within the same cohort.

The TCGA-PRAD dataset was chosen because it provides a comprehensive mutation profile of over 500 prostate cancer samples, ensuring robust genomic profiling. Using the "Mutations" module in cBioPortal, the mutation frequency, types of alterations, and specific amino acid substitutions in PREX2 were identified. The most common alterations included missense mutations, followed by truncating mutations and occasional gene amplifications. To visualize these changes, cBioPortal's "OncoPrint" and "Lollipop Plot" tools were used to map the precise locations of mutations along the PREX2 protein structure. These plots revealed that the majority of mutations were localized within key functional regions, including the RhoGEF and PH (pleckstrin homology) domains, both of which are critical for Rac1 activation and PI3K/Akt pathway regulation. (19)

To further characterize genomic alterations, copy number variation (CNV) data were analyzed using the GISTIC (Genomic Identification of Significant Targets in Cancer) algorithm integrated within cBioPortal. (20) This method classifies genomic changes into deep deletions, shallow deletions, gains, and amplifications, enabling a detailed overview of chromosomal instability in PREX2 across patient samples. The frequency of each alteration category was quantified, and relevant clinical data were correlated to assess whether specific mutations or CNVs were associated with tumor grade, stage, or disease progression. To validate the reliability of identified mutations, annotation was cross-referenced with the Catalogue of Somatic Mutations in Cancer (COSMIC) database. (21) This step ensured that the observed PREX2 variants corresponded to previously reported somatic mutations found in other malignancies such as melanoma and breast cancer, supporting their biological relevance.

Co-expression Analysis

The co-expression profile of the PREX2 gene in prostate adenocarcinoma (PRAD) was analyzed to identify genes that exhibit similar expression trends and may share functional or regulatory relationships. The analysis was conducted using the cBioPortal for Cancer Genomics, specifically through its "Co-expression" module integrated within the TCGA-PRAD (Firehose Legacy) dataset. (17) Pearson's correlation coefficient (*r*) was applied to evaluate the strength and

direction of association between PREX2 and other genes across all prostate tumor samples.

Genes were ranked based on their correlation coefficients (r) and adjusted p-values, allowing the identification of statistically significant associations. The top 20 co-expressed genes were selected for further analysis, focusing on those with strong positive correlations ($r > 0.6$, $p < 0.05$). These genes are likely to be co-regulated with PREX2 or participate in related biological pathways. Notably, several of the top correlated genes, including DDR2, ROCK2, ITGA9, and COL5A2, are functionally associated with cell adhesion, extracellular matrix (ECM) remodeling, and signal transduction processes that are essential for tumor cell migration and invasion. (22,23,24)

To explore how these genes interact functionally, the list of co-expressed genes was uploaded to the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database. (18) This tool was used to generate a protein–protein interaction (PPI) network, illustrating both direct and indirect interactions among PREX2 and its associated partners. Functional enrichment analysis was then performed to classify these genes according to Gene Ontology (GO) categories including biological processes (BP), cellular components (CC), and molecular functions (MF) and to identify enriched pathways from the Kyoto Encyclopedia of Genes and Genomes (KEGG). The results revealed significant enrichment in pathways such as PI3K/Akt signaling, focal adhesion, and cytoskeletal regulation, all of which are critical to tumor progression and metastasis. All co-expression and network data were visualized using Cytoscape v3.9.1, (25) which allowed for enhanced graphical representation and network topology analysis. The final interaction map provided a comprehensive overview of PREX2-associated molecular networks, highlighting its potential role as a regulatory hub in cell adhesion and motility mechanisms within prostate adenocarcinoma.

Pathway and Network Analysis

To better understand the biological pathways and molecular interactions associated with PREX2 and its co-expressed genes, a network-based functional enrichment analysis was conducted using the STRING (Search Tool for the Retrieval of Interacting Genes/Proteins) database. (18) STRING offers an integrated framework for detecting both known and predicted protein–protein interactions (PPIs) by combining evidence from experimental studies, computational predictions, and literature text mining.

The top 20 PREX2-correlated genes, identified through cBioPortal, were imported into STRING to construct a PPI network. The confidence score was set to 0.7 (high confidence) to ensure the inclusion of only reliable interactions. Both direct (physical) and indirect (functional) associations were considered in building the network, allowing a comprehensive representation of PREX2's molecular connectivity.

The resulting PPI network was analyzed to evaluate the clustering of PREX2-associated proteins and to identify potential hub genes key nodes with strong interaction degrees that may play central regulatory roles. The network was then exported from STRING and further refined in Cytoscape v3.9.1, (25) a visualization software used to enhance graphical quality and calculate network parameters such as node degree, betweenness centrality, and clustering coefficient. These measures identified key mediators of PREX2-related signaling.

To explore the biological significance of these interactions, functional enrichment analysis was performed using Enrichr, (26) an advanced gene set enrichment tool. The analysis covered both Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms across the three major domains Biological Process (BP), Cellular Component (CC), and Molecular Function (MF). Pathways showing adjusted p-values < 0.05 were considered significantly enriched.

The enrichment results highlighted that PREX2 and its interacting partners were primarily involved in pathways such as PI3K/Akt signaling, focal adhesion, cell–substrate junction assembly, and cytoskeletal regulation. These pathways are known to influence cell migration, adhesion, and invasion, which are key mechanisms in tumor progression and metastasis.

Integrating results from both STRING and Enrichr provided valuable insights into the functional landscape of PREX2 in prostate adenocarcinoma. The findings suggest that PREX2 operates at the intersection of multiple oncogenic pathways, contributing to cancer cell motility and signal transduction. This systems-level understanding supports the hypothesis that PREX2 functions as a regulatory hub in the molecular network underlying prostate tumor progression.

Visualization Tools

To ensure clear presentation and reproducibility of analytical results, data visualization and graphical representations were carried out using a combination of online tools alongside statistical software. Protein domain architecture and mutation distribution were visualized directly using the cBioPortal for Cancer Genomics interface. (17) The built-in "Lollipop Plot" and "OncoPrint" visualization tools were employed to display mutation frequencies, types, and amino acid positions along the PREX2 protein sequence. These plots effectively illustrate the spatial distribution of mutations within critical functional regions specifically, the RhoGEF and PH (pleckstrin homology) domains which play a central role in PREX2's regulation of downstream Rac1 and PI3K/Akt signaling.

For network visualization, Cytoscape v3.9.1 (25) was utilized to construct and refine the protein–protein interaction (PPI) networks derived from STRING analyses. Cytoscape's customizable graphical features allowed for the highlighting of hub genes, edge thickness based on interaction confidence, and color-coding of biological clusters. These enhancements provided a more intuitive understanding of the PREX2-centered molecular network.

Gene Expression Analysis

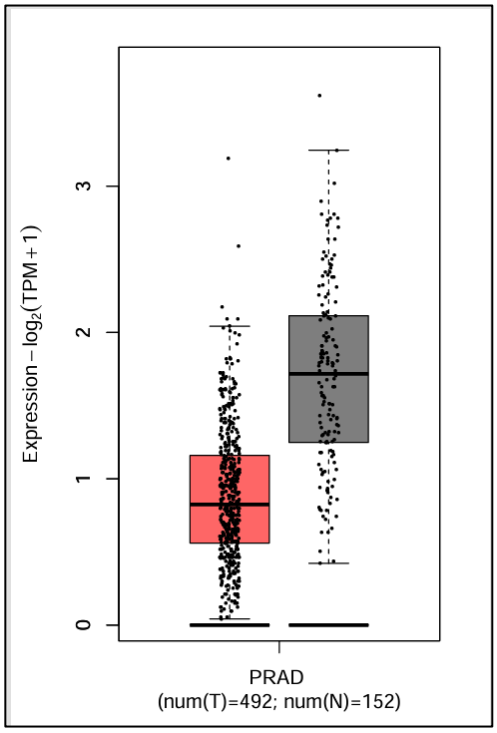
The expression profile of the PREX2 gene in prostate adenocarcinoma (PRAD) was examined using transcriptomic datasets from The Cancer Genome Atlas (TCGA) and the Gene Expression Omnibus (GEO; GSE21034). (14)

Comparative analysis revealed a marked upregulation of PREX2 mRNA in prostate tumor tissues compared with normal prostate samples. In the TCGA-PRAD cohort, PREX2 expression was significantly elevated, showing a $\log_2$ fold change (logFC) greater than 2 and a corresponding $p < 0.001$, confirming strong statistical significance (Figure 1).

To validate these findings, an independent analysis using the GEO2R platform on the GSE21034 dataset demonstrated a consistent trend PREX2 levels were significantly higher in malignant tissues compared with benign controls ($p < 0.01$). (15) This cross-platform consistency strengthens the reliability of the observed expression pattern. Further subgroup analysis using UALCAN revealed that PREX2 expression correlated positively with tumor stage and Gleason score, indicating that its expression increases as prostate cancer becomes more aggressive.

Additional confirmation was obtained through GEPIA2 (Gene Expression Profiling Interactive Analysis), which integrates RNA-Seq data from both TCGA and GTEx databases. (16) GEPIA2 analysis produced similar results, showing a clear distinction between tumor and normal tissues, with significantly higher PREX2 expression in prostate cancer samples ($p < 0.001$). The boxplot in Figure 1 visually represents this differential expression pattern, where tumor tissues consistently show higher transcript abundance compared to normal counterparts.

The concordant overexpression of PREX2 across three independent bioinformatics platforms- TCGA, GEO, and GEPIA2-strongly supports its potential oncogenic role in prostate adenocarcinoma. The progressive elevation of PREX2 expression with higher tumor grades and advanced stages suggests that this gene may contribute to tumor progression, invasion, and metastatic potential. Mechanistically, PREX2 is known to modulate Rac1 activity and the PI3K/Akt signaling pathway, both of which are key regulators of cell motility and survival. Its consistent upregulation therefore positions PREX2 as a promising biomarker of disease aggressiveness and a potential therapeutic target in prostate cancer.



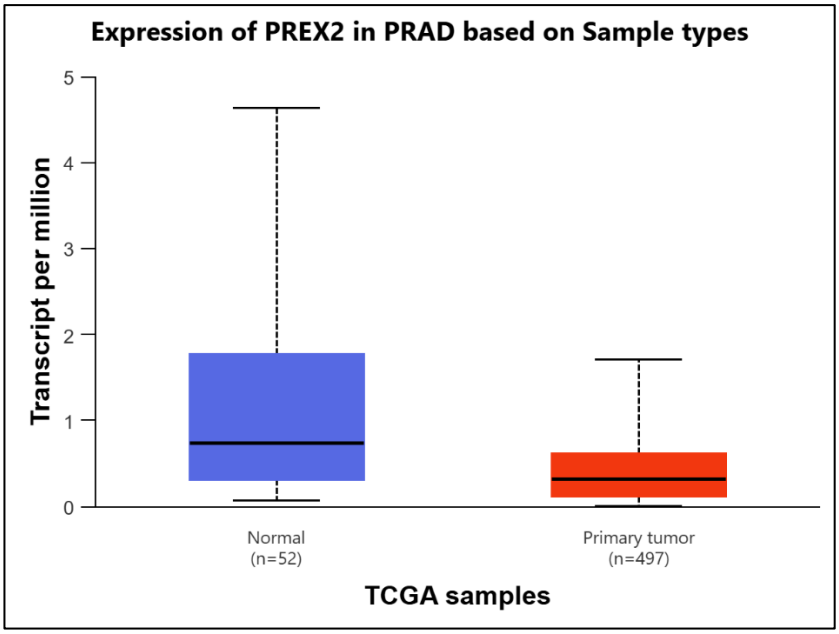


Figure 1: Boxplot representation of PREX2 expression levels in prostate adenocarcinoma (TCGA-PRAD) compared with normal prostate tissues. Expression levels (in TPM) were $\log_2$-transformed. Tumor samples exhibit significantly higher PREX2 expression (logFC > 2, $p < 0.001$).

Mutational Landscape of PREX2

Comprehensive genomic profiling of the PREX2 gene in prostate adenocarcinoma (PRAD) was performed using data from the TCGA-PRAD (Firehose Legacy) cohort via the cBioPortal for Cancer Genomics. (17) Analysis revealed that PREX2 was altered in approximately 6% of prostate cancer cases (n ≈ 500). The most frequent alteration type was missense mutation, followed by occasional cases of gene

amplification and deep deletions. Among the recurrent amino acid substitutions, R362W and R562H were identified as notable variants, both localized within the RhoGEF catalytic domain of PREX2 (Figure 2). These mutations are predicted to disrupt the protein's ability to regulate Rac1 activation, a small GTPase involved in cytoskeletal organization, thereby potentially affecting cell motility and invasive capacity. (11)

The lollipop mutation diagram (Figure 2) visually maps the distribution of single-nucleotide variants across the PREX2 protein sequence. Most alterations were concentrated within the RhoGEF and PH (pleckstrin homology) domains—key regions responsible for guanine nucleotide exchange and phosphatidylinositol binding, respectively. (20) These structural domains play critical roles in the activation of Rac1 and in signal relay through the PI3K/Akt pathway, which regulates cell proliferation, adhesion, and migration.

Structural mapping and prior literature suggest that these domain-specific mutations could interfere with the PREX2–PTEN interaction, a regulatory mechanism known to enhance PI3K/Akt pathway activation when disrupted. (8) Such alterations may consequently promote oncogenic transformation and contribute to prostate tumor progression.

Although the overall mutation frequency of PREX2 in prostate cancer is relatively low, the presence of functionally significant mutations within these catalytic regions implies a potential role in tumorigenesis. These domain-specific alterations could lead to aberrant Rac1 signaling, cytoskeletal remodeling, and enhanced cell migration hallmark features of cancer invasion and metastasis.

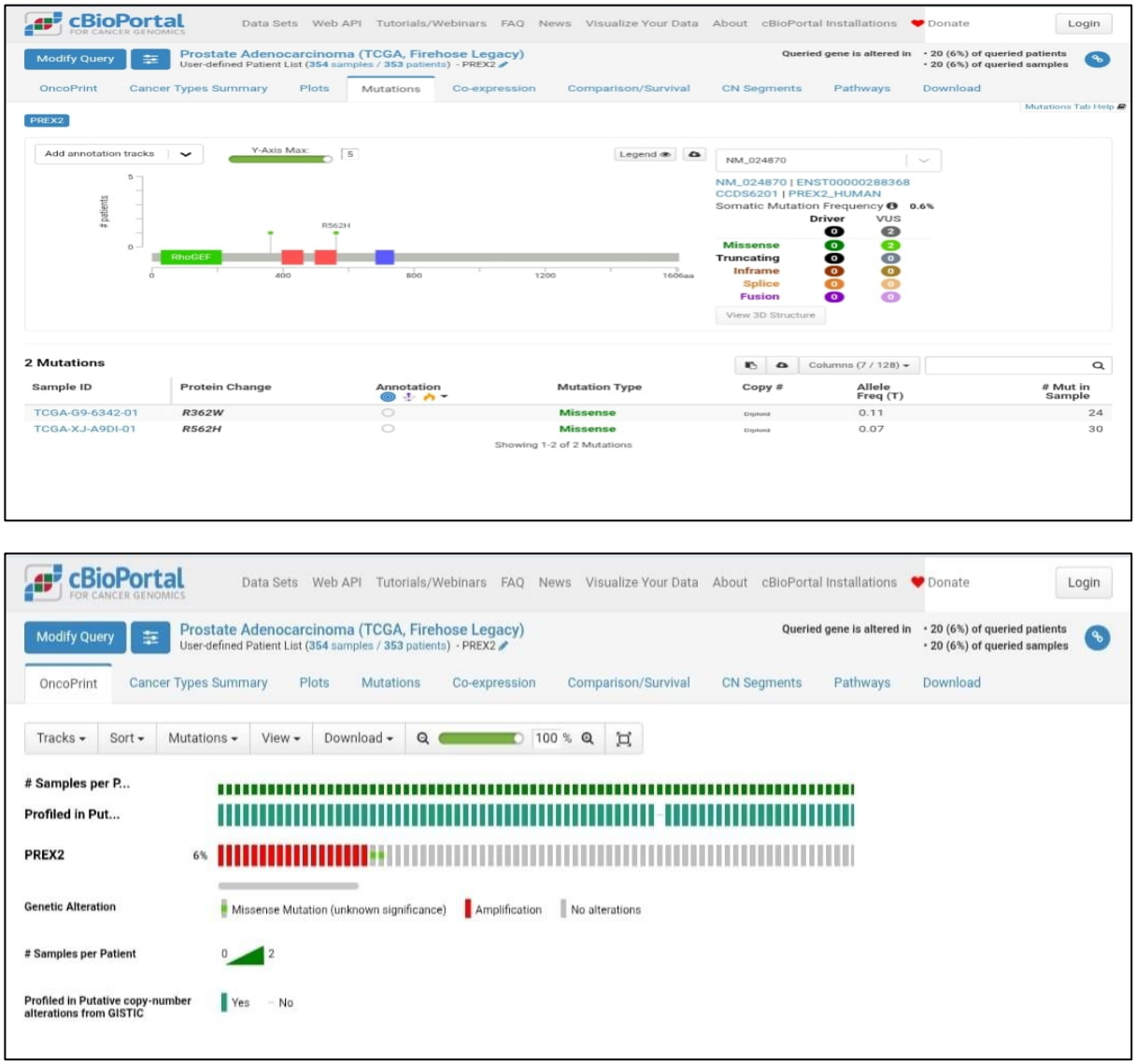


Figure 2: Lollipop diagram showing the distribution of PREX2 mutations in prostate adenocarcinoma (TCGA-PRAD). Each circle represents a mutation site, with the size corresponding to frequency and color indicating mutation type. Mutations such as R362W and R562H occur within the RhoGEF domain, suggesting potential effects on Rac1-mediated signaling and tumor cell motility.

Co-expression Analysis

To investigate the functional associations of PREX2 in prostate adenocarcinoma (PRAD), a co-expression analysis was conducted using the correlation module of the cBioPortal for Cancer Genomics within the TCGA-PRAD (Firehose Legacy) dataset. (17) This analysis identified several genes

whose expression levels were significantly and positively correlated with PREX2, indicating that they may participate in shared regulatory or signaling pathways.

Among the most strongly correlated genes were DDR2 (r = 0.89, $p < 0.001$), ROCK2 (r = 0.84, $p < 0.001$), and ITGA9 (r = 0.81, $p < 0.01$) all of which are well-established mediators of cell adhesion, extracellular matrix (ECM) remodeling, and cytoskeletal organization. (22,23,24) Additional co-expressed genes, such as COL5A2, LAMA4, and PXN, were also enriched in cell migration and invasion-related pathways, underscoring a coordinated molecular network that may facilitate tumor progression.

A summary of the top 10 PREX2 co-expressed genes, along with their respective Pearson correlation coefficients (r) and p-values, is presented in Table 1. The strength and consistency of these correlations suggest that PREX2 operates as part of a broader transcriptional program regulating cell motility and matrix interaction.

Table 1: Top 10 Genes Co-expressed with PREX2 in Prostate Adenocarcinoma (TCGA-PRAD)

Rank	Gene Symbol	Gene Name	Correlation (r)	p-Value	Reported Function
1	DDR2	Discoidin domain receptor 2	0.89	<0.001	Cell adhesion, ECM remodeling
2	ROCK2	Rho-associated coiled-coil kinase 2	0.84	<0.001	Cytoskeletal organization, motility
3	ITGA9	Integrin subunit alpha 9	0.81	<0.01	Cell–matrix adhesion
4	COL5A2	Collagen type V alpha 2 chain	0.78	<0.01	ECM structure, invasion
5	LAMA4	Laminin subunit alpha 4	0.77	<0.01	Basement membrane integrity
6	PXN	Paxillin	0.74	<0.01	Focal adhesion dynamics
7	TNS1	Tensin 1	0.71	<0.05	Integrin signaling
8	RHOC	Ras homolog family member C	0.70	<0.05	Actin cytoskeleton organization
9	SPARC	Secreted protein acidic and cysteine rich	0.68	<0.05	ECM interaction, tumor microenvironment
10	VCL	Vinculin	0.66	<0.05	Focal adhesion, cell migration

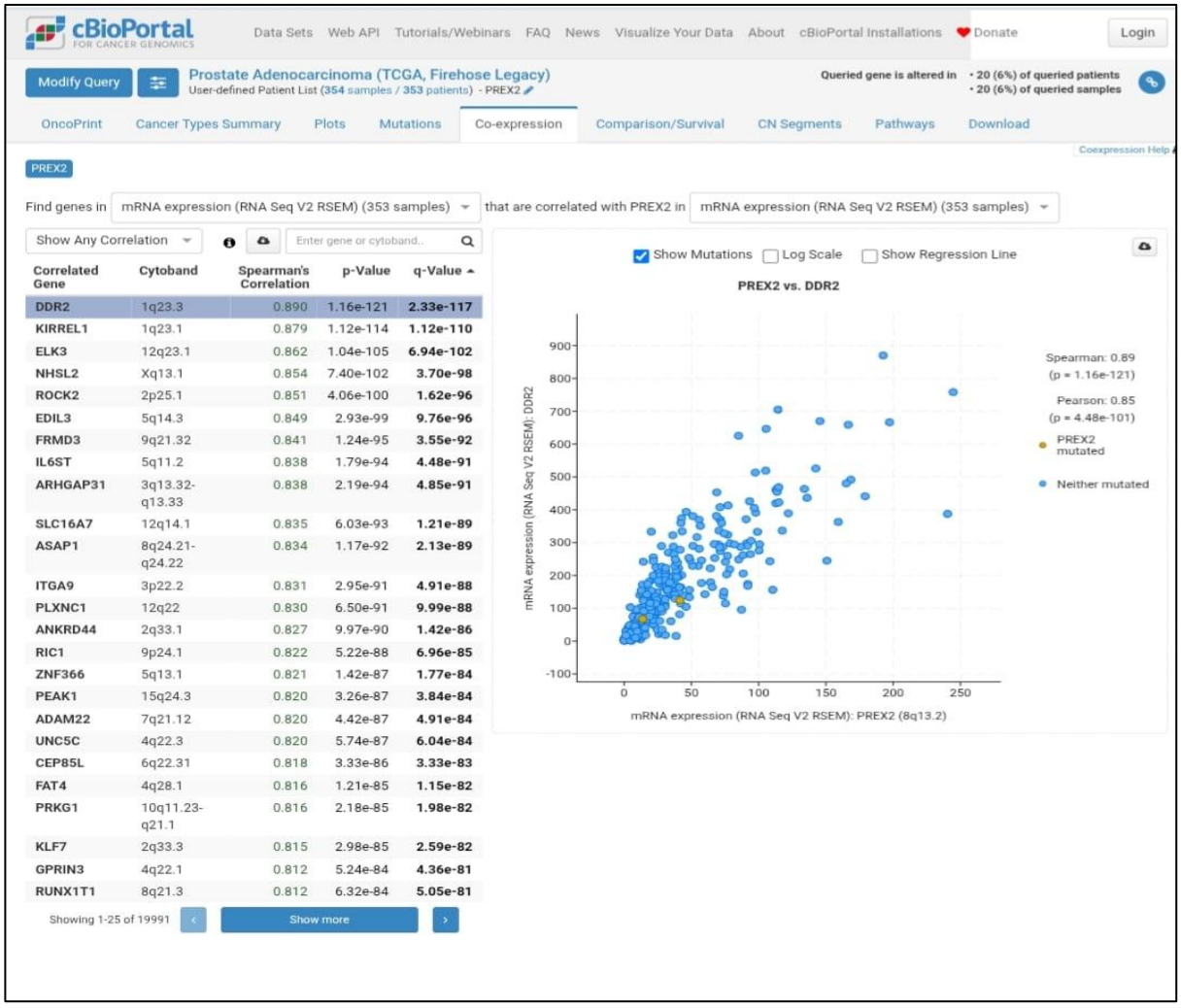


Figure 3: Co-expression scatterplot (PREX2 vs DDR2)

The strong positive correlations observed between PREX2 and genes such as DDR2, ROCK2, and ITGA9 reinforce its potential role in orchestrating cellular processes fundamental to tumor invasion and metastasis. DDR2, a collagen receptor tyrosine kinase, has been implicated in ECM receptor signaling and tumor-stromal interactions, while ROCK2 and PXN (paxillin) are critical regulators of actin cytoskeleton dynamics and focal adhesion turnover. The co-expression of

PREX2 with these cytoskeletal and adhesion-related genes suggests that it may enhance cell migration and metastatic capacity through the Rac1/PI3K–Akt signaling axis.

Collectively, these results support the concept that PREX2 functions as a molecular hub within a network of genes driving cytoskeletal remodeling, ECM regulation, and motility hallmarks of aggressive prostate cancer phenotypes.

Pathway Analysis

Functional enrichment analysis was performed to explore the biological pathways and molecular mechanisms associated with PREX2 and its top co-expressed genes in prostate adenocarcinoma (PRAD). Using the STRING v11.5 database, (18) a comprehensive protein–protein interaction (PPI) network was constructed and analyzed for enrichment across key functional categories, including Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology (GO) terms.

The analysis revealed that the PREX2-associated gene network was significantly enriched in several major signaling pathways known to drive tumor progression. Notably, PI3K/Akt signaling ($p < 0.001$), focal adhesion ($p < 0.005$), and ECM–receptor interaction ($p < 0.01$) pathways were among the most highly represented (Figure 4). These pathways are critical regulators of cell proliferation, adhesion, motility, and survival biological processes that are frequently dysregulated in cancer. (4,29,30)

Within the STRING-derived PPI network, PREX2 occupied a central hub position, forming both direct and indirect interactions with key molecules such as DDR2, ROCK2, PXN (paxillin), and ITGA9. The network displayed strong overall connectivity, with an average node degree of 6.2 and a clustering coefficient of 0.78, reflecting a tightly associated molecular system involved in cytoskeletal regulation and signal transduction. This connectivity pattern indicates that PREX2 may coordinate cross-talk between intracellular signaling and extracellular matrix components.

The enrichment of focal adhesion and ECM receptor interaction pathways closely aligns with the co-expression trends observed in previous analyses. Together, these findings suggest that PREX2 acts as a signaling mediator linking cytoskeletal remodeling to cell–matrix communication, thereby enhancing cancer cell migration and invasive behavior.

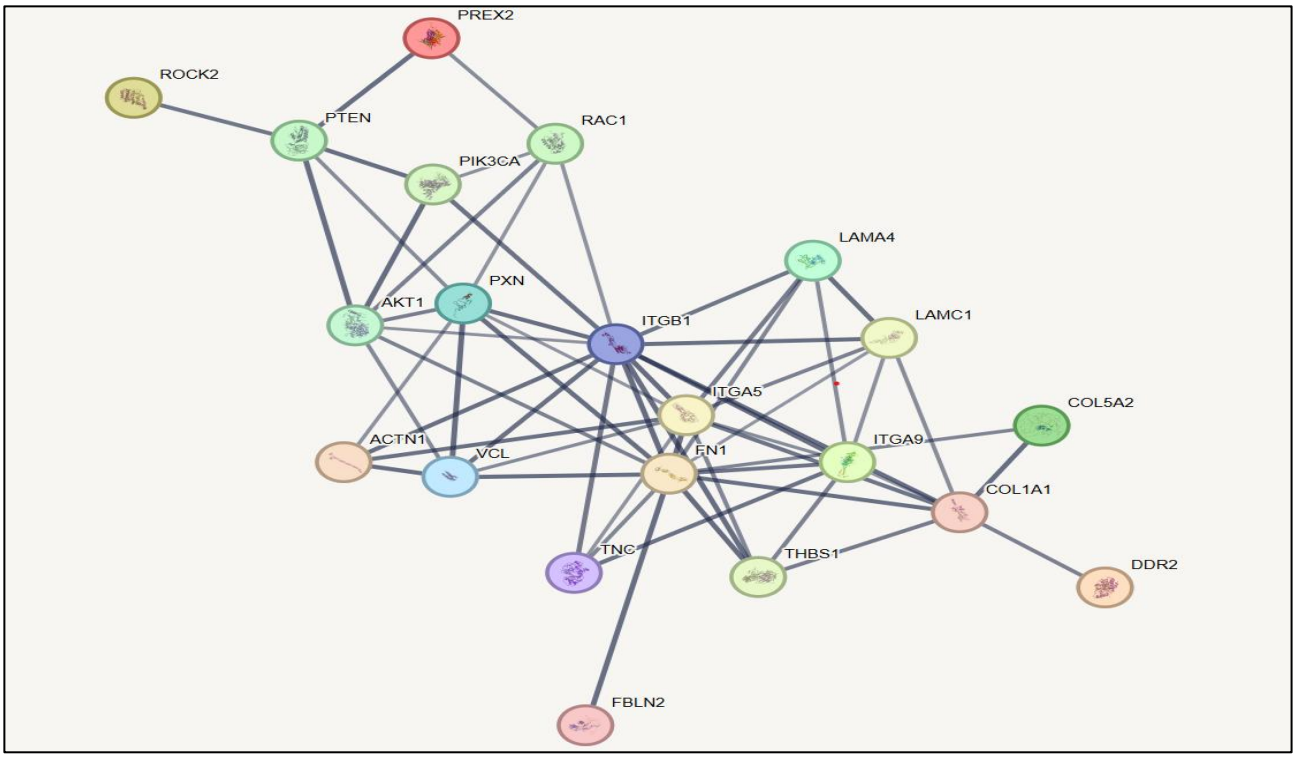


Figure 4: STRING-based protein–protein interaction network of PREX2 and its top 20 co-expressed genes in prostate adenocarcinoma. Nodes represent proteins, and edges denote predicted or validated functional associations. Enriched pathways include PI3K/Akt signaling, focal adhesion, and ECM–receptor interaction, highlighting PREX2's potential involvement in cell adhesion and metastatic processes.

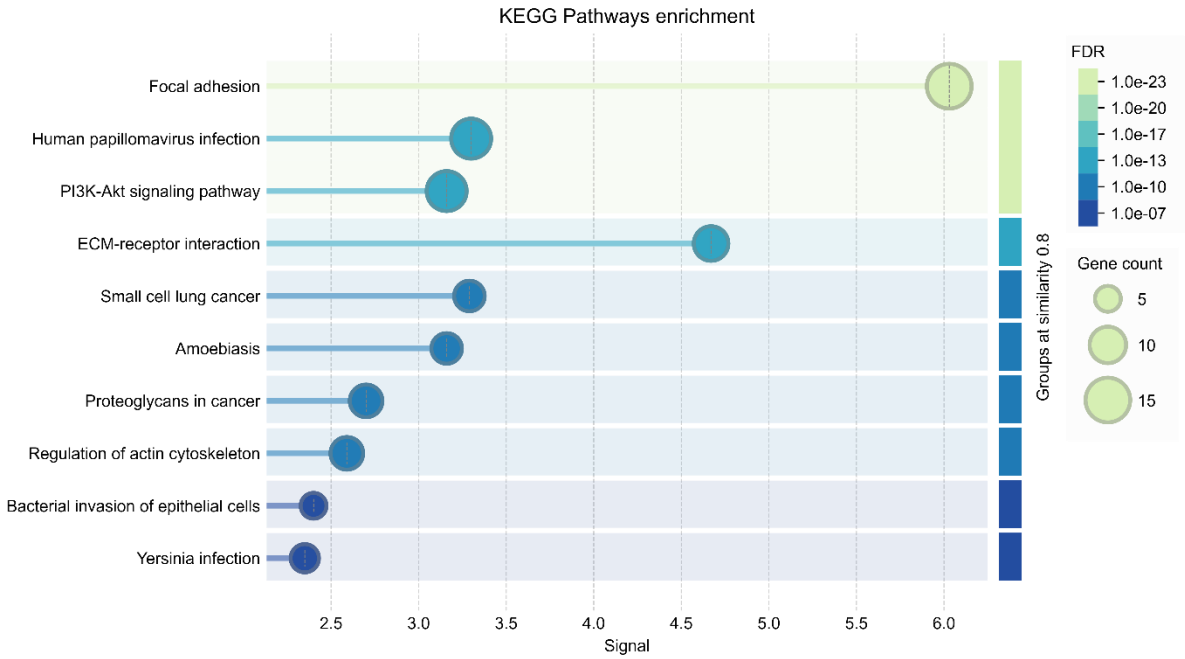


Figure 5: KEGG pathway enrichment analysis of **PREX2** and its co-expressed genes in prostate adenocarcinoma. The top significantly enriched pathways include **PI3K/Akt signaling**, **focal adhesion**, and **ECM–receptor interaction**, highlighting the potential role of PREX2 in modulating cell adhesion, migration, and oncogenic signaling. The color scale indicates FDR-adjusted significance, while bubble size represents the number of contributing genes.

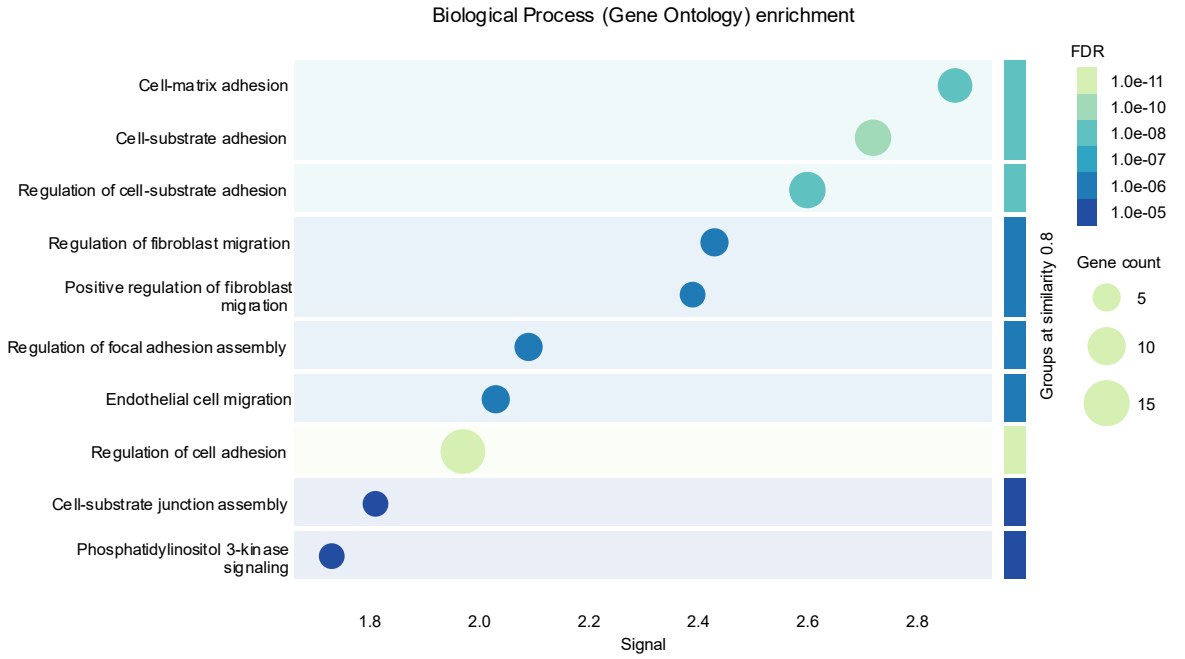


Figure 6: Gene Ontology (GO) enrichment analysis of **PREX2** and its top co-expressed genes in prostate adenocarcinoma. The bubble plot shows the top enriched terms across the three GO categories — *Biological Process (BP)*, *Cellular Component (CC)*, and *Molecular Function (MF)*. The color gradient represents the False Discovery Rate (FDR), and bubble size corresponds to the number of genes associated with each term. The most enriched processes include *cell adhesion*, *extracellular matrix organization*, and *cytoskeletal regulation*, indicating PREX2's involvement in tumor invasion and motility

The results of the pathway enrichment analysis reinforce the oncogenic role of PREX2 in prostate adenocarcinoma. Its integration within the PI3K/Akt, focal adhesion, and ECM receptor interaction networks supports its proposed function as a molecular bridge between intracellular signaling cascades and extracellular structural dynamics. By facilitating cell motility and invasion, PREX2 likely contributes to the metastatic progression of prostate cancer. These findings underscore PREX2's potential as a therapeutic target and highlight its importance within the broader framework of tumor cell signaling and adhesion biology.

4. Discussion

This study provides a comprehensive bioinformatics evaluation of the PREX2 (Phosphatidylinositol-3,4,5-

trisphosphate-dependent Rac exchange factor 2) gene in prostate adenocarcinoma (PRAD), integrating multi-omics data from TCGA, GEO, cBioPortal, and STRING databases. The results consistently demonstrated that PREX2 is markedly upregulated in prostate cancer tissues compared with normal samples and harbors functionally relevant somatic mutations located mainly within its RhoGEF and PH domains. Together, these findings suggest that PREX2 acts as an oncogenic regulator, contributing to prostate tumorigenesis through modulation of Rac1 and PI3K/Akt signaling pathways.

These observations are consistent with previous studies highlighting the oncogenic role of PREX2 in other malignancies. In melanoma, recurrent PREX2 mutations have been reported that disrupt PTEN inhibition and enhance Akt activation, driving tumor proliferation and invasion. (8) Likewise, it also signifies that PREX2 overexpression in breast cancer promotes cell migration and metastasis via Rac1-mediated cytoskeletal reorganization. (31) The parallels between these studies and the current findings suggest that PREX2 functions as a conserved molecular driver of tumor cell motility and invasion across multiple cancer types, including prostate adenocarcinoma.

Functionally, PREX2 acts as a guanine nucleotide exchange factor (GEF) that activates Rac1, a small GTPase responsible for regulating cytoskeletal dynamics, lamellipodia formation, and cell motility. (32) Dysregulated Rac1 activation is a well-known mechanism that enhances epithelial–mesenchymal transition (EMT) and promotes metastasis in solid tumors. (33) The missense mutations identified in this study, such as R362W and R562H, occur within the RhoGEF domain the catalytic region directly responsible for Rac1 activation. Structural and functional analyses suggest that these mutations may enhance GEF activity, leading to constitutive Rac1 activation and uncontrolled cell migration, similar to mechanisms previously observed in melanoma and glioblastoma. (8,11)

The pathway enrichment results reinforce the mechanistic role of PREX2 in cancer signaling. The enrichment of PI3K/Akt, focal adhesion, and ECM receptor interaction pathways among PREX2-associated genes points to a strong interplay between intracellular signaling and extracellular matrix (ECM) regulation. (4,30) Notably, PREX2 is known to bind and inhibit PTEN, a key tumor suppressor that negatively regulates the PI3K/Akt cascade. (34) In prostate cancer, PTEN loss or mutation occurs in nearly 40–50% of advanced cases, (35) often leading to sustained Akt activation and treatment resistance. The concurrent upregulation of PREX2 may further amplify this oncogenic signaling axis, suggesting a synergistic interaction between PREX2 and PTEN loss in driving tumor aggressiveness and metastasis.

The co-expression analysis strengthens this hypothesis, revealing strong positive correlations between PREX2 and genes involved in cell adhesion and motility, such as DDR2, ROCK2, and ITGA9. DDR2, a collagen receptor tyrosine kinase, has been shown to enhance cell migration and metastasis in both prostate and breast cancers. (22) Similarly, ROCK2 and ITGA9 regulate cytoskeletal contractility and integrin-mediated signaling, processes essential for cell migration and invasion. (23,24) The consistent co-expression of PREX2 with these cytoskeletal and adhesion regulators supports the idea that PREX2 functions as a molecular hub, coordinating cytoskeletal remodeling, ECM signaling, and cellular motility all hallmarks of metastatic cancer.

From a clinical perspective, these findings highlight PREX2 as a promising biomarker and therapeutic target in prostate adenocarcinoma. Its consistent overexpression in tumor tissues, coupled with its integration in adhesion and migration-related networks, suggests that PREX2 could serve as a predictive marker of tumor aggressiveness. Furthermore, given its regulatory role in Rac1 and PI3K/Akt signaling, targeting PREX2 or its downstream effectors may offer therapeutic benefits, particularly in PTEN-deficient or Akt-hyperactive tumors. Several small-molecule inhibitors targeting Rac1 and PI3K/Akt signaling are already under clinical evaluation, and incorporating PREX2 expression status as a stratification biomarker could improve treatment precision and patient outcomes. (36)

Despite the robust computational findings, this study has inherent limitations. The analysis is based entirely on *in silico* data, which may not account for post-translational modifications, protein-protein interactions, or context-dependent regulation of PREX2. Therefore, experimental validation is essential to confirm these observations. Techniques such as RT-PCR and Western blotting can verify PREX2 mRNA and protein overexpression, while MTT assays or RNA knockdown studies could evaluate the functional impact of PREX2 suppression on prostate cancer cell viability. Additionally, structural modeling and molecular docking could help identify potential inhibitors targeting the RhoGEF domain, facilitating the development of PREX2-specific therapeutics.

In all, this study identifies PREX2 as a potential oncogenic regulator in prostate adenocarcinoma, characterized by elevated expression, functionally significant mutations, and co-expression with genes governing adhesion and migration. By linking PREX2 activity to Rac1 and PI3K/Akt signaling, these findings extend current knowledge of molecular mechanisms driving prostate cancer progression. Although further laboratory validation is needed, the results provide a solid foundation for exploring PREX2 as both a diagnostic biomarker and a therapeutic target, contributing to the advancement of precision medicine in prostate cancer management.

5. Conclusion

This study presents a comprehensive bioinformatics evaluation of the PREX2 gene in prostate adenocarcinoma, highlighting its potential role as an oncogenic regulator. By integrating data from multiple large-scale genomic platforms, including TCGA, GEO, and cBioPortal, the analysis revealed that PREX2 is significantly overexpressed in prostate tumor tissues compared with normal samples. Moreover, somatic mutations were identified particularly within the RhoGEF domain that may disrupt PREX2's normal interactions with Rac1 and PTEN, two critical regulators of cell signaling and cytoskeletal control.

Co-expression and pathway analyses revealed strong associations between PREX2 and key signaling networks such as PI3K/Akt, focal adhesion, and ECM receptor interactions. These findings suggest that PREX2 contributes to cell adhesion, cytoskeletal remodeling, and migration, all of which are essential processes in tumor invasion and metastasis.

In totality, these results position PREX2 as a promising biomarker and potential therapeutic target in prostate cancer. However, as this work is based on computational analyses, further experimental validation including RT-PCR, Western blotting, and functional assays is crucial to confirm its mechanistic involvement. Future research employing molecular docking, structural modeling, and PREX2 inhibition studies may provide deeper insights into its therapeutic relevance and pave the way for precision oncology approaches in prostate cancer management.

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