Experimed 2024; 14(3): 215-220

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1505817

Determining Prostate Cancer-Related Pathways and the Role of the *RPH3AL* Gene

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Cite this article as: Seven D, Bayrak OF. Determining prostate cancer-related pathways and the role of the *RPH3AL* gene. Experimed 2024; 14(3): 215-220.

ABSTRACT

Objective: Prostate cancer is the fifth leading cause of death worldwide. Treatment modalities for advanced prostate cancer include androgen deprivation therapy (ADT), chemotherapy, radiotherapy, and targeted therapy. Transcriptomic profiling in prostate cancer enhances our understanding of the disease at the molecular level, facilitating more accurate diagnosis and personalized treatment choices, and ultimately improving patient outcomes. Identifying new therapeutic biomarkers for prostate cancer is important for developing targeted therapy options. This study aimed to elucidate the pathways associated with prostate cancer and identify differentially expressed genes.

Materials and Methods: An RNA-seq dataset, GSE210205, was used to reveal transcriptomic differences between prostate cancer and benign prostate cell lines. GEO2R analysis, GSEA analysis, WebGestalt analysis, and GEPIA analyses were performed to generate differentially expressed genes, identify enriched pathways, and investigate gene expression in prostate cancer.

Results: Pathways such as Wnt/β-catenin signaling, DNA IR-induced double-strand breaks, cellular response via ATM, Type II interferon signaling, and TGF-β signaling were enriched in the prostate cancer transcriptome. Among the five most over-expressed genes, *RPH3AL* was the most prominent.

Conclusion: RPH3AL is a potential biomarker for prostate cancer based on transcriptomic profiling. Further investigation is required to validate the role and potential of this agent as a therapeutic target.

Keywords: Prostate cancer, transcriptomic profiling, *RPH3AL, TGF-β*

INTRODUCTION

Prostate cancer is the second most frequently diagnosed cancer in men and the fifth leading cause of death globally, with 1.6 million new diagnoses and 366,000 deaths annually attributed to this condition. Risk factors for prostate cancer include age, African American ethnicity, and a family history of the disease, with potential influences from diet and other factors (1). The introduction of widespread screening for prostate-specific antigen (PSA) led to a high increase in the incidence of prostate cancer. In cases of localized prostate cancer, in which the tumor remains confined within the prostate gland and has not metastasized to adjacent tissues or distant organs, primary treatment modalities typically include radical prostatectomy (RP) and whole-prostate radiation therapy. As the disease progresses to more severe stages, involving local invasion into nearby tissues and metastasis to distant sites, systemic medical therapies become predominant. Common sites of metastasis in prostate cancer include the bones, lymph nodes, liver, and lungs. The presence of metastasis significantly affects the prognosis and treatment strategy of prostate cancer patients (2). In advanced stages, androgen deprivation therapy (ADT) serves as the cornerstone treatment, often followed by radiotherapy, chemotherapy, immunotherapy, and targeted therapy. The widespread adoption of these interventions underscores the importance of effectively managing and addressing the potential complications associated with each

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Submitted: 27.06.2024 **Revision Requested:** 16.08.2024 **Last Revision Received:** 23.10.2024 **Accepted:** 06.12.2024 **Published Online:** 11.12.2024

treatment modality (3). Unraveling the molecular pathogenesis of cancer requires a better understanding of the genetic and molecular mechanisms responsible for its onset, progression, and dissemination. This knowledge is essential for developing targeted therapies, predicting outcomes, and improving personalized treatment strategies. Transcriptomic profiling in prostate cancer enhances our understanding of the disease at the molecular level, facilitating more accurate diagnosis and personalized treatment choices, and ultimately improving patient outcomes (4). Prostate cancer initiation and progression are driven by a complex interplay of genetic, epigenetic, and environmental factors. Identifying the pathways involved in prostate cancer progression is crucial for understanding disease progression and developing targeted therapies.

To better understand cancer predisposition, many researchers have focused on Genome-Wide Association Studies (GWAS). These studies on genetic susceptibility identified the rs7212943 variant, located within the *RPH3AL* gene, as a potential regulatory variant involved in the regulation of exocytosis in endocrine and exocrine cells (5). Additionally, the single nucleotide polymorphisms (SNPs) rs461251 and rs684232, which contain cis-regulatory elements, are associated with promoters that interact with the *RPH3AL* gene through H3K27Ac modifications in prostate cancer (6). Downregulation of the *RPH3AL* gene has been observed in bladder cancer and is associated with the development of muscle-invasive tumors. To the best of our knowledge, the expression of this gene has not been reported in prostate cancer.

In this study, we identified pathways associated with prostate cancer pathogenesis using an RNA-seq dataset, focusing on the expression of *RPH3AL*.

Materials and Methods

Prostate Cancer Dataset

To elucidate the molecular dynamics underlying the aggressive phenotype of prostate cancer compared with benign prostate tissue, the following cell lines were utilized: BPH-1, an immortalized cell line derived from benign prostatic hyperplasia, is frequently used as a human model for studying prostate growth and physiology (7). DU 145, an epithelial cell line, was isolated from the brain of a 69-year-old Caucasian male with prostate cancer, whereas PC-3 was derived from a bone metastasis of prostate cancer (8, 9). The Gene Expression Omnibus (GEO) database provided RNA-seq data for the aforementioned cell lines (accession number GSE210205). This dataset includes 4 replicates of each BPH-1, DU145, and PC3 cell lines. We employed both Differential Expression (via GEO2R) and Gene Set Enrichment Analysis (GSEA) analysis techniques to evaluate the GEO data.

GEO2R analysis

GEO2R is an analytical tool designed to examine gene expression data retrieved from the GEO database. It is particularly valuable for conducting comparative analyses of gene expression across different experimental conditions or groups. Using GEO2R, we performed differential expression analysis to identify differentially expressed genes in tumor cells compared with benign control cells, with a false discovery rate of 0.001. Following the tool's guidelines, raw counts were used to assess gene expression using DeSeq2, with normalization of median ratios to normalize sequencing depth and RNA composition. As the samples were from a specific study, normalization for sequence depth had a minimal impact (10). The analysis included volcano plots. The volcano plot, in turn, visualizes differentially expressed genes by plotting statistical significance (-log10(p value)) against the magnitude of change (log2(fold change)), allowing an intuitive display of genes that are both statistically significant and have substantial gene expression changes. Differentially expressed genes were identified by filtering the expression data, with a p value threshold of <0.001 and a log2 fold change value of ≥0.5 based on default settings, previous studies, and upon inspecting the size of the resulting gene set that were differentially expressed between tumor and benign cells.

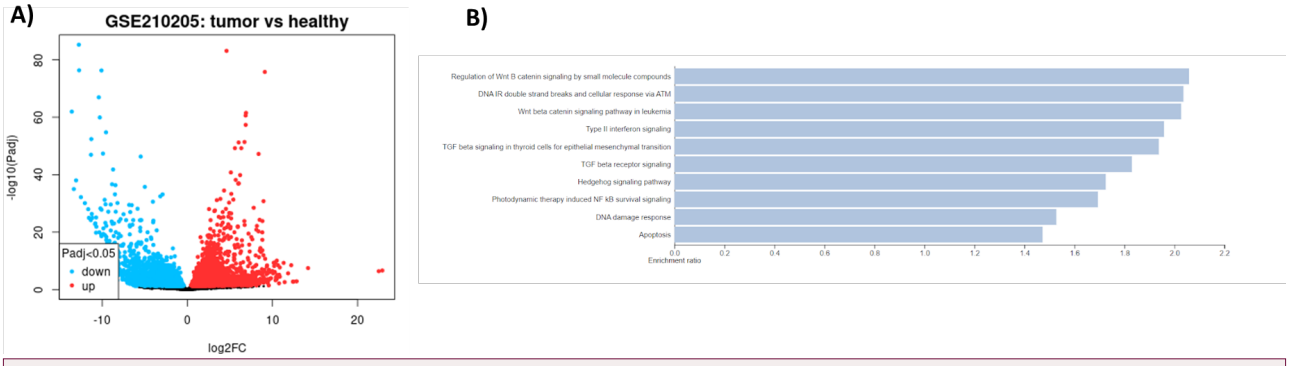

Figure 1. The volcano plot illustrates differentially expressed genes, with upregulated genes represented by red dots and downregulated genes represented by blue dots. B) The bar graph presents the results of the Over-Representation Analysis (ORA), highlighting the top 10 enriched pathways. The x-axis displays the enrichment ratio, whereas the y-axis lists the pathways in descending order of enrichment.

Gene Set Enrichment Analysis of RNA-seq Data

GSEA is a computational technique used to assess whether predefined sets of genes are over-represented in a large set of genes, with possible associations with different phenotypes. The data analysis was performed using the GSEA tool (version 4.1.0). The gene sets used for GSEA were obtained from Database C4 of MSigDB (http://www.broad.mit.edu/cancer/software/gsea). C4 refers to a set of gene sets that are computationally defined by mining large collections of cancer-oriented microarray data (11). The specific parameters employed in the GSEA were as follows: the number of permutations was set to 100, and the type of permutation was configured to the gene set to address any potential issues arising from a limited sample size.

WebGestalt Analysis

The WEB-based Gene SeT AnaLysis Toolkit (WebGestalt) is a robust and versatile platform for GSEA and various other forms of gene set enrichment analysis. This approach empowers researchers to input a list of genes and evaluate their enrichment across biological contexts, including signaling pathways, disease associations, and gene ontology categories. In WebGestalt, it was performed over-representation analysis (ORA) using wikipathways cancer with the following parameters: the organism of interest was Homo sapiens, and only gene symbols from the gene list were used (p=0.001).

GEPIA

Gene Expression Profiling Interactive Analysis (GEPIA) is a web-based platform that integrates RNA sequencing data from the Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) project. It allows users to explore and analyze gene expression patterns in tumor and normal tissues, offering insights into the functional relationships, prognostic significance, and potential molecular interactions across various cancer types and healthy tissue counterparts. The mRNA expression of *RPH3AL* was determined for prostate cancer. GEPIA also facilitates survival analysis based on gene expression levels, employing the log-rank test, also known as the Mantel–Cox test, to evaluate hypotheses. This analysis allows users to assess the correlation between gene expression and patient survival outcomes (12).

RESULTS

The dataset has 2566 differentially expressed genes with a p<0.001. 1128 of the genes were downregulated while 1438 were upregulated. A volcano plot generated from the Geo2R analysis illustrates the statistical significance (-log10 p value) against the magnitude of the expression difference (log2 fold change) (Figure 1A). Regulation of Wnt Beta catenin signaling, DNA IR double-strand breaks, cellular response via ATM, Type II interferon, and TGF beta signaling were the most enriched pathways according to the Webgestalt analysis (Figure 1B).

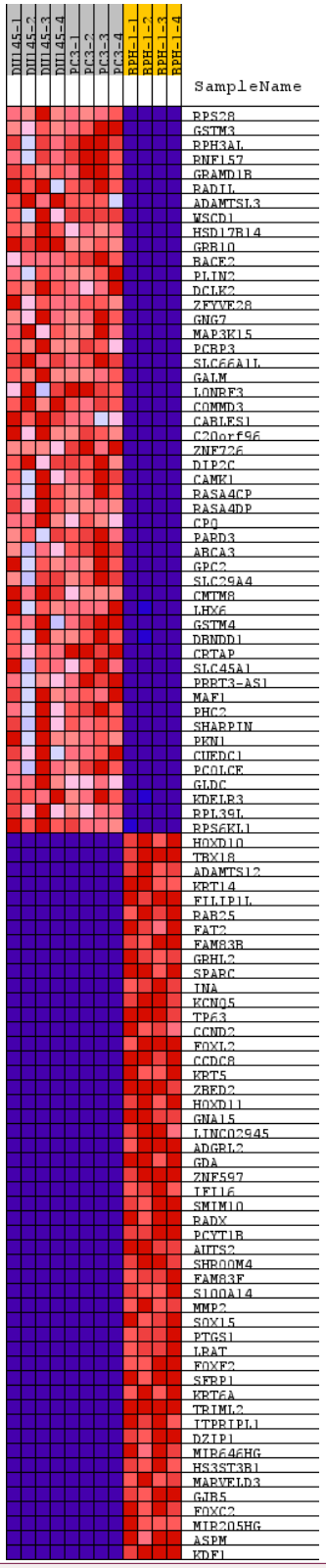

Figure 2. Heatmap of differentially expressed genes (DEGs) derived from GSE210205. Red indicates high gene expression and purple represents downregulated expression.

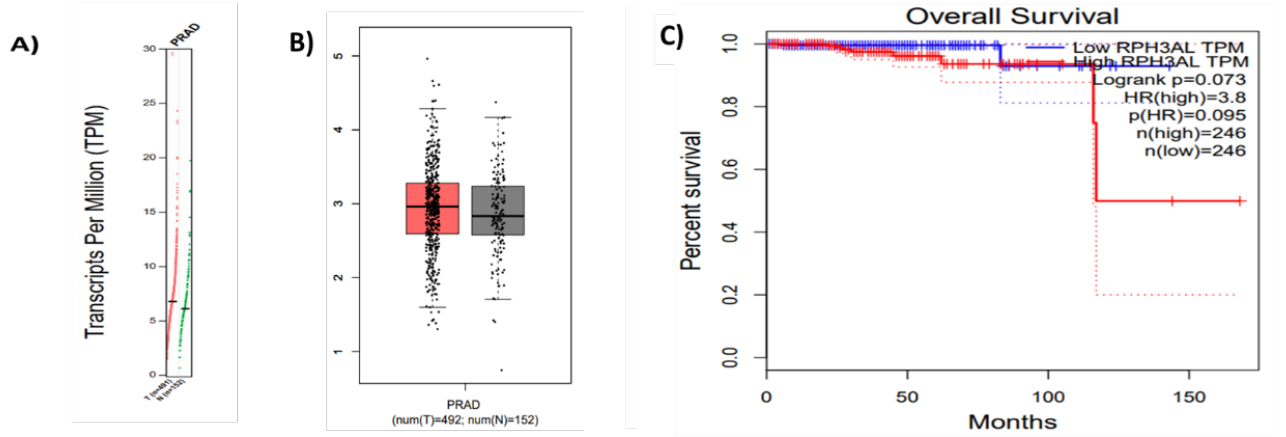

Figure 3. A) *RPH3AL* gene read count in healthy and prostate cancer samples. Green dots represent healthy prostate tissue, while red dots indicate cancerous tissue, showing increased levels of the *RPH3AL* transcript in cancer samples. B) Expression levels of *RPH3AL* in tumor and healthy tissue. Boxplot showing the increased level of *RPH3AL* mRNA levels compared with healthy tissue. C) Overall survival graph for prostate cancer patients in relation to RPH3AL gene expression, demonstrating that higher levels of *RPH3AL* are associated with decreased survival rates.

The heatmap generated by GSEA is shown in Figure 2. Among the five most over-expressed genes, *RPH3AL* was identified. The association between *RPH3AL* and prostate cancer has not been previously investigated. Previously, this gene was considered a biomarker of colorectal cancer. Therefore, we decided to conduct an in-depth analysis of this gene in patients with prostate cancer.

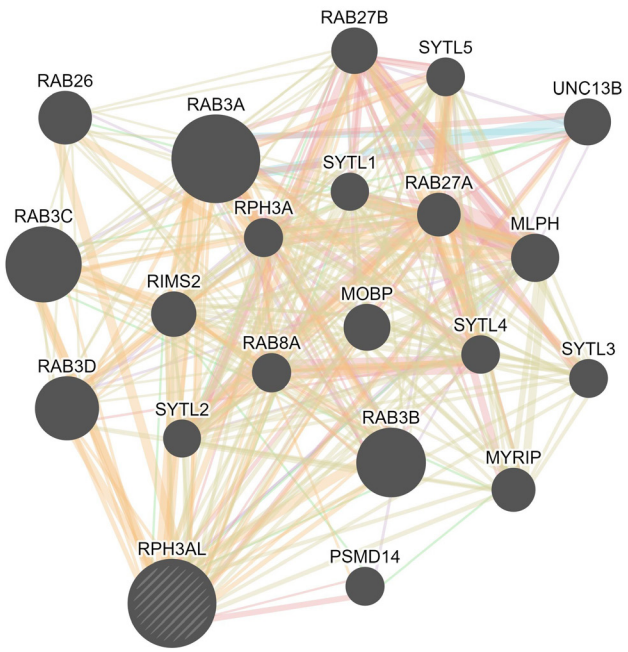

Figure 4. *RPH3AL* gene interaction network generated using GeneMANIA analysis. The network illustrates various types of interactions associated with the RPH3AL gene: pink lines represent physical interactions, purple lines indicate co-expression, green lines show genetic interactions, and yellow lines denote predicted interactions.

GEPIA analysis revealed that tumor samples exhibited higher expression of the *RPH3AL* gene compared with benign prostate tissue (Figure 3A). The mRNA expression levels in tumor and normal tissues were analyzed using the GEPIA database (http://gepia.cancer-pku.cn), which incorporates data from 492 tumor samples and 152 normal samples derived from the TCGA and GTEx datasets. The expression levels of *RPH3AL* in patients with prostate cancer were specifically evaluated. Boxplots were used to visualize the expression levels in both tumor and normal tissues, and differential expression analysis was conducted using one-way ANOVA (Figure 3B). Additionally, higher expression of the *RPH3AL* gene was associated with a lower survival rate in prostate cancer (Figure 3C).

We analyzed *RPH3AL* gene interactions using GeneMANIA (Figure 4). The database prediction revealed that *RPH3AL* exhibited physical interactions with members of the RAB family, which are small GTPases involved in regulating intracellular membrane trafficking.

DISCUSSION

The management of prostate cancer has evolved significantly over the past decade because of substantial advances in understanding the genomic landscape and underlying biology of prostate cancer. However, the heterogeneity, particularly in advanced prostate cancer, presents a challenge in combating diverse cancer cell populations. Identifying appropriate therapeutic targets is vital for effectively treating prostate cancer and improving patient outcomes. This study aimed to identify the responsible pathways and potential biomarkers associated with prostate cancer by analyzing the GSE210205 dataset. By comparing benign and metastatic prostate cancer cell lines, we sought to identify significant differences that may inform therapeutic targets and diagnostic markers. Our specific objectives include elucidating the molecular mechanisms

driving prostate cancer progression and identifying critical genes and pathways that could function as biomarkers or therapeutic targets.

The expression of Wnt ligands and secreted Wnt antagonists is frequently dysregulated in prostate cancer, leading to outcomes that often do not correlate with the anticipated effects of these proteins on the stability of β-catenin. Prostate cancer commonly exhibits aberrant expression and mislocalization of β-catenin (13,14). Consistent with these observations, the most significantly enriched pathway identified was the regulation of Wnt/β-catenin signaling. In mammalian cells, the occurrence of double-strand breaks (DSBs) triggers a robust cellular response, including checkpoint signaling and repair mechanisms, or cell death through apoptosis. Central to this process is the MRN (MRE11/RAD50/NBS1) complex, which binds to DSBs and facilitates the activation of the Ataxia Telangiectasia Mutated (ATM) protein. ATM, a critical kinase related to phosphatidylinositol 3-kinase (PI3K), plays a pivotal role in orchestrating the DNA damage response (DDR) (15). In prostate cancer cell lines, an increase in DNA ionizing radiation (IR)-induced double-strand breaks and subsequent cellular response through the ATM pathway was noted. Previous studies have implicated members of this pathway, such as BRCA1 and BRCA2 genes (16), although a direct connection to this specific pathway has not been established. This study is the first to demonstrate an association between prostate cancer, IR-induced double-strand breaks, and cellular response via the ATM pathway. Type II interferon has been identified as an enriched pathway in prostate cancer. This pathway begins with binding of IFN-gamma to its receptor, initiating a phosphorylation cascade involving members of the JAK and STAT protein families (17). Previous studies have suggested interferons as a promising therapeutic approach for advanced prostate cancer (18). Recently, Hagiwara et al. provided evidence that MUC1 may functionally contribute to the activation of the type II interferon pathway in prostate cancer (19).

Finally, enrichment of TGF-β signaling was identified in the prostate cancer dataset. Recently, there has been significant interest in inhibiting TGF-β activity, blocking its receptor binding, and disrupting signaling pathways using small molecule inhibitors. These approaches represent burgeoning research areas with the aim of targeting the tumor microenvironment as a novel therapeutic strategy for prostate cancer (20). Our study highlights TGF-β as a promising therapeutic target for advanced prostate cancer, in line with prior research.

We also identified the most significantly differentially expressed genes. Among the top five highlighted over-expressed genes, *RPH3AL* (Rabphilin 3A Like (without C2 domains)) also called NOC2, was over-expressed in tumor cell lines and exhibited minimal expression in benign prostate cell lines. The existing literature provides limited information about this gene. *RPH3AL* encodes a protein that regulates calcium-ion-dependent exocytosis in both endocrine and exocrine cells (21). It was initially identified in medulloblastoma tumors by cloning a tumor suppressor region (22). Recently, Lv et al. identified differentially methylated probes (DMP) for Alzheimer's disease, and RPH3AL was found to be glia-specific DMPs (23). In a genetic investigation of kidney function, a relationship was found between the glomerular filtration rate and the *RPH3AL* gene variant (24). In clinical practice, predicted TP53 gene mutations enhance clinical-genomic risk stratification by identifying more aggressive tumors. A study based on gene expression assessment found that TP53 mutation is associated with low expression of *RPH3AL*, whereas high expression indicates the wild-type TP53 (25). *RPH3AL* exhibited decreased levels of RNA and protein expression in breast cancer tissues compared with normal tissues, suggesting as a biomarker (26). Conversely, the detection of autoantibodies against Rabphilin-3A-like protein has been identified as a potential biomarker in the sera of colorectal cancer patients (27). Genemania analysis of the *RPH3AL* gene demonstrated that the RAB family closely interacts with it. Shibasaki et al. revealed the physical and functional interaction between Noc2 and Rab3 during exocytosis (28).

In addition to the analysis results of the GSE210205 dataset, which contains metastatic DU-145 and PC3 and benign prostate cell lines, GEPIA analysis also showed higher expression of *RPH3AL* in the tumor compared with the normal samples. The patients that exhibit higher expression of *RPH3AL* have a lower percentage of survival. Overall, these data suggest that *RPH3AL* gene acts as an oncogene in prostate cancer.

The results of this initial study indicate that the *RPH3AL* gene serves as a biomarker of prostate cancer progression. However, expression levels in patients and cell lines should also be analyzed using quantitative real-time reverse transcription-polymerase chain reaction (qRT–PCR) to validate these findings. The functional properties of this gene should also be investigated to better understand its impact on prostate cancer. This is the first study to address *RPH3AL* expression in patients with prostate cancer. Further studies are required to elucidate the functional properties of *RPH3AL* in prostate cancer.

Acknowledgment: Extend my deepest gratitude to Dr. Emrah Nikerel for his expert guidance and support in conducting the GSEA analysis.

Ethics Committee Approval: Ethics committee approval was excluded in this study because online bioinformatics tools are open sources and freely used in all research.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study – D.S.; Data Acquisition – D.S.; Data Analysis/Interpretation- D.S.; Drafting Manuscript – D.S.; Critical Revision of Manuscript – D.S., O.F.B.; Final Approval and Accountability – D.S., O.F.B.

Conflict of Interest: The authors declare no conflict of interest.

Financial Disclosure: The authors declare that they received no financial support for this study.

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