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Protein Kinase C Epsilon Overexpression in Prostate Adenocarcinoma is Associated with Oncogenesis

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Abstract

Background: *PKCε, an isozyme of serine-threonine kinase, has been implicated in epithelial cancer metastasis and progression. This study investigates the impact of the oncogenic PKCε, overexpressed abnormally in human Prostate tumor samples and cell lines, to understand its efficacy.* ***Methods:*** *The microarray dataset, GSE86257, was processed for normalization. The identification of upregulated and downregulated genes was based on FDR >1 and p <0.05 values. Cytoscape analysis and functional enrichment of significant genes were done. The identified genes were validated on the TCGA dataset and survival analysis was performed by Kaplan-Meier analysis.* ***Results:*** *A total of 1524 DEGs were identified with 728 upregulated genes and 818 downregulated genes. The two significant modules with MCODE score:9.0 and Venn analysis provided cyclin-dependent kinase inhibitor protein (CDK1), Cyclin B1 (CCNB1), Phospholipase C Gamma 1 (PLCG1), Cyclin Dependent Kinase 9 (CDK9), Phosphoinositide-3-Kinase Regulatory Subunit 3 (PIK3R3), H4 Clustered Histone 6 (H4C6), Phospholipase C Gamma 2 (PLCG2) as most interacting genes. TCGA data analysis and Prognostic analysis revealed CCNBI, CDK9, and PLCG1 associated with poor prognosis.* ***Conclusion:*** *PKCε regulates genes that are responsible for cancer progression. Therefore, targeting PKCε in Prostate cancer may serve as an important regulatory effect and may improve the prognosis of the disease.*

Keywords: *PKCε, Prostate Cancer, CCNB1, PLCG1, CDK9, TCGA, STRING.*

1. Introduction

Protein kinase C isozymes are the phorbol ester tumor promoters and have been widely implicated in cancer advancement. They belong to a family of serine/threonine kinases that are divided into three groups: classical (cPKCs α, βI, βII, and γ), novel (nPKCs δ, ε, η, and θ), and atypical (aPKCs ζ and λ/ι). The development and repression of the cancer phenotype are frequently associated with altered patterns of isozyme expression and activation state. Patients with invasive ductal breast cancer and non-small cell lung cancer (NSCLC) have been found to overexpress PKCε. Also, the PKCε levels were significantly higher in prostate cancer and its overexpression is associated with disease recurrence. Ras/Erk, phosphatidylinositol 3-kinase (PI3K)/Akt, nuclear factor κB (NF-κB), and Stat3 are mitogenic and survival pathways that are activated by PKCε. It is also reported as a regulator for cell motility, invasion, and epithelial-mesenchymal transition (EMT) in tumours. Transgenic overexpression of PKCε leads to preneoplastic lesions in the mouse prostate. Similarly, genetic ablation of the PKCε gene leads to spontaneous prostate tumor formation and metastases in TRAMP mice. Studies have shown that PKCε inhibition in cancer cell proliferation and xenografts reduces metastatic disease. This idiosyncratic functionality of PKCε isozymes reflects the capability to regulate growth-inhibitory signalling pathways and thus regulate oncogenic activities in prostate cancers. Studies has shown that, it is not present in the healthy, benign prostate epithelium, but it is highly expressed in the majority of human prostate tumors. All such emerging evidence links PKCε to prostate cancer progression; thus, understanding the PKCε molecular paradigm for tumor phenotype will reveal the functional interaction of the PKCε isozymes and its association with prostate oncogenesis.

2. Materials And Methods

Details of samples chosen from the dataset:

Microarray datasets GSE86257 were accessed for analysis through Gene Expression Omnibus (GEO database) http://www.ncbi.nlm.nih.gov/geo. The chip dataset GSE86257 included control samples with stable PKCε expression P8 parental cells and CaP8 parental cells. Similarly, P8 parental cells and CaP8 parental cells without stable PKC activity as treatment was processed. A set of three replicates of P8 parental cells (GSM2299136, GSM2299137, GSM2299138) & CaP8 parental cells (GSM2299139, GSM2299140, GSM2299141) were curated. Similarly, three replicates of control include P8 cell with stable PKC-ε expression (GSM2299142, GSM2299143, GSM2299144) & CaP8 cell with stable PKC-ε expression (GSM2299145, GSM2299146, GSM2299147) respectively were derived from the GEO database. Gene expression profiling was performed using Affymetrix Mouse Gene 1.0 ST Array.

Data pre-processing and normalization:

The samples were divided into two groups: the Control group, with PKCε in the regular expression, and the treated group, with PKCε overexpression expression in prostate cancer cell lines. For background correction, the initial dataset's quantile normalization and log transition were obtained. The online statistical tool GEO2R and the R/Bioconductor and Limma package v3.26.8 were used for raw reads processing. The collected data was then processed using Entrez's Gene ID converter to convert gene ID. To determine the differentially regulated genes (DEG's), $p<0.05$ and false discovery rate (FDR, >1) were considered. The tool used was 1GEO2R built-in with T-test and Benjamini and Hochberg methods. Among the gene sets, the upregulated set had logFC > 1 and $p <0.05$, whereas downregulated DEGs had logFC <-1 and $p <0.05$.

PPI network construction

The STRING v1026 database (http://string-db.org) was used for the retrieval of interacting gene. All upregulated and downregulated DEGs were used for constructing the PPI (Protein-protein interaction) networks. The confidence score >0.4 was taken to construct PPI networks and analyzed in CystoscapeTM, version 3.10 software.

Module identification and Enrichment analysis:

The PPI network was assessed by Molecular Complex Detection (MCODE) to form modular clusters through the vertex weighing method. The module analysis included degree cut-off 2, node score cut-off 0.2, k-core of 2, and maximum depth of 100. The significant modules have an MCODE score >5 and the number of nodes >10.

Identifying and analyzing significant hub genes:

Pearson's correlation test processed MCODE genes to identify the significant hub genes by analyzing five significant topological algorithms of closeness, degree, edge percolated component (EPC), maximal clique centrality (MCC), and maximum neighbourhood component (MNC). The most interacting significant hub genes were established by Venn analysis of the genes obtained from these five algorithms with the help of an online tool (http://bioin formatics.psb.ugent.be/webtools/Venn/).

Functional enrichment analysis:

Pathway enrichment of hub genes was executed with the Gene Set Analysis Toolkit (WebGestalt) and Metascape (https://metascape.org) by considering $p < 0.05$ as significant.

Validation through TCGA database:

The mRNA expression of the screened hub genes was validated using the TCGA database. The prostate adenocarcinoma expression datasets (PRAD, n=2387) from the TCGA database were explored. The data were plotted as a boxplot with Tukey's Honest Significant Difference (HSD) test used for p-values determination (***$p<0.001$; **$p<0.01$; *$p<0.05$; and ns (not significant).

Survival analysis of hub genes:

The survival analysis of hub genes in the Prostate Database was analyzed. Publicly available cancer microarray datasets was processed for meta-analysis of the predictive significance of genes between gene expression and clinical prognosis. A Kaplan-Meier plot represents the data analysis for the associated Cox proportional hazards model, which was found significant at $p<0.05$.

3. Results and Discussion

Identification of the DEGs, PPI network construction and MCODE analysis:

The microarray dataset (Table 01) was obtained from GEO database for experimental analysis and normalized for DEG identification. A total of 1542 DEGs (Fig.1a) were identified with the upregulated set of 724 genes in red color (Fig.1a) and 818 downregulated sets (Fig.1a) with blue color representation. The PPI network with a confidence score of 0.4 and p-value: < 1.0e-16, was generated and contain 483 nodes and 1170 edges (Fig.1b). The MCODE analysis revealed two significant Module 1 with score:9.0, of node:09, edges: 36 and Module 2 with score:6.167, of nodes:13, edges:37 (Fig.1c). The Venn analysis identified 7 (Fig.1d) most interacting genes which were identified as cyclin-dependent kinase inhibitor protein (CDK1), Cyclin B1 (CCNB1), Phospholipase C Gamma 1 (PLCG1), Cyclin Dependent Kinase 9 (CDK9), Phosphoinositide-3-Kinase Regulatory Subunit 3 (PIK3R3), H4 Clustered Histone 6 (H4C6), Phospholipase C Gamma 2 (PLCG2).

Table 01: The Microarray datasets obtained from GEO database.

Prostate cancer dataset	GSE86257	
	P8 parental cells	CaP8 parental cells
Control	GSM2299136	GSM2299139
	GSM2299137	GSM2299140
	GSM2299138	GSM2299141
Treatment (overexpression)	GSM2299142	GSM2299145
	GSM2299143	GSM2299146
	GSM2299144	GSM2299147

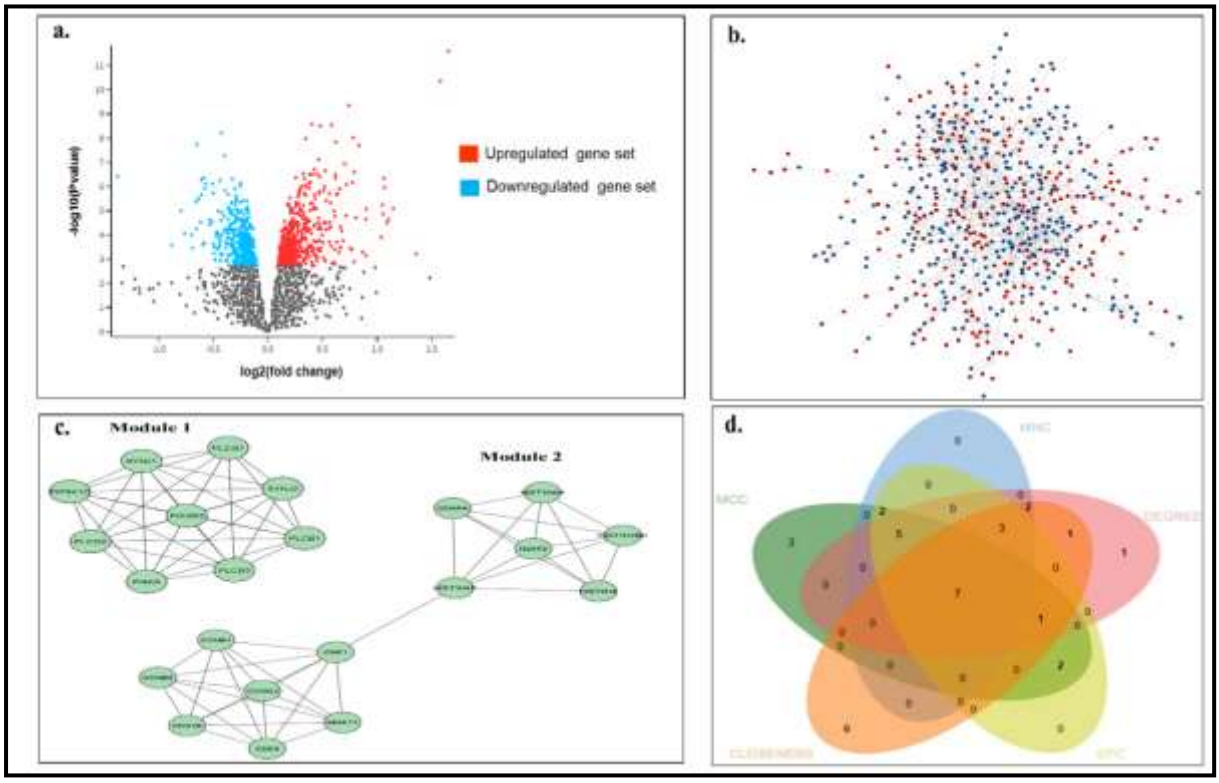

Fig:01: a) Volcanic plot representation of DEG's. Red represents the upregulated set, and blue represents the downregulated set. (LogFDR ≥1 and p-value≤0.05). b) PPI network of upregulated (Red) and downregulated (Blue) genes. c) The MCODE interacting hub genes. d) The Venn plot of the seven most interacting genes with five topological interactions.

Functional enrichment of DEGs: The biological significance of hub genes was established by analyzing enriched biological processes of lipid degradation (p <3.6e-2) and mitosis (p <9.9e-2). Upon molecular function analysis, the kinase activation was most significant (p <5.1e-2). Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathway enrichment analysis (Fig.02) shows that the hub genes PLCG1, PLCG2, PIK3R3 were enriched in VEGF Signalling, Fc epsilon RI signaling, and Non—Small cell lung carcinoma, whereas H4C6, CCNB1 and CDK1 were enriched in condensation of prophase chromosome.

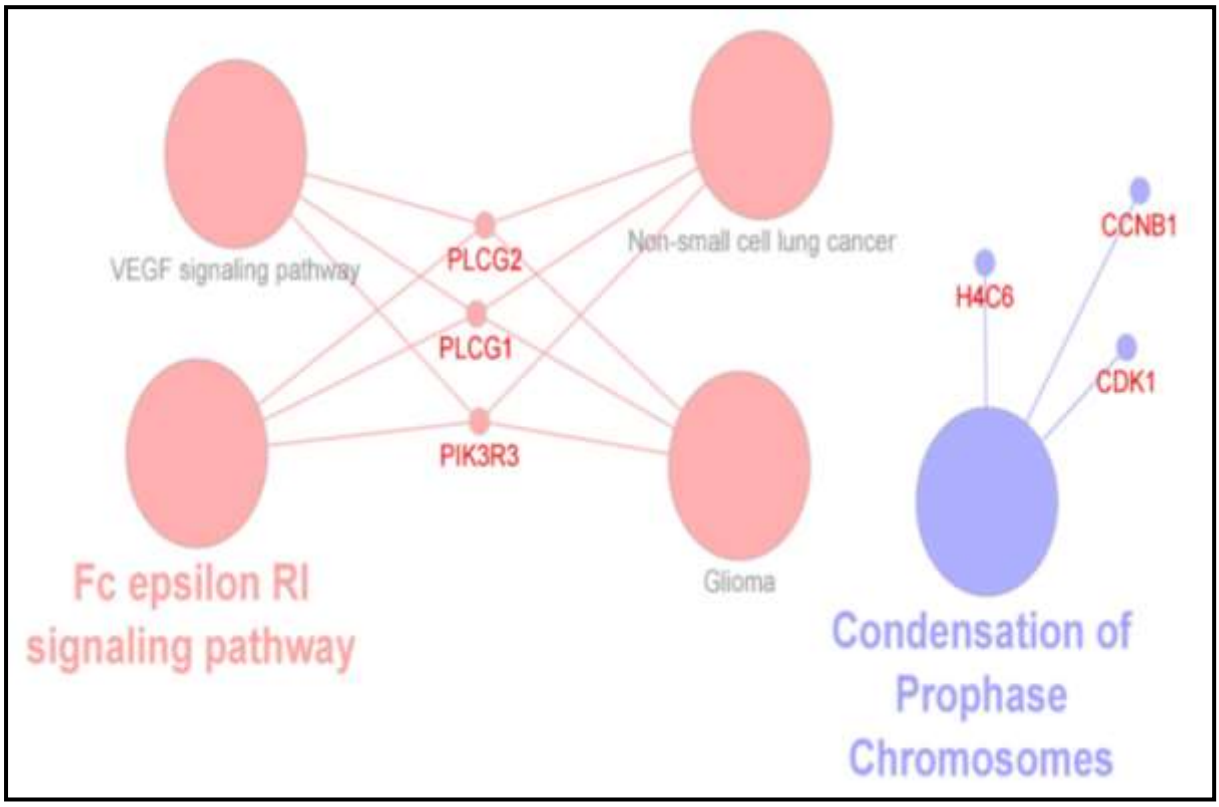

Fig 02: Functional annotation of KEGG pathways of hub genes.

Validation of hub genes through TCGA database analysis:

Upon TCGA database analysis, CDK1, CCNB1, PLCG1, and PIK3R3 expression were significantly upregulated (Fig.03) in the TCGA PRAD dataset. The mRNA expression of PLCG2 was significantly downregulated (Fig.03). The CDK9 shows similar expression (Fig.03) when compared with normal prostate mRNA levels. The H4C6 expression was not reported in the TGCA database.

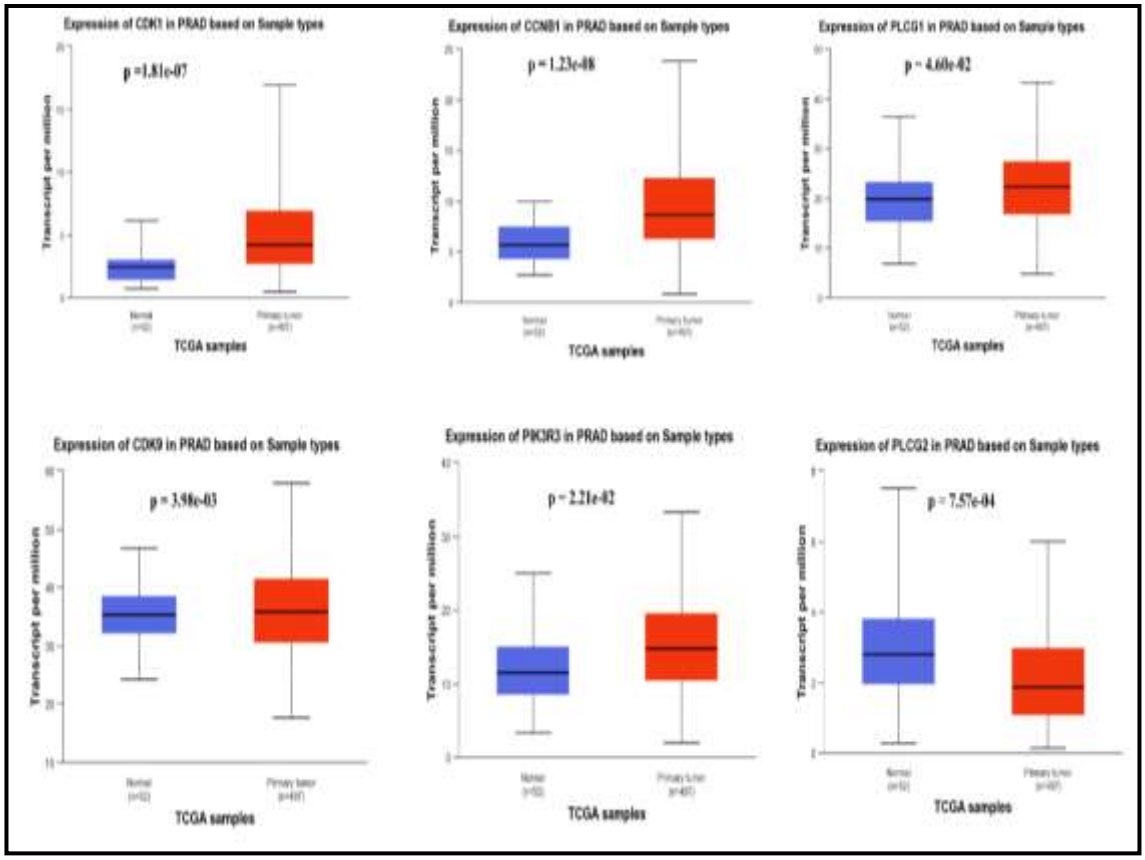

Fig 03: The mRNA expression represented in box plot of hub genes from TCGA PRAD database with significant p <0.005.

Prognostic value of hub genes: Each hub gene was evaluated with the correlation between expression and survival rates. The Cox p-value (Table 02, Fig 04) of CCNB1, CDK1, PLCG1 shows a significant Cox p-value from PRAD database. The CDK9, PLCG2 and PIK3R3 were found to have Cox p-values which were not significant (Table 02, Fig 04). The H4C6 cox p-value was not found in any dataset.

Table 02: The Cox p-value of hub genes with the datasets.

Gene	Cox p-value	Dataset
CCNB1	0.01	GSE13507
CDK1	0.02	GSE5287
CDK9	0.49	GSE13507
PLCG1	0.01	GSE13507
PLCG2	0.36	GSE13507
PIK3R3	0.78	GSE13507

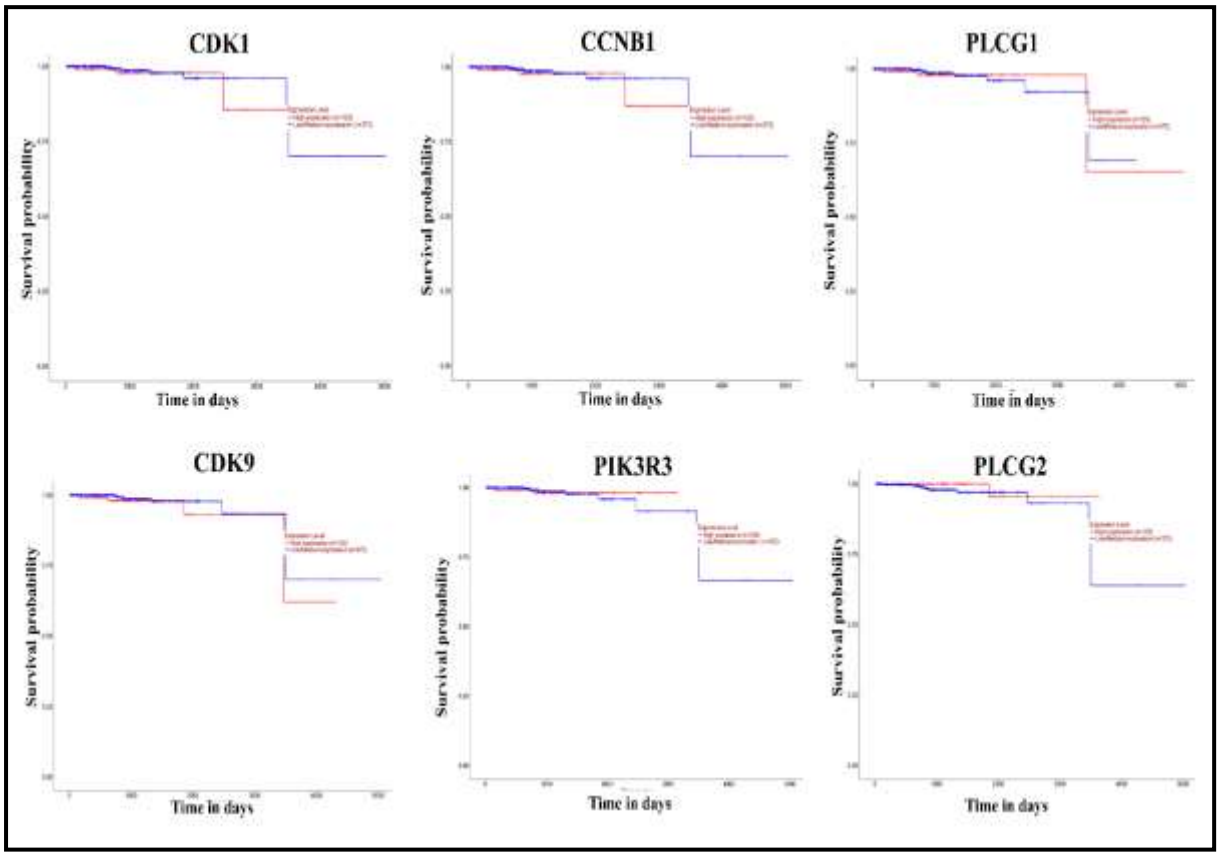

Fig 04: The comparative plot of expression and survival analysis (Kaplan-Meier plot) of hub genes.

The PKCε is involved in regulating metastasis in epithelial cancers such as prostate, lung, breast, and head and neck cancer. This regulatory effect has been exploited in many studies to identify the potential effect of this kinase in regulating signalling pathways causing tumor development and progression. Therefore, this study assessed the PKCε through bioinformatic analysis to uncover the mechanisms associated with prostate tumorigenesis. The clinical variability of Prostate cancer (PC) in clinical practice makes it difficult to analyze the metabolic profile of PC samples. To achieve this, two microarray profile datasets from groups with control PKCε activity and overexpressed PKCε activity were taken into account. By processing through various bioinformatics analyses, CCNB1, CDK1, and PLCG1 genes were recognized to be upregulated and associated with poor prognosis among PC patients.

CDK1 is a serine/threonine kinase known as cyclin-dependent kinases (CDKs). This kinase forms a complex with cyclin proteins essential for their activity. The KEGG enrichment analysis in this study showed the involvement of CDK1 as a catalytic subunit of the M-phase promoting factor responsible for the condensation of prophase chromosomes. CDK1 encourages transition in mitotic phases of G2/M and G1/S. Studies revealed that CDK1 activity triggers unrestrained cell proliferation in various cancers. A targeted miR-7 delivery among in-vivo experiments showed the inhibition of CDK1 as a therapeutic therapy for prostate cancer treatment.

Similarly, CCNB1 (Cyclin B1) is involved in mitosis via encoding for regulatory proteins. Malignancies such as breast cancer and non-small cell lung cancer overexpress the CCNB1 protein. CCNB1 expression was noticeably higher in PRAC when compared to adrenocortical adenoma. Its high expression is associated with poor outcomes among various cancer patients. Furthermore, many studies have shown that patients with high levels of CCNB1 expression are more likely to develop tumor metastases and have a poor prognosis. Consistent with these findings, this study shows that CCNB1 has a high expression with poor prognosis among PC cases.

The PKCε activation is influenced by another identified hub gene PLCG1. This gene is responsible for producing the second messenger's diacylglycerol (DAG) and inositol 1,4,5-trisphosphate, which activate protein kinase C (PKC) and raise intracellular calcium levels. These raised calcium levels are associated with growth-factor stimulation and hence high cancer metastatic rate. It is an essential oncogene, with high expression of protein in most malignant tumors, including liver, lung, and prostate cancer. PLCG1 controls the intracellular transmission of receptor-mediated tyrosine kinase activators and intracellular signalling cascades. Also, KEGG analysis shows VEGFA activation via PLCG1.

Our study indicates the significance of PKCε in regulating prostate cancer. The genes identified through analysis are responsible for tumor progression. These genes are found to be overexpressed in PRAD datasets and also has poor survival outcome. The study aimed to identify the efficacy of targeting PKCε as a druggable target for efficiently reducing PC tumorigenesis. The results obtained indicate the genes that are responsible for PC metastasis. Therefore, targeting PKCε may reduce the expression of these genes, hence reducing prostate cancer tumorigenesis.

4. Conclusion

Targeted Prostate cancer therapy is necessary to control the tumor from metastasizing and recurrence. PKCε is a good candidate for targeted therapy as its inhibition affects many downstream oncogenes responsible for tumor proliferation and metastasis.

Conflict of interest: All authors read and approved the final manuscript. None of the authors declare any conflict of interest or competing interest.

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