Journal of Applied Biology & Biotechnology Vol. 13(1), pp. 202-212, Jan-Feb, 2025
Available online at http://www.jabonline.in
DOI: 10.7324/JABB.2024.189230

Bioinformatics analysis reveals PIH1D1 as an important prognostic marker in breast cancer

Dhiraj Kumar Singh[1], Bimal Jit[1], Rashmi Gupta[1], Amit Kumar Verma[2], Riyaz Ahmad Mir[1*]

[1]Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India.
[2]Department of Bioscience, Jamia Millia Islamia, New Delhi, India.

ARTICLE INFO

Article history:
Received on: June 02, 2024
Accepted on: September 15, 2024
Available Online: November 15, 2024

Key words:
Breast cancer, R2TP/PAQosome, PIH1D1 expression, bioinformatics, TCGA, TNM plot, TIMER 2, GEPIA, UALCAN

ABSTRACT

R2TP (Rvb1, Rvb2, Tah1, and Pih1) complex was initially discovered as a Heat shock protein 90 associated multimeric protein complex in yeast and is highly conserved in mammals. In Human R2TP complex is known by different names including RUVBL1 for Rvb1, RuvBL2 for Rvb2, RPAP3 (RNA polymerase II-associated protein 3) for Tah1 and PIH1D1 for Pih1, and known to be a specialized Co-chaperone of Hsp-90 protein. This multimeric-protein complex is involved in the assembly and maturation of several multisubunit complexes including RNA polymerase II, small nucleolar ribonucleoproteins, and complexes containing phosphatidylinositol-3-kinase-like kinases. Nevertheless, evidence in the clinical setting is scanty with respect to the expression of PIH1 domain containing 1 (PIH1D1) and its prognostic values in breast cancer (BRCA). Exploring the function of PIH1D1 in BRCA could reveal its therapeutic and translational potential in BRCA. In the current study, we analyzed PIH1D1 expression levels across cancers with the help of a bioinformatics tool and tumor immune estimation resource, and compared with normal and BRCA tissues using tumor, normal, and metastasis plot. Disease-free and overall survival analysis was done by survival plot, in gene expression profiling interactive analysis web server. Furthermore, histological analysis of PIH1D1 protein expression was done with the help of the Human Protein Atlas Database. Clusters and interaction analysis were done using cell line expression cluster toll in Human Protein Atlas. The result of our study shows that PIH1D1 expression was higher in almost all cancer groups and significantly increased in BRCA. Additionally, we found a positive statistical correlation between PIH1D1 and CD4+ and CD8+ T cell infiltration, which is an indication of the immunotherapeutic potential of PIH1D1. The expression of chemokines and receptors was shown to be significantly positively linked with Chemokine (C-C motif) ligand 14 when PIH1D1 was increased. Furthermore, the UALCAN (University of ALabama at Birmingham CANcer) data analysis results also revealed that PIH1D1 was significantly associated with tumor metastasis, menopausal status, grade, and different stages of patients with BRCA. In conclusion, the current study provides the preliminary proof of concept regarding the biomarker potential of PIH1D1. However, further *in vivo* and *in vitro* mechanistic studies in detail are required to explore the therapeutic potential of PIH1D1 in BRCA.

INTRODUCTION

Breast cancer (BRCA) is the second most common cancer in women after skin cancer. The disease is associated with the family history and renders the most complex pathological gynecological malignancy in women. Though the change in breast or lump formation is the early symptom, lacking a suitable biomarker of specificity and sensitivity rendered the prognosis as well as a treatment option. Signs of BRCA include a lump or change in the size or shape of the breast. According to the World Health Organization, BRCA is the most common type of cancer in India, accounting for roughly 18% of all cancer cases and 27% of all malignancies in women globally. Of all the cancers that affect humans, BRCA is the one that is easiest to identify and cure [1]. There are certain factors that affect prognosis (chance of recovery) and treatment options. In BRCA, the stage is based on the size and location of the primary tumor, the spread of cancer to nearby lymph nodes or other parts of the body, tumor grade, and whether certain biomarkers are present [2]. Although conventional therapeutic strategies for BRCA, including surgery, radiotherapy and chemotherapy, targeted therapies, and more recently immunotherapies dramatically prolonged the survival of BRCA patients, the incidence and mortality rates of some subtypes continuously increased in recent years and the trend even varies depending on the race, age, or region [3,4]. A variety of biomarkers, including DNA (genes), proteins, and hormones, are employed in the diagnosis, monitoring, and treatment of many illnesses. These biomarkers are utilized in drug target development, prognosis and therapy prediction, diagnosis, and recurrence [5]. Identification of novel biomarkers in BRCA is critical for accurate prognosis analysis and therapeutic efficacy prediction. Stage IV BRCAs, in particular, are detrimental metastatic breast cancers (MBCs). MBCs are rarely

*Corresponding Author
Riyaz Ahmad Mir, Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India.
E-mail: riyaz978 @ gmail.com

curative, so their 5-year survival rate (26%) is much lower than localized cancer (99%) [6,7]. In the past, a number of bioinformatics analyses have been conducted to identify key differentially expressed genes and enriched biological pathways or to evaluate the expression of a few specific genes in BRCAs, but such analysis using transcriptomes of MBCs have not been satisfactorily performed. Therefore, it is urgently needed to discover novel molecular biomarkers, therapeutic targets, or prognostic evaluation index for cervical cancer. The extensive application of bioinformatics databases have facilitated the discovery of new biomarkers for cancer management [7,8]. The R2TP complex is conserved from yeast to humans and appears specialized in the assembly of protein and RNP complexes. It is involved in many cellular processes like small nucleolar ribonucleoprotein biogenesis, RNA polymerase, or PIKK signaling [9]. It is composed of four different proteins: RUVBL1/Rvb1, RUVBL2/Rvb2, PIH1 domain containing 1 (PIH1D1)/Pih1, and RPAP3/Tah1 (human/yeast). RUVBL1/Rvb1 and RUVBL2/Rvb2 belong to the AAA+ family of ATPases and their relevant biological arrangement appears to be an alternating hetero-hexamer/dodecamer. PIH1D1/Pih1 and RPAP3/Tah1 heteromerize and are believed to function as an adapter to recruit clients, as well as a bridge between Heat shock protein 90 (HSP90) and the RUVBLs. Structural studies highlighted the C-terminal domain of RPAP3 as the recruiting module for RUVBLs in the human R2TP complex. In mammals, the R2TP further associates with a set of prefoldin proteins, which together form the PAQosome [10]. PIH1D1/Pih1 encompasses two domains (PIH1-N and PIH1-C). The PIH1-N region is a phospho-peptide binding domain that binds DpSDD/E consensus sites. A number of PIH1D1 phosphorylation-dependent binding partners were found using proteomic and *in silico* screening. One of them, mediated by a motif similar to the DpSDD sequence, had shown direct phosphorylation-dependent interaction with a protein human ecdysoneless (ECD), which was associated with the stabilization of the tumor suppressor p53 [11]. It has been demonstrated in the past that the ECD protein mediates the interaction of the R2TP complex with HSP90 by PIH1D1 and chooses which intracellular molecules the chaperone complex regulates [12]. Possibly this interaction may lead to an increase in the expression of PIH1D1 and ECD. Breast and pancreatic tumors exhibit considerable overexpression of ECD, and these over expressions are directly correlated with unfavorable prognostic indicators and poor patient survival [13]. PIH1-C is a CS domain, a motif found in several HSP90/Hsp82 co-chaperones, and is required for Tah1 and RPAP3 binding [14]. Despite the intensive study of PIH1D1 in many cancers, there is limited research regarding PIH1D1 in BRCA. However, it remains unclear how this molecular chaperone complex contributes to oncogenesis. Currently, research groups and pharmaceutical companies are searching for and developing inhibitors of R2TP/PAQosome as a promising chemotherapeutic target for cancers. However, the expression and prognostic values of PIH1D1 in BRCA have not been well-studied. By studying the role of PIH1D1 in BRCA, it might provide a novel biomarker for drug development and repositioning of this cancer. Herein, we have studied the expression patterns, interactive analysis, correlation expression, and survival of a biomarker PIH1D1 in BRCA patients using the OncoDB, gene expression profiling interactive analysis (GEPIA), University of ALabama at Birmingham CANcer (UALCAN), databases.

MATERIALS AND METHODS

2.1. PIH1D1 Expression Levels Across Cancers

We analyzed PIH1D1 expression levels across cancers with the help of Tumor IMmune Estimation Resource (TIMER 2) from TCGA data. TIMER is a comprehensive resource for systematic analysis of immune infiltrates across diverse cancer types. Gene DE module was used that allows us to study the differential expression between tumor and adjacent normal tissues for any gene of interest across all TCGA tumors. Distributions of gene expression levels are displayed using box plots. The statistical significance computed by the Wilcoxon test is annotated by the number of stars (*: p-value <0.05; **: p-value < 0.01; ***: p-value < 0.001).

2.2. PIH1D1 Expression Levels in Normal and BRCAs Tissues

Analysis of PIH1D1 expression in normal and BRCA tissues was done using tumor, normal, and metastasis (TNM) plots, which are used for differential gene expression analysis. The normal and tumor analysis provides detailed analysis for genes of interest in specific tissue types using gene chip data. It can be used for various kinds of analysis includes: gene versus gene correlation, gene ontology (GO) analysis, GO for differentially expressed genes, gene versus all genes correlation, compare tumor, normal and metastasis, multigene analysis, and so on.

2.3. Disease-Free and Overall Survival (OS)

Disease-free and OS were analyzed by Survival Plot, in the GEPIA web server. It is a newly developed interactive web server for analyzing the RNA sequencing expression data of 9,736 tumors and 8,587 normal samples from the TCGA and the GTEx projects, using a standard processing pipeline. GEPIA provides customizable functions such as tumor/normal differential expression analysis, profiling according to cancer types or pathological stages, patient survival analysis, similar gene detection, correlation analysis, and dimensionality reduction analysis.

2.4. TCGA Data Analysis of PIH1D1 in BRCA

The UALCAN data analysis portal is a comprehensive, user-friendly, and interactive web resource for analyzing cancer OMICS data. It is built on PERL-CGI with high-quality graphics using javascript and CSS. UALCAN is designed to provide easy access to publicly available cancer OMICS data (TCGA, MET500, CPTAC, and CBTTC), and also allow users to identify biomarkers, *in silico* validation of potential genes of interest, provide graphs and plots depicting expression profiles and patient survival, evaluate epigenetic regulation of gene expression by promoter methylation, and perform pan-cancer gene expression analysis. Here, we evaluated the expression of PIH1D1 in BRCA by TCGA analysis.

2.5. Histological Analysis of PIH1D1 Protein in BRCA

Histological analysis of PIH1D1 protein expression was done with the help of the Human Protein Atlas Database. The Human Protein Atlas Database has multiple sections, and each section focuses on a particular feature of the genome-wide analysis of human proteins. Here, we analyzed tissue-specific protein expression of PIH1D1, and protein level in Ductal carcinoma and in lobular carcinoma. We also analyzed the expressional pattern of PIH1D1 in different BRCA cell lines with different cell line categories.

2.6. Expression Clusters and Interaction Analysis

Clusters and interaction analysis were done using cell line expression cluster toll in humans. The interactive uniform manifold approximation and projection plot displays the 72 gene clusters resulting from Louvain clustering of gene expression across all cell lines. General information about the different clusters regarding annotation and a number of included genes is shown upon mouse-over, while clicking on a specific cluster in the table will highlight the selected cluster in

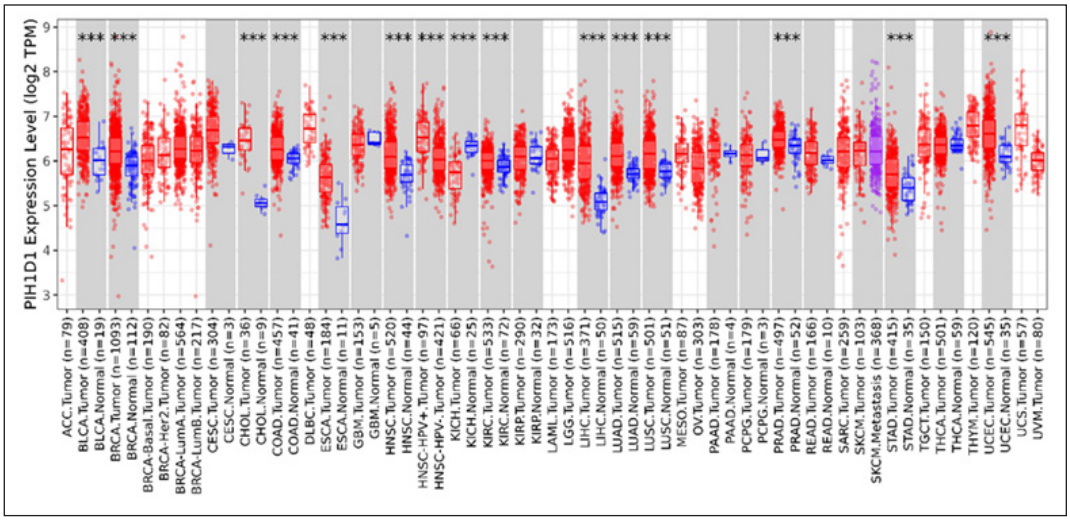


Figure 1. Expression levels of PIH1D1 mRNA across cancers from TCGA data in TIMER 2. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

the plot and display the general information together with a heat-map of the RNA expression across all cell lines for corresponding genes and a GO tree-map showing the enrichment of GO-terms in the cluster, where applicable.

The protein–protein interaction network for each gene is based on data from the IntAct database and enables exploration of the interaction partners and their features including predicted and experimental subcellular location and tissue specificity. Interaction data is available for 11,351 genes. More information about the genes in the network is displayed by mouse-over and the network can be extended by clicking on a gene of interest.

2.7. Correlation of PIH1D1 and Immune Cells

Correlation of immune cells with PIH1D1 was done using TIMER2.0, It is a webserver that provides immune infiltrates, which is used for systematical analysis across diverse cancer types. It also allows users to generate high-quality figures dynamically to explore tumor immunological, clinical, and genomic features comprehensively. Here, we tried to explore the correlation of PIH1D1 expression with immune infiltration level in BRCA cancer. This webserver was just applied a linear regression here. If the data is not linear, there might be a discrepancy between the Rho and the slope of the line.

2.8. Relations Between Lymphocytes, Immunomodulators, and Chemokines with Expression of PIH1D1

The relation between lymphocytes, immunomodulators, and chemokines with an expression of PIH1D1 was done using the tumor or immune system interaction database (TISIDB). TISIDB is a web portal for tumor and immune system interaction, which integrates multiple heterogeneous data types. TISIDB allows users to interrogate the function of a specific gene in tumor–immune interplay through literature mining and high-throughput data analysis. Moreover, TISIDB offers a friendly interface for users to browse, search, and download data. These data are integrated into 10 categories of information for each gene. We believe TISIDB would become a valuable resource for cancer immunology research and therapy.

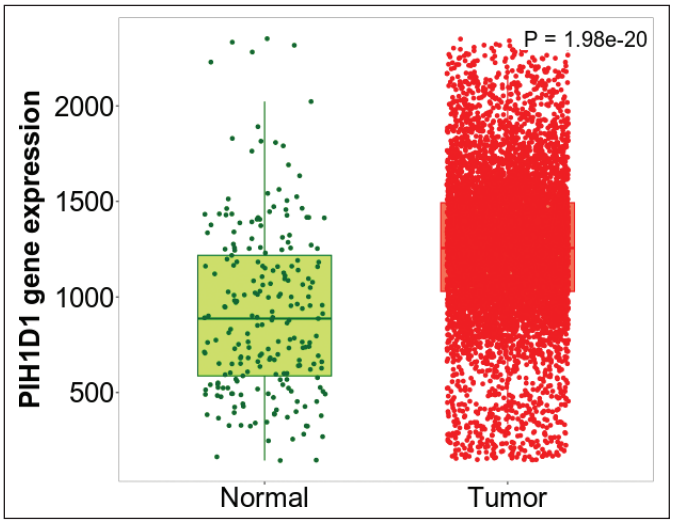


Figure 2. The expression of the PIH1D1 in BRCA compared with normal tissues.

RESULTS

3.1. PIH1D1 Expression Levels Across Cancers

We used the TIMER 2. database to compare the expression levels of PIH1D1 mRNA across cancers and its mRNA expression with that in corresponding normal tissues. Figure 1 presents the expression of the PIH1D1 across cancers. The results showed that PIH1D1 expression was higher in almost all cancer groups than in normal tissues, including the bladder, brain, breast, and head and neck. However, the mRNA expression of PIH1D1 was significantly downregulated in kidney renal clear cell carcinoma.

3.2. PIH1D1 Expression Levels in Normal and BRCAs Tissues

TNM datasets were used to compare the expression level of PIH1D1 in BRCA with those in corresponding normal tissues using Gene chip-based data. Figure 2 presents the expression of the PIH1D1

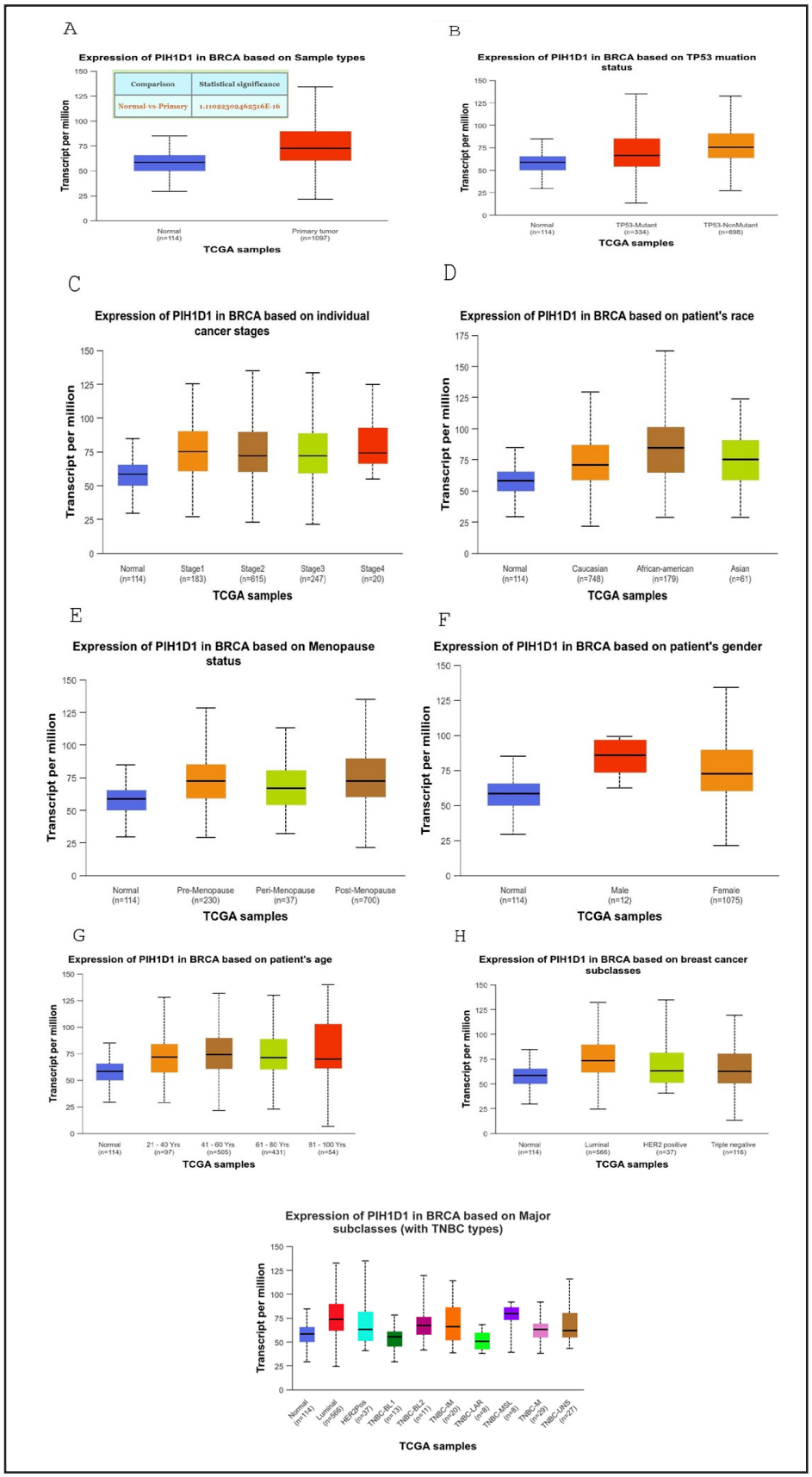


Figure 3. UALCAN cancer database analyzed clinicopathological parameters for BRCA. (A). **PIH1D1** expression in primary tumor sample types, (B). In TP53 mutation state and nonmutant state in BRCA, (C). Tumor stage $p < 0.001$, (D). In race, $p > 0.05$, (E). **PIH1D1 gene** expression in menopause state, (F). Gender wise in BRCA, (G). Age wise and (H). luminal subclass as compare to normal, and (I). PIH1D1 expression based on major subclass with TNBC.

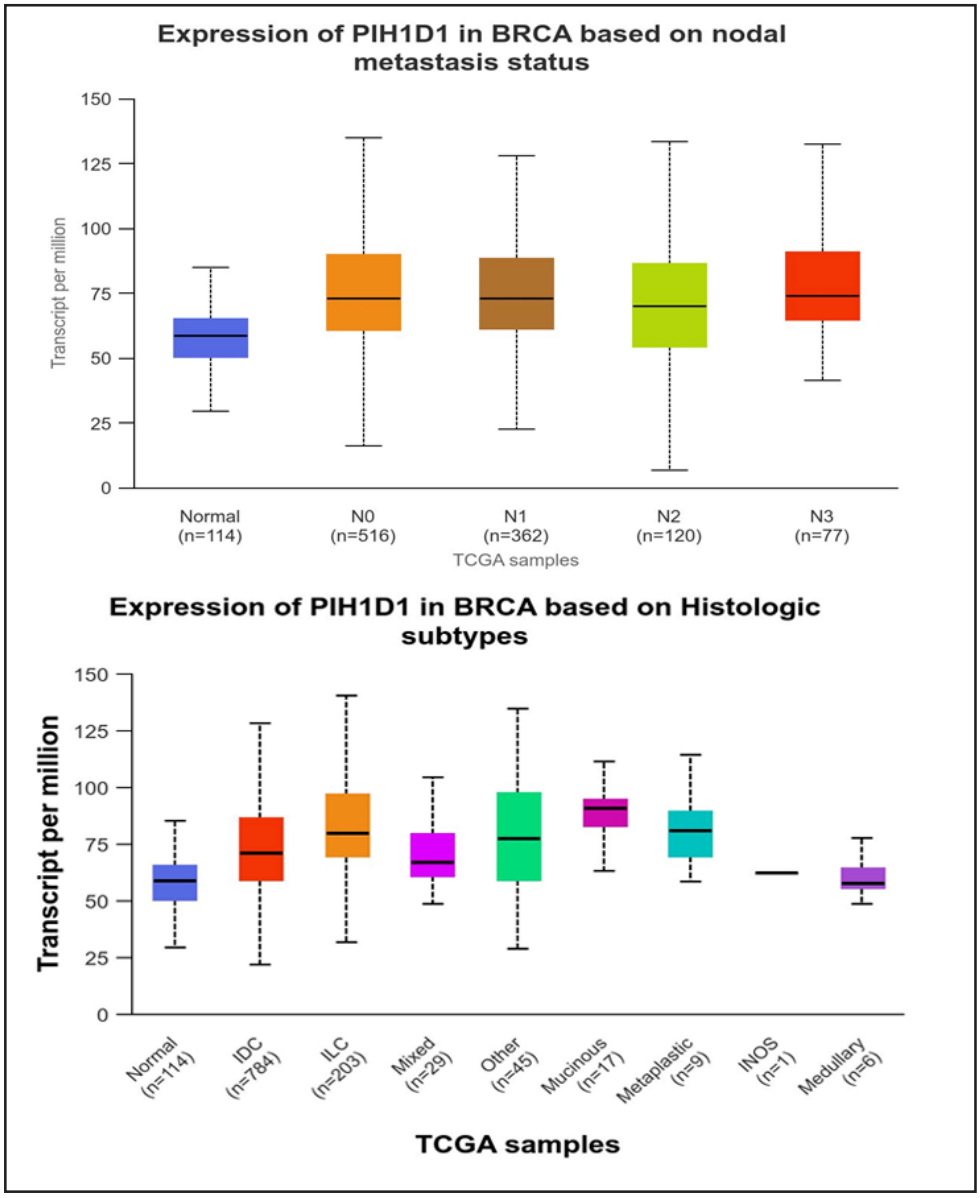


Figure 4. PIH1D1 expression in node and mucinous: (A). PIH1D1 expression in lymph node metastasis, whereas (B). PIH1D1 expression in mucinous (2% of all breast carcinomas) of histologic subtypes.

in BRCA compared with normal tissues. PIH1D1 was significantly upregulated in BRCA tissues.

3.3. Association of PIHD1 Expression with Clinicopathological Parameters with BRCA

We utilized the UALCAN cancer database to analyze clinicopathological parameters for BRCA. PIH1D1 expression was significantly increased in primary tumor sample types in comparison to normal (Fig. 3A), Similarly, PIH1D1 expression was significantly increased in the TP53 mutation state and maximum at the nonmutant state in BRCA (Fig. 3B). PIH1D1 expression was observed increase in tumor stage as compared to normal of BRCA (Fig. 3C, $p < 0.001$), whereas race (Fig. 3D, $p > 0.05$) also observed increase.

Expression of PIH1D1 was significantly increased in the menopause state (Fig. 3E), Gender wise in BRCA, males were shown to have increased PIH1D1 expression as compared to females (Fig. 3F), and also observed expression of PIH1D1 increased in 40–60 years old patients (Fig. 3G), and Luminal subclass (Fig. 3H and I) as compare to normal. Using UALCAN, we saw an increase in PIH1D1 expression in lymph node metastasis (Fig. 4A, $p < 0.001$), whereas in mucinous (2% of all breast carcinomas) of histologic subtypes (Fig. 4B).

3.4. PIH1D1 Expression and its Association with Overall and Disease-Free Survival

Survival analysis were done in BRCA based on PIH1D1 gene expression levels (Fig. 5). OS was presented (Fig. 5A) and suggested that increased time point of survival had more fluctuation in PIH1D1 gene expression. During 100 months, survival can be seen with low expression and later on high, whereas (Fig. 5B) disease-free survival has lower PIH1D1 gene expression to survive longer [8].

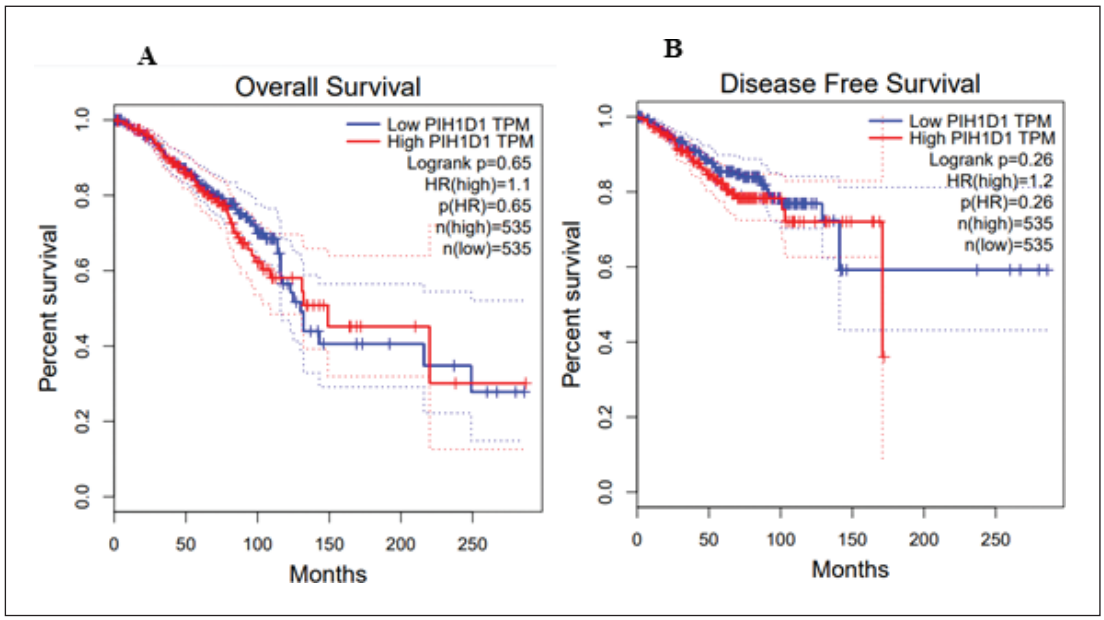


Figure 5. Survival analysis based on PIH1D1 gene expression in BRCA: (A). Expression of PIH1D1 in OS, (B). Disease-free survival analysis in BRCA.

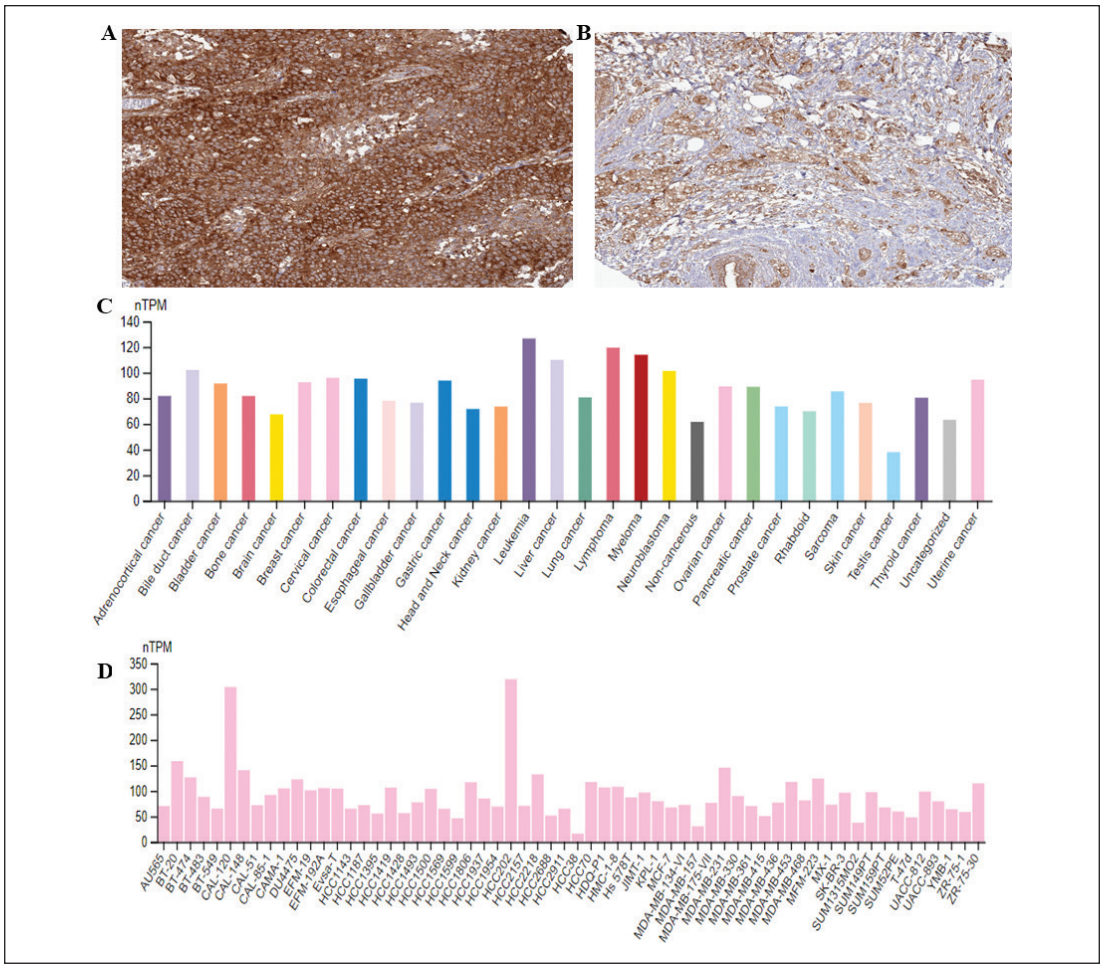
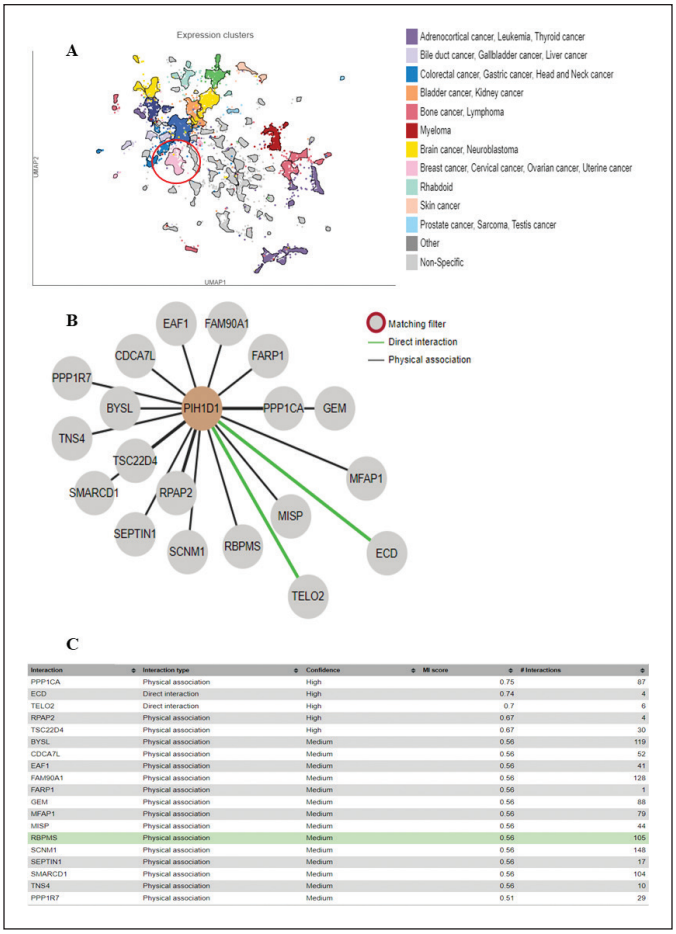


Figure 6. Histological analysis of PIH1D1 protein: (A). Showing protein level in Ductal carcinoma, (B). Showing protein level in lobular carcinoma, (C). Expressional pattern of PIH1D1 in different cell line categories, and (D). Different BRCA cell lines showing PIH1D1 protein expression.



Interaction	Interaction type	Confidence	MI score	# Interactions
PPP1CA	Physical association	High	0.75	87
ECD	Direct interaction	High	0.74	4
TELO2	Direct interaction	High	0.7	6
RPAP2	Physical association	High	0.67	4
TSC22D4	Physical association	High	0.67	30
BYSL	Physical association	Medium	0.56	119
CDCA7L	Physical association	Medium	0.56	52
EAF1	Physical association	Medium	0.56	41
FAM90A1	Physical association	Medium	0.56	128
FARP1	Physical association	Medium	0.56	1
GEM	Physical association	Medium	0.56	88
MFAP1	Physical association	Medium	0.56	79
MISP	Physical association	Medium	0.56	44
RBPMS	Physical association	Medium	0.56	105
SCNM1	Physical association	Medium	0.56	148
SEPTIN1	Physical association	Medium	0.56	17
SMARCD1	Physical association	Medium	0.56	104
TNS4	Physical association	Medium	0.56	10
PPP1R7	Physical association	Medium	0.51	29

Figure 7. Expression clusters and interaction analysis: (A). Picture depicted clusters expression of PIH1D1 protein, (B and C). Associating partner of PIH1D1 either physical or direct.

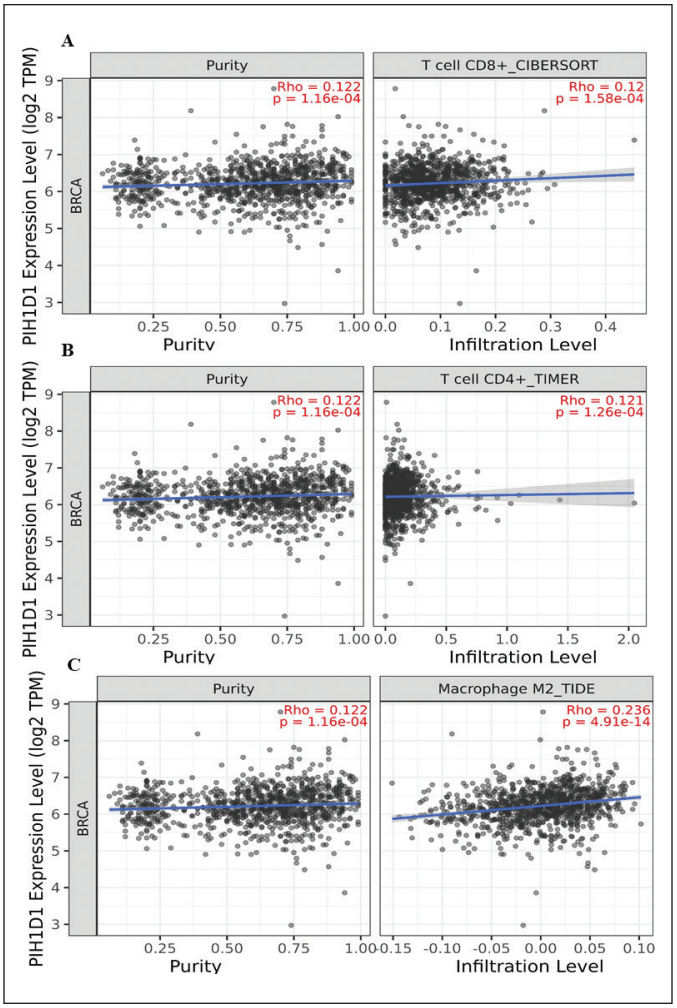


Figure 8. Correlation between immune cells and PIH1D1. (A–C). The graph represents the positive correlation between T Cell CD8+, T Cell CD4+, and macrophage M2 cells and PIH1D1 (Spearman's correlation performed for the analysis).

3.5. Histological Analysis of PIH1D1 Protein in BRCA

Histological analysis of PIH1D1 protein expression was done using the Human Protein Atlas Database. In this analysis, we found a strong level of intensity of PIH1D1 protein in both Ductal carcinoma, as well as in lobular carcinoma (Fig. 6A and B). In addition to this, we observed the expression of PIH1D1 above the detectable and marginal value in different cell line categories which cannot be ignored (Fig. 6C). Among different BRCA cell lines, PIH1D1 protein expression shown maximum in CAL-120 and HCC202 (Fig. 6D).

3.6. Expression Clusters and Interaction Analysis of PIH1D1 in Pan-Cancer

In this study, we analyzed clusters and interaction patterns of PIH1D1 in pan-cancer and observed that PIH1D1 protein expresses in a cluster in many different cancers such as adrenocortical cancer, bile duct cancer, gallbladder cancer, bladder cancer, and kidney cancer but highly observed in colorectal cancer, brain cancer and BRCA (Fig. 7A). We also observed PIH1D1 directly interact with ECD and Telo2 and physically associate with ELL associated factor 1 (EAF1), RNA polymerase II-associated protein 2 (RPAP2), cell division cycle-associated 7-like protein (CDCA7L), Tensin-4 (TNS4), and FARP1 (FERM, RhoGEF, and pleckstrin domain-containing protein 1) (Fig. 7B and C).

3.7. Correlation of PIH1D1 Protein with Purity and Infiltration Level of Immune Cells in Breast Cancer

We observed a statistically significant positive association between PIH1D1 and infiltration of $CD4^+$ and $CD8^+$ T cells. Moreover, significant positive correlations also were found with Macrophage M2 based on the XCELL algorithm (Fig. 8A–C).

3.8. Correlation Analysis of Lymphocytes, Immunomodulators, and Chemokines with Expression of PIH1D1

3.8.1. Correlation analysis of lymphocyte markers with expression of PIH1D1

We observed a statistically significant positive association between PIH1D1 and infiltration of CD4+ and CD8+ T cells. These findings suggest PIH1D1 has potential prognosticator value and therapeutic role in BRCA. In addition, we revealed the association between PIH1D1 and immune cell infiltrates (Fig. 9A). Here, we observed a significant positive correlation between PIH1D1 and monocytes (Fig. 9B) whereas (Fig. 9C) negative with T-regulatory cells in tumor-infiltrating lymphocytes (TILs).

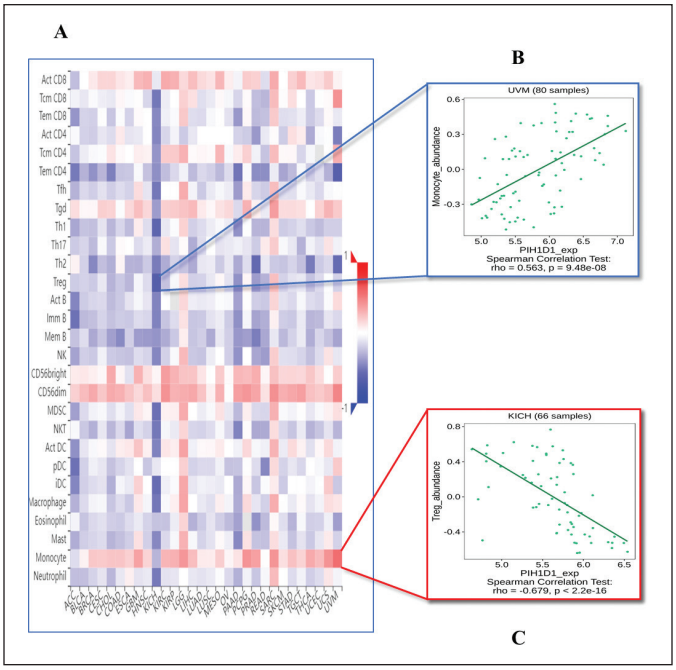


Figure 9. Expression of PIH1D1 and TILs: (A). Association of TILs abundances with PIH1D1 level; (B and C) most significant TILs with the negative and positive Spearman's correlation with PIH1D1 levels, respectively.

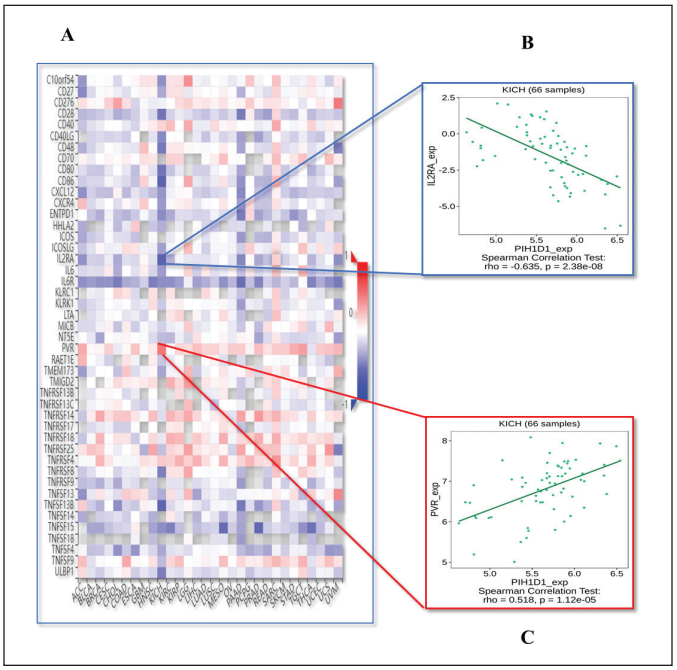


Figure 11. Expression of PIH1D1 and immunostimulator: (A). Association of immunostimulator abundances with PIH1D1 level; (B and C). The most significant immunostimulator with the negative and positive Spearman's correlation with PIH1D1 levels, respectively.

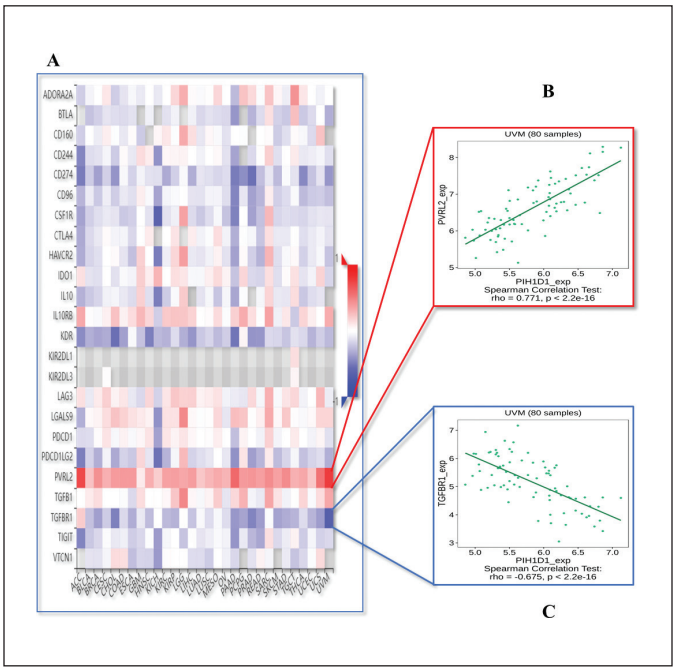


Figure 10. Expression of PIH1D1 and immunoinhibitor: (A). Association of immunoinhibitor abundances with PIH1D1 level; (B and C). Most significant immunoinhibitor with the positive and negative Spearman's correlation with PIH1D1 levels, respectively.

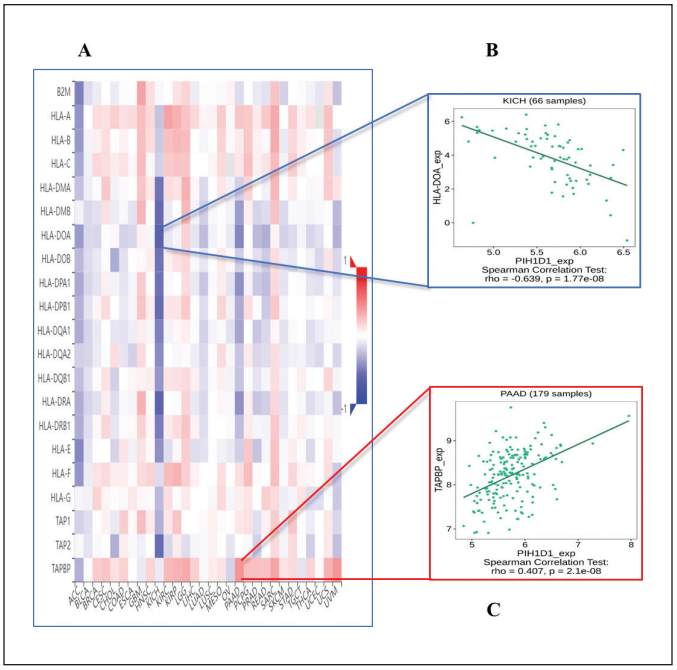


Figure 12. Expression of PIH1D1 and MHC molecule: (A). Association of MHC molecule abundances with PIH1D1 level; (B and C). Most significant MHC molecule with the negative and positive Spearman's correlation with PIH1D1 levels, respectively.

3.8.2. Correlation analysis of immunomodulators markers with expression of PIH1D1

Furthermore, we analyzed the association of immune modulators with the expression of PIH1D1 protein and found a strong correlation with Poliovirus receptor-related 2 (PVRL2) but a negative correlation between PIH1D1 and transforming growth factor beta receptor 1 (TGFBR1) in immunoinhibitor (Fig. 10A–C).

There was also found significant positive correlation of PIH1D1 with PVR but negative correlations with IL2RA in immunostimulators (Fig. 11A–C). We also found a positive association between PIH1D1

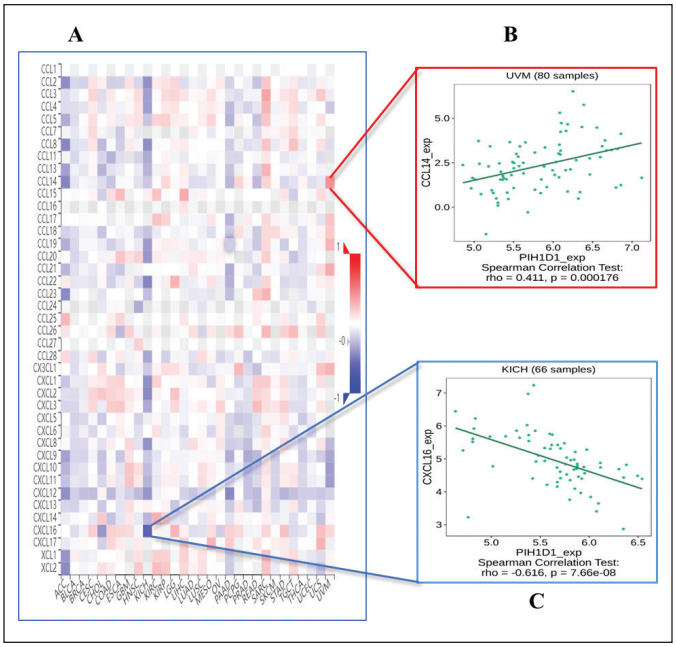


Figure 13. Expression of PIH1D1 and chemokines: (A). Association of chemokines abundances with PIH1D1 level; (B and C). Most significant chemokines with a positive and negative Spearman's correlation with PIH1D1 levels, respectively.

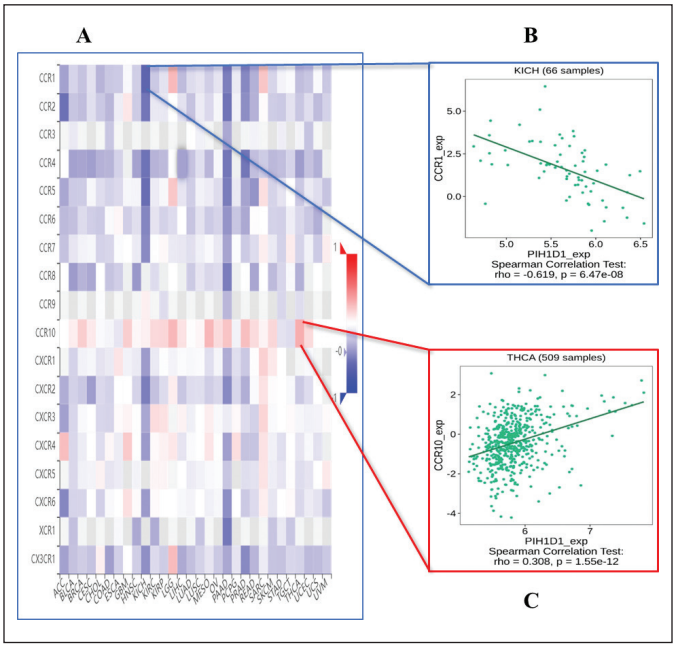


Figure 14. Expression of PIH1D1 and receptors: (A). Association of receptors abundances with PIH1D1 level; (B and C). Most significant receptors with a negative and positive Spearman's correlation with PIH1D1 levels, respectively.

and TAP binding protein (TAPBP) and a negative with HLA-DOA in MHC molecules (Fig. 12A–C).

3.8.3. Correlation analysis of chemokines and receptors with under the expression of PIH1D1

We analyzed chemokines and receptors expression under the influences of PIH1D1 and we observed association of Chemokine (C-C motif) ligand 14 (CCL14) and CXCL16 (CXC chemokine ligand 16) chemokines were significantly positive and negative correlated with PIH1D1 protein (Fig. 13A–C), whereas receptors CCR10 and CCR1 were shown as positive and negative correlated with PIH1D1 protein (Fig. 14A–C).

DISCUSSION

PIH1D1 is a component of R2TP complex relatively unstable protein which is stabilized by Tah1 (RPAP3). R2TP significantly expresses higher in cancer cells and also stabilizes the expression of mTOR [14]. Although the role of PIH1D1 in the tumorigenesis and prognosis of several cancers has been partially confirmed, further bioinformatics analysis of BRCA has yet to be performed. Importantly, the current study focuses on the analysis of exploring the expression and prognostic values of PIH1D1 in BRCA. We hope that the current findings will contribute to providing new ideas for the clinical diagnosis, prognosis assessment, and targeted therapy of BRCA. In our study, UALCAN datasets and the GEPIA database revealed that the expression of PIH1D1 was significantly higher in BRCA than in normal tissues. PIH1D1 expression was significantly related to tumor stage ($p < 0.001$) and lymph node metastasis. A high PIH1D1 expression was significantly associated with better OS in patients with BRCA.

In this study, we compared the expression levels of PIH1D1 mRNA across different cancers with respect to the corresponding normal tissues. The results showed PIH1D1 mRNA expression was higher in almost all cancer groups than normal tissues, including the bladder, brain, head and neck, and BRCA. Similar evidence was observed by Kamano *et al.* [15] in cancer. The exact cause regarding the upregulation of PIH1D1 in different cancers with respect to normal tissue is unknown; however, it may be assumed that PIH1D1 might play an oncogenic role in BRCA. Furthermore, our evidence indicates the association of PIH1D1 with different clinical parameters such as patient age, race, menopause status, BRCA subclasses, Individual cancer stages, and gender. We also observed significant expression on major subclasses with TN (TRIPLE NEGATIVE) BRCA. Similarly, the expression pattern was observed with Mutant and nonmutant TP53 BRCA samples.

Our study revealed after clusters and interaction analysis that PIH1D1 directly interacts with ECD and Telo2 and physically associates with EAF1, RPAP2, CDCA7L, TNS4, FARP1, and many more. A similar study was observed in Mir *et al.* [13]. On the basis of this evidence, we can suggest PIH1D1 is also involved in DNA replication and cell cycle regulation [13]. We observed a statistically significant positive association between PIH1D1 and infiltration of CD4+ and CD8+ T cells. These findings suggest PIH1D1 has potential prognosticator value and therapeutic role in BRCA. Tumor immunogenicity is not a universal feature of tumor formation; however, the presence of tumor-specific CD4 and CD8 T cells in tumor tissue is viewed as a positive prognostic indicator when considered combined. One important goal of immune checkpoint blocking, tumor immunogenicity induction, is yet only sometimes accomplished [16]. In addition, we revealed the association between PIH1D1 and immune cell infiltrates. Under normal conditions, the immune system can identify and eliminate tumor cells, but tumor cells can manipulate immune cells to elude the immune system's monitoring [17]. Here, we observed a significant positive correlation between PIH1D1 and monocytes whereas negative with T-regulatory cells in TILs. TILs have been identified as an independent predictor of survival and cancer sentinel node status [18].

We found a negative correlation between PIH1D1 and TGFBR1 but a strong correlation with PVRL2 in immunoinhibitors. Similar

recent research indicates PVRL2 expression is higher in cancerous tissues compared to normal tissues, with breast, endometrial, lung, and ovarian malignancies showing the greatest expression [19]. Indoleamine 2,3-dioxygenase (IDO1), colony-stimulating factor 1 receptor (CSF1R), V-set domain-containing T-cell activation inhibitor 1 (VTCN1), kinase insert domain receptor (KDR), LGALS9 (Galectin-9), TGFBR1, TGFB1, IL10RB, and PVRL2 were shown to be substantially expressed in pancreatic cancer (PC) tissues in recent *in silico* research utilizing the TCGA database. Additionally, the research showed that patients with PC had considerably higher levels of IDO1, CSF1R, VTCN1, KDR, LGALS9, TGFBR1, TGFB1, IL10RB, and PVRL2 mRNA than in normal tissues [20].

There was also found significant positive correlation of PIH1D1 with PVR but negative correlations with IL2RA in immunostimulators. We also found a positive association between PIH1D1 and TAPBP and a negative with HLA-DOA in MHC molecules. Previous studies have revealed that PVR is associated with the triple-negative BRCA molecular subtype and poor survival in BRCA patients, and shown PVR mRNA expression levels significantly associated with shorter OS [21].

However, we analyzed chemokines and receptors expression under the influences of PIH1D1 and found an association of CCL14 and CXCL16 chemokines were significantly positive and negatively correlated. Recently, it was observed that the tumor-infiltrating B cells, CD4+ and CD8+ T cells, macrophages, neutrophils, and dendritic cells all had a significant connection with CCL14 [22]. Recent research showed that CXCL16 played a direct role in the migration and invasiveness of BRCA cells and that its low expression or downregulation facilitated their invasion and migration [23]. We also observed that receptors CCR10 and CCR1 were shown as positively and negatively correlated with PIH1D1 protein. One chemokine receptor, CCR10, can be activated by CCL27 or CCL28 and is typically expressed by melanocytes, plasma cells, and skin-homing T lymphocytes. Numerous tumor tissues have shown CCR10 expression, and CCR10's roles in cancer have been investigated. Malignant melanoma cells overexpress CCR10, and this interaction between CCL27 and CCR10 can promote the development and migration of melanoma cancer cells [24]. Uncertainty surrounds the function of CCR10 activation in BRCA cells.

CONCLUSION

In summary, According to this data, BRCA has higher levels of PIH1D1 expression than normal tissues. The results indicate that PIH1D1 could be used for early detection of BRCA and may be used as an indicators of prognosis. Moreover, PIH1D1 may become the target of immunotherapy for BRCA in the future. However, our current research has some limitations. All the data analyzed in this study are derived from bioinformatics databases. Further *in vivo* and *in vitro* studies need to explore the biomarker potential of PIH1D1 in BRCA in patient tissues.

ACKNOWLEDGMENTS

DK is thankful to AIIMS New Delhi-110029 and Jamia Millia Islamia, New Delhi 110025 for providing the necessary facilities to carry out this piece of work.

CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

FUNDING

There is no funding to report.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to s ubmit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declares that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

REFERENCE

1. Breast Cancer. Breast cancer information & overview. Available via https://www.cancer.org/cancer/types/breast-cancer.html (Accessed 24 February 2024)
2. DeVita VT. Breast cancer therapy: exercising all our options. N Engl J Med 1989;320:527–9; http://doi.org/10.1056/NEJM198902233200812
3. Kulkarni A, Stroup AM, Paddock LE, Hill SM, Plascak JJ, Llanos AAM. Breast cancer incidence and mortality by molecular subtype: statewide age and racial/ethnic disparities in New Jersey. Cancer Health Disparities 2019;3:e1–17; http://doi.org/10.9777/chd.2019.1012
4. Youlden DR, Cramb SM, Yip CH, Baade PD. Incidence and mortality of female breast cancer in the Asia-Pacific Region. Cancer Biol Med 2014;11:101–15; http://doi.org/10.7497/j.issn.2095-3941.2014.02.005
5. Ahmad A, Imran M, Ahsan H. Biomarkers as biomedical bioindicators: approaches and techniques for the detection, analysis, and validation of novel biomarkers of diseases. Pharmaceutics 2023;15(6):1630. http://doi.org/10.3390/pharmaceutics15061630.
6. Howlader N. Noone cancer of the breast (Invasive). Age-adjusted SEER incidence rates by year, race and sex. National Cancer Institute, Bethesda, MD, pp 1975–2017, 2020.
7. Kim J. *In silico* analysis of differentially expressed genesets in metastatic breast cancer identifies potential prognostic biomarkers. World J Surg Oncol 2021;19:188; http://doi.org/10.1186/s12957-021-02301-7
8. Ma H, Liu Z, Li H, Guo X, Guo S, Qu P, *et al.* Bioinformatics analysis reveals MCM3 as an important prognostic marker in cervical cancer. Comput Math Methods Med 2021;2021:8494260; http://doi.org/10.1155/2021/8494260
9. Rivera-Calzada A, Pal M, Muñoz-Hernández H, Luque-Ortega JR, Gil-Carton D, Degliesposti G, *et al.* The structure of the R2TP complex defines a platform for recruiting diverse client proteins

to the HSP90 molecular chaperone system. Struct Lond Engl 1993;2017:(25):1145–52.e4; http://doi.org/10.1016/j.str.2017.05.016
10. Houry WA, Bertrand E, Coulombe B. The PAQosome, an R2TP-based chaperone for quaternary structure formation. Trends Biochem Sci 2018;43:4–9; http://doi.org/10.1016/j.tibs.2017.11.001
11. Hořejší Z, Stach L, Flower TG, Joshi D, Flynn H, Skehel JM, *et al.* Phosphorylation-dependent PIH1D1 interactions define substrate specificity of the R2TP cochaperone complex. Cell Rep 2014;7:19–26; http://doi.org/10.1016/j.celrep.2014.03.013
12. Ammons S. Role of ecdysoneless in ERBB2/HER2 mediated breast oncogenesis. Theses and Dissertations, University of Nebraska Medical Center, Omaha, NE, 2016.
13. Mir RA, Bele A, Mirza S, Srivastava S, Olou AA, Ammons SA, *et al.* A novel interaction of ecdysoneless (ECD) protein with R2TP complex component RUVBL1 is required for the functional role of ECD in cell cycle progression. Mol Cell Biol 2016;36:886–99; http://doi.org/10.1128/MCB.00594-15
14. Kakihara Y, Kiguchi T, Ohazama A, Saeki M. R2TP/PAQosome as a promising chemotherapeutic target in cancer. Jpn Dent Sci Rev 2020;56:38–42; http://doi.org/10.1016/j.jdsr.2019.08.001
15. Kamano Y, Saeki M, Egusa H, Kakihara Y, Houry WA, Yatani H, *et al.* PIH1D1 interacts with mTOR complex 1 and enhances ribosome RNA transcription. FEBS Lett 2013;587:3303–8; http://doi.org/10.1016/j.febslet.2013.09.001
16. Ostroumov D, Fekete-Drimusz N, Saborowski M, Kühnel F, Woller N. CD4 and CD8 T lymphocyte interplay in controlling tumor growth. Cell Mol Life Sci 2018;75:689–713; http://doi.org/10.1007/s00018-017-2686-7
17. Muenst S, Läubli H, Soysal SD, Zippelius A, Tzankov A, Hoeller S. The immune system and cancer evasion strategies: therapeutic concepts. J Intern Med 2016;279:541–62; http://doi.org/10.1111/joim.12470
18. dos Santos FDM, da Silva FC, Pedron J, Furian RD, Fortes C, Bonamigo RR. Association between tumor-infiltrating lymphocytes and sentinel lymph node positivity in thin melanoma. An Bras Dermatol 2019;94:47–51; http://doi.org/10.1590/abd1806-4841.20197414
19. Oshima T, Sato S, Kato J, Ito Y, Watanabe T, Tsuji I, *et al.* Nectin-2 is a potential target for antibody therapy of breast and ovarian cancers. Mol Cancer 2013;12:60; http://doi.org/10.1186/1476-4598-12-60
20. Fan Y, Li T, Xu L, Kuang T. Comprehensive analysis of immunoinhibitors identifies LGALS9 and TGFBR1 as potential prognostic biomarkers for pancreatic cancer. Comput Math Methods Med 2020;2020:6138039; http://doi.org/10.1155/2020/6138039
21. He J, Navarrete S, Jasinski M, Vulliamy T, Dokal I, Bessler M, *et al.* Targeted disruption of Dkc1, the gene mutated in X-linked dyskeratosis congenita, causes embryonic lethality in mice. Oncogene 2002;21:7740–4; http://doi.org/10.1038/sj.onc.1205969
22. Gu Y, Li X, Bi Y, Zheng Y, Wang J, Li X, *et al.* CCL14 is a prognostic biomarker and correlates with immune infiltrates in hepatocellular carcinoma. Aging 2020;12:784–807; http://doi.org/10.18632/aging.102656
23. Fang Y, Henderson FC, Yi Q, Lei Q, Li Y, Chen N. Chemokine CXCL16 expression suppresses migration and invasiveness and induces apoptosis in breast cancer cells. Mediators Inflamm 2014;2014:e478641 http://doi.org/10.1155/2014/478641
24. Lin H, Sun S, Lu X, Chen P, Chen C, Liang W, *et al.* CCR10 activation stimulates the invasion and migration of breast cancer cells through the ERK1/2/MMP-7 signaling pathway. Int Immunopharmacol 2017;51:124–30; http://doi.org/10.1016/j.intimp.2017.07.018

How to cite this article:
Singh DK, Jit B, Gupta R, Verma AK, Mir RA. Bioinformatics analysis reveals PIH1D1 as an important prognostic marker in breast cancer. J Appl Biol Biotech 2025; 13(1):202–212. DOI: 10.7324/JABB.2024.189230