Identification of Prognostic Biomarkers and Independent Indicators Among PFDN1/2/3/4/5/6 in Liver Hepatocellular Carcinoma

Yin-Hai Dai

Shaanxi University of Chinese Medicine

Fuping Li (✉ 1627442702@qq.com)

Shaanxi University of Chinese Medicine <https://orcid.org/0000-0003-2138-3330>

Wei-Jie Kong

Shaanxi University of Chinese Medicine

Xue-Qin Zhang

Sichuan University

Mao Wang

Shaanxi University of Chinese Medicine

Hai-Long Ma

Shaanxi University of Chinese Medicine

Qi Wang

Shaanxi University of Chinese Medicine

Primary research

Keywords: hepatocellular carcinoma, prefoldin protein, biomarker, prognosis, Oncomine, Kaplan-Meier plotter

Posted Date: August 24th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-725619/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Previous studies have proved that the aberrant expressions of PFDNs (Prefoldin) family proteins were correlated with several human cancer. However, the specific functions of PFDNs in liver hepatocellular carcinoma(LIHC) remain unknown. The study aimed to identify the prognostic biomarkers and independent indicators among PFDN1/2/3/4/5/6 in liver hepatocellular carcinoma.

Methods: We used these databases including Oncomine, Ualcan, GEPIA2, Human Protein Atlas, The Cancer Genome Atlas, Kaplan-Meier plotter, cBioPortal, STRI-

NG and TIMER and the software of Cytoscape in our study.

Results: PFDN1/2/3/4/5/6 were highly expressed in LIHC tissues. The mRNA expression levels of PFDN1/2/3/4/5/6 were relevant to tumor grades.PFDN1/3/4/5 expressions significantly changed in different cancer stages. The protein expression levels of PFDNs were higher in LIHC tissue than normal liver tissue. Moreover, High mRNA expressions of PFDN1/2/3/4 were associated with shorter OS of LIHC patients. In multivariate analysis,high expressions of PFDN1/2/4 were independently correlated with poorer OS of LIHC patients. In our findings,55% of patients with LIHC had genetic mutations on PFDNs. Besides, there were significant associations between the expressions of PFDN1/2/3/4/5 and six types of infiltrated immune cells(B cells, CD4+T cells, CD8+T cells, neutrophil, macrophage, and dendritic cells).

Conclusions:PFDN1/2/3/4 were potential prognostic markers to suggest poor OS of LIHC patients. In addition, high PFDN1/2/4 expressions were independent prognostic factors in OS for LIHC patients.

Background

Liver Hepatocellular Carcinoma (LIHC) is the most common type of primary liver cancer, which seriously threatens human health. According to the global cancer data survey in 2020, liver cancer has been the fourth common cause of all cancer-related death globally[1]. Even though the therapies of LIHC are developing and improving,including earlier diagnosis and more useful therapeutic methods(especially the advent of checkpoint inhibitors,multikinase inhibitors,and antiangiogenics) in the previous decades, it appears to very poor prognosis and survival in LIHC patients who were detected advanced stages[2]. All in all, there are still some inadequacies in present biomarkers that predict prognosis because of melanoma heterogeneity, Therefore, further knowledge is needed to discover new diagnostic and prognostic biomarkers, and LIHC patients can benefit from those.

Prefoldin (PFDN) is a hexameric chaperone complex, which is composed of two groups:α subunit group(PFDN3(also named VBP1),PFDN5),and β subunit group (PFDN1,PFDN2,PFDN4,PFDN6). It plays an important role in transferring proteins to eukaryotic cytoplasmic chaperone proteins(c-CPN) and preventing protein misfolding[3]. Previous studies have proved that the aberrant expressions of PFDNs family proteins were correlated with several human cancers, including gastric cancer[4], lung cancer[5,6], colorectal cancer[7], breast cancer[8], and liver cancer [9].

To date, only a limited number of studies have proved that the abnormal expressions of some PFDNs family proteins have some clinical values in LIHC. For instance, Al-Yhya N et al.[10] found that the downregulation of histone deacetylase 1 and 3 (HDAC1/3) inhibited the proliferation of hepatocellular carcinoma cells by reducing the expressions of PFDN2/6. By analyzing the copy number alterations of chromosome 20q, Wang D et al.[11] found that PFDN4 was a potential prognostic indicator that was associated with poor survival in LIHC patients. However, the specific functions of PFDNs in LIHC remain unknown. In this study, we aimed to show that the expressions and mutations of PFDNs in hepatocellular carcinoma by comprehensive bioinformatical analysis and found potential biomarkers which may have profound significance in the therapy of advanced LIHC patients.

Materials And Methods

ONCOMINE database

ONCOMINE(<http://www.oncomine.org>), a robust database, an integrated data-mining platform, which provides a powerful set of analysis functions that compute gene expression features, cluster and gene-set modules[12]. In our study, data were obtained to assess the expressions of PFDN1/2/3/4/5/6 in liver cancer. Different mRNA expression levels were compared using Student's t-test, and the parameters were set as: P value = 0.01, fold change = 1.5, gene rank = 10%, and data type = mRNA.

The Cancer Genome Atlas(TGCA)

The Cancer Genome Atlas(<https://portal.gdc.cancer.gov/>) is a landmark cancer genomics program that provides comprehensive data about 36 types of human cancers, including genome variation, mRNA expression, and methylation[13]. In our study, The clinical data of 374 LIHC patients were acquired from TGCA, and their basic clinical characteristics were presented in supplementary Table 1.

GEPIA 2

GEPIA 2 (<http://gepia2.cancer-pku.cn/#index>) is a web-based tool, which provides different modules to analyze normal and cancer gene expression profiling[14]. In this study, we explored the mRNA expression levels of PFDNs in LIHC tissues and normal liver tissue and the association and the association between PFDNs expression and cancer staging by "Expression DIY" module of GEPIA 2. Moreover, we selected 50 most frequently altered neighboring genes of the PFDNs family proteins via the "Similar Genes Detection" module.

UALCAN

UALCAN(<http://ualcan.path.uab.edu>) is a comprehensive online tool, which provides different modules to analyze TGCA cancer transcriptome data[15]. In this study, the data about mRNA expressions of PFDNs in different grades were extracted by using the "TGCA Analysis" module and the "Liver Hepatocellular Carcinoma" dataset. Different mRNA expression levels of PFDNs were compared using Student's t-test, P-value ≤ 0.05 was deemed significant.

Human Protein Atlas(HPA)

HPA (<https://www.proteinatlas.org/>) is a freely available database, which contains RNA-sequencing data of 32 different tissues and provides millions of immunohistochemistry images about all major tissues in the human body[16].In our study, the immunohistochemistry images were performed for comparison for the protein expression levels of PFDNs between LIHC tissues and normal liver tissues.

Kaplan-Meier plotter

Kaplan-Meier plotter (<http://kmplot.com/>) is a web-based meta-analysis tool, which is used to evaluate the impact of thousands of genes(mRNA,miRNA, protein) on survival in 21 cancer types [17–18].In our study, to evaluate the effect of mRNA expressions of PFDNs on survival in LIHC, we selected OS, RFS, PFS, and DSS as evaluation indicators and performed their survival curves by using Kaplan-Meier plotter, some statistical parameters including hazard ratio(HR), 95% confidence intervals(CIs), and P values were showed in these survival curves. P-value $\leq$ 0.05 was deemed significant.

cBioPortal

CBioPortal (<http://www.cbioportal.org/>) is a comprehensive online tool, which based on the TCGA database. Users are able to explore, visualize, and analyze cancer transcriptome data by using it[19–20].In this study, we selected the dataset of Liver Hepatocellular Carcinoma (TCGA, Firehose Legacy) which contained 360 complete samples of 442 total, and analyzed genetic alterations, expression heatmap, and co-expression of the PFDNs proteins by this dataset.mRNA expression z-Scores (microarray) with a z-Score threshold ± 1.8

STRING

STRING (<https://string-db.org/>) is an efficient web-based tool, which can predict proteins functional interactions in more than 5,000 organisms [21]. In our study, we established the PPI network among the 6 PFDNs family proteins and another PPI network for PFDNs and their 50 frequently neighboring genes by using the tool of STRING.

DAVID 6.8

DAVID6.8 (<https://david.ncifcrf.gov/>) is an efficient web-based tool, which provides a comprehensive gene list annotation and analysis function[22]. In our study, the functions of PFDNs and their 50 frequently altered neighboring genes were analyzed by analysis of GO and KEGG via the “Start Analysis” module.GO enrichment analysis was composed of three parts: biological processes(BP), cellular components (CC), and molecular functions(MF).

Cytoscape

Cytoscape (<https://cytoscape.org/>) is a friendly and convenient software for visualizing molecular interaction networks and biological pathways and integrating those networks with annotations[23].In this

study, we established a PPI network for the PFDNs proteins and their 50 neighboring genes using it and screened nine hub genes via the “CytoHubba” plugin. Lastly, the picture of PPI was presented in a style of grid layout.

TIMER

TIMER (<https://cistrome.shinyapps.io/timer/>) is an intuitive tool, which contains a comprehensive source for systematical analysis of immune infiltrates across various tumor types[24–25]. In this study, we analyzed the correlation between the expressions of PFDNs and six types of infiltrated immune cells by using “Gene” module.

Statistical methods

All statistical analysis was performed via the XIAN TAO platform(www.xiantao.love). Before the statistical analysis, we chose RNA seq data from the format of level 3 HTSeq-FPKM(Fragments per Kilobase per Million), and then, we converted the RNAseq data to the format of TPM(transcripts per million reads) and finally took the log2 transformation. In this study, the statistical method of Cox regression analysis including univariate analysis and multivariate analysis was used to assess the impact of clinical characteristics and PFDNs expressions on the survival of LIHC patients. P-value $\leq$ 0.05 was deemed significant.

Results

Study Characteristics

Our study explored the associations between PFDN1/2/3/4/5/6 and liver hepatocellular carcinoma in many aspects including transcriptional levels, protein levels, genes mutation, clinical significance,protein-protein interactions, GO/ KEGG enrichment analysis, and immune cells infiltration. The flow chart of the entire study design was presented in Fig. 1.

mRNA levels of PFDNs in LIHC patients

We first analyzed the transcriptional levels of PFDNs in LIHC and normal liver tissues by using the Oncomine database (<http://www.oncomine.org>). The results were shown in Fig. 2 and Table 1.the transcriptional levels of PFDN2/3/4/5/6 were significantly increased in LIHC.In Roessler Liver 2 dataset[26], PFDN2 was increased in hepatocellular carcinoma (fold change = 1.793, p = 1.29E-51) versus normal liver tissues.Roessler Liver statistics showed that PFDN2 was up-regulated in hepatocellular carcinoma (fold change = 1.584, p = 5.65E-6) compared to normal liver tissues[26]. Likewise, Wurmbach Liver statistics showed that the expression of PFDN2 was higher in hepatocellular carcinoma than normal liver tissues (fold change = 1.613,p = 6.64E-5)[27]. Roessler Liver 2 statistics showed that PFDN3 was increased in hepatocellular carcinoma (fold change = 2.364,p = 4.76E-66) versus normal liver tissues[26]. Similarly, Chen et al.[28]reported that PFDN3 was up-regulated in hepatocellular carcinoma (fold change = 1.635, p = 3.54E-15) compared to normal liver tissues.

In Roessler Liver dataset[26], PFDN4 was increased in hepatocellular carcinoma (fold change = 3.253, $p = 6.11E-11$) versus normal liver tissues. Likewise, Roessler Liver 2 statistics showed that the expression of PFDN4 was higher in hepatocellular carcinoma than normal liver tissues (fold change = 2.758, $p = 6.50E-7$ [26]. Mas et al.[29] showed that PFDN5 was up-regulated in cirrhosis (fold change = 1.696, $p = 8.38E-12$) compared to normal liver tissues. Additionally, in Chen Liver dataset[28], PFDN6 was increased in hepatocellular carcinoma (fold change = 1.651, $p = 4.82E-14$) versus normal liver tissues. Similarly, Roessler Liver statistics showed that PFDN6 was higher in hepatocellular carcinoma than normal liver tissues (fold change = 1.540, $p = 1.14E-5$)[26].

Table 1
Significant changes of the expression of PFDNs in mRNA level between LIHC and normal liver tissues(ONCOMINE)

	Types of LIHC VS normal liver tissue	Fold Change	P value	T-test	Ref
PFDN1	NA	NA	NA	NA	NA
PFDN2	Hepatocellular Carcinoma	1.793	1.29E-51	17.327	Roessler Liver[26]
	Hepatocellular Carcinoma	1.584	5.65E-6	5.015	Roessler Liver 2[26]
	Hepatocellular Carcinoma	1.613	6.64E-5	4.569	Wurmbach Liver [27]
PFDN3	Hepatocellular Carcinoma	2.364	4.76E-66	20.410	Roessler Liver 2[26]
	Hepatocellular Carcinoma	1.635	3.54E-4	8.514	Chen Liver[28]
PFDN4	Hepatocellular Carcinoma	3.253	6.11E-11	8.970	Roessler Liver[26]
	Hepatocellular Carcinoma	2.758	6.50E-71	21.896	Roessler Liver 2[26]
PFDN5	Cirrhosis	1.696	8.38E-12	9.762	Mas Liver [29]
PFDN6	Hepatocellular Carcinoma	1.651	4.82E-14	8.088	Chen Liver[28]
	Hepatocellular Carcinoma	1.540	1.14E-5	4.972	Roessler Liver[26]
NA: Not Available					

Linkages of mRNA levels of PFDNs with clinicopathological parameters of

LIHC patients

We explored the mRNA expressions of PFDNs in LIHC and normal liver samples via the GEPIA2 database (<http://gepia2.cancer-pku.cn/#index>). The results showed that the expression levels of PFDN1/2/3/4/5/6 were higher in LIHC tissues than normal liver tissues (Fig. 3A, B). In addition, it was also confirmed that the expressions of PFDNs were higher in LIHC tissues than paracancerous ones based on a matched comparison of 50 samples (Fig. 3C). Subsequently, we evaluated the associations between the expressions of PFDNs family proteins and tumor grades and cancer staging by using the GEPIA2 database and the UALCAN database (<http://ualcan.path.uab.edu>). From Fig. 4A, we could see that the mRNA expressions of 6 PFDNs family proteins were significantly correlated with tumor grades. The highest mRNA levels of PFDN1/3/4/5/6 were found in grade 4, while the highest mRNA level of PFDN2 was discovered in grade 3. Likewise, from Fig. 4B, we could see that the mRNA levels of PFDN1/3/4/5 significantly changed ($p < 0.05$) in different stages, whereas PFDN2 and PFDN6 did not ($p > 0.05$).

We also analyzed the immunohistochemistry (IHC) of 6 PFDNs family proteins in LIHC tissues and normal liver tissues by using the HPA database (<https://www.proteinatlas.org/>). The result showed that medium or high expressions of PFDN1/2/3/4/5 were detected in LIHC tissues, whereas medium or low protein expressions of them were found in normal liver tissues (Fig. 5A). PFDN6 protein was not detected in LIHC tissue and normal liver tissue. All in all, the protein expression levels of PFDNs were higher in LIHC tissue than normal liver tissue.

The prognostic value of PFDNs in LIHC patients

We evaluated the prognostic value of PFDNs in the survival of LIHC via the online tool of Kaplan-Meier Plotter (<http://kmplot.com/>). The curves of overall survival (OS), relapse-free survival (RFS), progression-free survival (PFS) and disease-specific survival (DSS) were showed in Fig. 5B. High mRNA expressions of PFDN1/2/3/4 were relevant to poor OS ($p < 0.05$). Besides, high mRNA expressions of PFDN2/3/4 were related to poor RFS and DSS ($p < 0.05$), and high expression of PFDN6 was associated with poor RFS ($p < 0.05$). However, the expression of PFDN5 was not significantly correlated with the survival of LIHC patients ($p > 0.05$).

PFDN1/2/4 were independently correlated with prognosis in LIHC patients

After we showed the significant correlation between PFDN1/2/3/4/6 expressions with the survival of LIHC patients by K-M survival analysis, and then, we attempted to analyze the independent prognostic value of PFDNs in OS and PFS for hepatocellular carcinoma patients. The clinical data of 374 LIHC patients were acquired from The Cancer Genome Atlas (TCGA, <https://portal.gdc.cancer.gov>) database, and their basic clinical characteristics were presented in Supplementary Table 1. Subsequently, we used the statistical method of Cox regression analysis to assess these data. The results were shown in Supplementary Table 2 and Supplementary Table 3. Univariate analysis indicated that high T stage (HR = 2.109, $p < 0.001$), high expression of PFDN1 (HR = 1.454, $p < 0.035$), PFDN2 (HR = 1.460, $p < 0.032$), PFDN3 (HR = 1.277, $p = 0.016$), and PFDN4 (HR = 1.789, $p = 0.001$) were significantly associated with poorer OS of LIHC patients. Besides, high T stage (HR = 2.384, $p < 0.001$), high expression of PFDN1 (HR = 1.390, $p < 0.028$) and PFDN4 (HR = 1.494, $p = 0.007$) were significantly relevant to poorer PFS of LIHC patients. In

multivariate analysis, we could see that T stage (HR = 2.178, $p < 0.001$), PFDN1 (HR = 1.187, $p < 0.017$), PFDN2 (HR = 1.3–71, $p < 0.021$), and PFDN4 (HR = 1.465, $p < 0.024$) were independently correlated with poorer OS of LIHC patients; T stage (HR = 2.349, $p < 0.001$) was independently related to poorer PFS of LIHC patients. Taken together, high PFDN1/2/4 expressions were independent prognostic factors in OS for LIHC patients.

Genetic mutations and co-expression of PFDNs in LIHC patients

Genetic mutations and co-expression of the PFDNs family proteins were analyzed via the cBioPortal database (<http://www.cbioportal.org/>). As were shown in Fig. 6A and B. In total, 55% (199/360) of patients with LIHC had at least two genetic mutations. PFDN1, PFDN2, PFDN3, PFDN4, PFDN5 and PFDN6 were altered in 12%, 35%, 14%, 14%, 8%, and 13% of the 360 complete samples (Fig. 6A). Similarly, the degree of genetic mutations of PFDNs family proteins was presented in the Expression Heatmap (Fig. 6B), the frequency of PFDN2 mutation was highest among PFDNs in LIHC. Moreover, we analyzed the correlations among the 6 PFDNs family numbers, from Fig. 6C, we could see that there were meaningful and positive associations as follows: PFDN1 with PFDN2/4/5; PFDN2 with PFDN1/4/5/6; PFDN4 with PFDN1/2/5/6; PFDN5 with PFDN1/2/4/6; PFDN6 with PFDN2/4/5.

Genetic mutations and expression heatmap of PFDNs in LIHC patients (cBioPortal). (C) The correlations among different expressions of the 6 PFDNs family proteins (cBioPortal). (D) PPI network among the 6 PFDNs family proteins (STRING). (E) PPI network for PFDNs and their 50 frequently neighboring genes (STRING). (F) Nine hub genes including RPL24, RPL37, RPL37A, RPS15, ZNF317, MYS6B, LSM7, PNAJC7, and ATP5Q2 were presented in PPI network (Cytoscape).

Established protein-protein interaction (PPI) network and analyzed the functions and pathways of PFDNs in LIHC patients

Firstly, we selected 50 most frequently altered neighboring genes of the 6 PFDNs proteins by using the GEPIA2 database. And then, we established the PPI network among the 6 PFDNs family proteins (Fig. 6D) and another PPI network for PFDNs and their 50 frequently neighboring genes (Fig. 6E) by using the tool of STRING (<https://string-db.org/>). Lastly, we screened out nine hub genes in these frequently neighboring genes by using the software of Cytoscape (<https://cytoscape.org/>). The result was showed in Fig. 6F, nine hub genes including RPL24, RPL37, RPL37A, PRS15, ZNF317, MYS6B, LSM7, PNAJC7, and ATP5Q2 were tightly related to the alterations of PFDNs.

Subsequently, we predicted the functions of PFDNs and their 50 frequently altered neighboring genes by analysis of GO and KEGG using DAVID6.8 (<https://david.ncifcrf.gov/>). The GO enrichment analysis showed GO:00000398 (mRNA splicing via spliceosome), GO:0006364 (rRNA processing), GO:000184 (nucleic acid-transcribed mRNA catabolic process), GO:0006457 (protein folding), GO:0005634 (nucleus), GO:0005737 (cytoplasm), GO:0005654 (nucleoplasm), GO:0005515 (protein binding), GO:0003723 (RNA binding) and GO:0044822 (poly(A) RNA binding) were meaningful modulated by alterations of the 6 PFDNs family proteins (Fig. 7A, B, C). Moreover, KEGG pathway

enrichment analysis indicated that hsa03010 (Ribosome), hsa03040(Spliceosome) and hsa03013(RNA transport) were significantly related to alterations of the 6 PFDNs family proteins(Fig. 7D).

Immune cell infiltration of PFDNs in LIHC patients

Lastly, we attempted to analyse the correlation between 6 PFDNs family proteins and infiltrated immune cells by using the TIMER database (<https://cistrome.shinyapps.io/timer/>). The results were shown in Fig. 8. PFDN1 expression was positively correlated with the infiltration of B cells (Cor = 0.319, $p = 1.46E-9$), CD8⁺T cells (Cor = 0.273, $p = 1.63E-7$), CD4⁺T cells (Cor = 0.357, $p = 9.07E-12$), macrophage (Cor = 0.404, $p = 7.72E-15$), neutrophil (Cor = 0.358, $p = 7.12E-12$) and dendritic cells (Cor = 0.351, $p = 2.79E-11$) (Fig. 8A). PFDN2 expression was positively associated with the infiltration of B cells (Cor = 0.153, $p = 4.42E-3$), CD4⁺T cells (Cor = 0.137, $p = 1.12E-2$), macrophage (Cor = 0.126, $p = 1.98E-2$) and neutrophil (Cor = 0.123, $p = 2.19E-2$) (Fig. 8B). PFDN3 expression was positively related to the infiltration of B cells (Cor = 0.353, $p = 1.61E-11$), CD8⁺T cells (Cor = 0.32, $p = 3.35E-09$), CD4⁺T cells (Cor = 0.312, $p = 3.35E-9$), macrophage (Cor = 0.421, $p = 4.72E-16$), neutrophil (Cor = 0.453, $p = 6.84E-19$) and dendritic cells (Cor = 0.453, $p = 1.23E-18$) (Fig. 8C). PFDN4 expression was positively relevant to the infiltration of B cells (Cor = 0.316, $p = 2.05E-9$), CD8⁺T cells (Cor = 0.329, $p = 4.37E-10$), CD4⁺T cells (Cor = 0.204, $p = 1.36E-4$), macrophage (Cor = 0.419, $p = 6.67E-16$), neutrophil (Cor = 0.265, $p = 5.63E-7$) and dendritic cells (Cor = 0.346, $p = 5.16E-11$) (Fig. 8D). PFDN5 expression was positively correlated with the infiltration of B cells (Cor = 0.244, $p = 4.82E-6$), CD8⁺T cells (Cor = 0.338, $p = 1.36E-10$), macrophage (Cor = 0.244, $p = 5.15E-6$), neutrophil (Cor = 0.132, $p = 1.40E-2$) and dendritic cells (Cor = 0.278, $p = 1.93E-7$) (Fig. 8E). However, PFDN6 was not significantly correlated with the above six types of infiltrated immune cells (all $p > 0.05$) (Fig. 8F).

Discussion

A few prior studies have indicated that the PFDNs family proteins are associated with several human cancers[4,5,6,8]. In addition, the significant prognostic values of some of PFDNs have been proved in few tumor types including gastric cancer[30] and colorectal cancer[7]. However, up to now, the specific mechanisms of the PFDNs in liver hepatocellular carcinoma have not been completely illuminated. In this paper, we selected six PFDNs family numbers including PFDN1/2/3/4/5/6 and explored their mRNA expressions and prognostic values in LIHC. Of course, we hope our findings will provide useful assistance for the diagnosis and treatment of liver cancer.

Among the PFDNs, the most research was found for PFDN1 in several cancers. For instance, D Wang et al.[6] found that overexpression of PFDN1 enhanced the migratory capacity of lung cancer cells by decreasing cyclinA expression. Report from Cheng Z et al. showed that PFDN1 played a crucial role in metastasis of gastric cancer cells via the Wnt/ β -catenin pathway[4]. However, reports about the status of PFDN1 in LIHC were poor. In this study, higher mRNA and protein expressions of PFDN1 were found in hepatocellular carcinoma than normal liver tissues. Moreover, PFDN1 expression was significantly relevant to tumor grades and cancer staging. High mRNA expression of PFDN1 was associated with poor OS of LIHC patients, which also functioned as an independent prognostic factor for poorer OS in LIHC

patients. Interesting, PFDN1 expression was positively correlated with the infiltration of B cells, CD8⁺T cells, CD4⁺T cells, macrophage, neutrophil, and dendritic cells. By taking the above findings into account, our study revealed that PFDN1 played an oncogenic role in LIHC.

PFDN2 dysregulation has been found in several carcinomas. Babak N et al.[8]found that the aberrant PFDN2 expression promoted breast cancer progression by increasing Taxane resistance to breast cancer. A few studies showed that PFDN2 dysregulation was tightly related to liver cancer[31,32].In our study, the mRNA expression of PFDN2 was higher in LIHC tissues than normal liver tissues. Moreover, PFDN2 expression was significantly associated with tumor grades,but did not correlate with cancer staging. High mRNA expression of PFDN2 was relevant to poor OS, RFS and DSS in LIHC, which also severed as an independent prognostic factor for poorer OS in LIHC patients, and the frequency of PFDN2 mutation was highest among PFDNs in LIHC.Furthermore, PFDN2 expression was positively related to the infiltration of B cells,CD4⁺T cells, macrophage, and neutrophil. Taken together, our study showed that PFDN1 played an oncogenic role in LIHC.

PFDN3(VBP1) was reported significantly related to the progression of hepatocellular carcinoma by interacting with Hepatitis B virus X protein(HBx)[33]. However, the prognostic value of PFDN3 in hepatocellular carcinoma is unknown to date.In our study, higher mRNA and protein expressions of PFDN3 were found in hepatocellular carcinoma than normal liver tissues. Moreover, PFDN3 expression was significantly related to tumor grades and cancer staging.High mRNA expression of PFDN3 was relevant to short OS, RFS, and DSS in LIHC. Interesting, PFDN3 expression was positively associated with the infiltration of B cells, CD8⁺T cells, CD4⁺T cells, macrophage, neutrophil, and dendritic cells. All results seemed to indicate that PFDN3 may function as a prognostic biomarker in LIHC patients.

Similar to PFDN2, PFDN4 dysregulation has been found in several malignancies. Report form C Collins et al.[34]revealed that PFDN4 was a driving gene in the development of breast cancer. Norikatsu M et al. [35]showed that low expression of PFDN4 was related to poor survival in colorectal cancer patients. In our study, higher mRNA and protein expressions of PFDN4 were found in LIHC tissues than normal liver tissues. Furthermore, PFDN4 expression was significantly associated with tumor grades and cancer staging. High mRNA expression of PFDN4 was relevant to poor OS, RFS, and DSS in LIHC, which also functioned as an independent prognostic factor for poorer OS in LIHC patients. Moreover, PFDN4 expression was positively correlated with the infiltration of B cells, CD8⁺T cells, CD4⁺T cells, macrophage, neutrophil and dendritic cells. By taking the above findings into account, our study showed that PFDN4 played an oncogenic role in LIHC.

PFDN5 has been widely known to correlate with neurological diseases by previous studies[3]. However, few studies reported the relationship between PFDN5 expression with human cancers.In our study, the mRNA and protein expressions of PFDN5 were higher in LIHC tissues than normal liver tissues.Moreover, PFDN5 expression was significantly relevant to tumor grades and cancer staging, but its expression had no meaningful association with survival prognosis in LIHC patients. In addition, PFDN5 expression was positively correlated with the infiltration of B cells, CD8⁺T cells, macrophage,neutrophil, and dendritic

cells. Taken together, the specific role of PFDN5 in human cancers still needs to be investigated by further knowledge.

Like PFDN5, very few studies on PFDN6 have been done in human malignancies. Report from Nasrin D-N et al.[36] showed that PFDN6 had prognostic value in children with acute lymphoblastic leukemia(ALL). In our study, PFDN6 expression level was higher in LIHC tissues than normal liver tissues. Moreover, PFDN6 expression was significantly associated with tumor grades, but did not correlate with cancer staging. High mRNA expression of PFDN6 was relevant to poor RFS. However, PFDN6 expression was not significantly related to the above six types of infiltrated immune cells.

In our study, there were some limitations should be acknowledged. Firstly, we did not explore the underlying mechanisms between PFDNs and LIHC. Secondly, the analysis between mRNA level and immune cell infiltration can reflect a few aspects of immune status, but not all, patients with LIHC. Moreover, there are still more large-scale and multi-center clinical studies to be conducted to validate our findings.

Conclusion

In conclusion, our findings showed that PFDN1/2/3/4 were potential prognostic markers to suggest poor OS of LIHC patients. In addition, high PFDN1/2/4 expressions were independent prognostic factors in OS for LIHC patients.

Abbreviations

LIHC: Liver Hepatocellular Carcinoma ; PFDN: Prefoldin ; PPI: protein-protein interaction; c-CPN: eukaryotic cytoplasmic chaperone proteins; OS: overall survival RFS: relapse-free survival; PFS: progression-free survival; DSS: disease-specific survival; IHC: immunohistochemistry; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes

Declarations

Ethical approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used during the current study are available from the corresponding author on reasonable request

Author contributions

FL and XZ wrote manuscript,WK,MW,HM and QW assisted in analysis work,FL and YD designed the study.The authors all approved of the final manuscript to be submitted to “Cancer Cell International”.

Conflicts of interest

The authors declare no competing interests

Funding

This study was supported by the Special Scientific research project of Education Department of Shaanxi Province(grant number:17JK0209) and the discipline innovation-team project of Shaanxi university of Chinese Medicine(grant number: 2 019-SY03).

Acknowledgements

Not applicable

References

1. Siegel R L, Miller K D, Fuchs H E, et al. Cancer Statistics, 2021[J]. CA Cancer J Clin, 2021,71(1):7–33.
2. Forner A, Reig M, Bruix J. Hepatocellular carcinoma[J]. Lancet, 2018,391(10127):1301–1314.
3. Mo S J, Zhao H C, Tian Y Z, et al. The Role of Prefoldin and Its Subunits in Tumors and Their Application Prospects in Nanomedicine[J]. Cancer Manag Res, 2020,12:8847–8856.
4. Zhou C, Guo Z, Xu L, et al. PFND1 Predicts Poor Prognosis of Gastric Cancer and Promotes Cell Metastasis by Activating the Wnt/ β -Catenin Pathway[J]. Onco Targets Ther, 2020,13:3177–3186.
5. Peñate X, Praena-Fernández J M, Romero P P, et al. Overexpression of Canonical Prefoldin Associates with the Risk of Mortality and Metastasis in Non-Small Cell Lung Cancer[J]. Cancers (Basel), 2020,12(4).
6. Wang D, Shi W, Tang Y, et al. Prefoldin 1 promotes EMT and lung cancer progression by suppressing cyclin A expression[J]. Oncogene, 2017,36(7):885–898.
7. Wang P, Zhao J, Yang X, et al. PFDN1, an indicator for colorectal cancer prognosis, enhances tumor cell proliferation and motility through cytoskeletal reorganization[J]. Med Oncol, 2015,32(12):264.
8. Nami B, Wang Z. Genetics and Expression Profile of the Tubulin Gene Superfamily in Breast Cancer Subtypes and Its Relation to Taxane Resistance[J]. Cancers (Basel), 2018,10(8).
9. Farooq M, Hozzein W N, Elsayed E A, et al. Identification of histone deacetylase 1 protein complexes in liver cancer cells[J]. Asian Pac J Cancer Prev, 2013,14(2):915–921.
10. Al-Yhya N, Khan M F, Almeer R S, et al. Pharmacological inhibition of HDAC1/3-interacting proteins induced morphological changes, and hindered the cell proliferation and migration of hepatocellular carcinoma cells[J]. Environ Sci Pollut Res Int, 2021.Apr 30; doi:10.1007/s11356-021-13668-1”

11. Wang D, Zhu Z Z, Jiang H, et al. Multiple genes identified as targets for 20q13.12-13.33 gain contributing to unfavorable clinical outcomes in patients with hepatocellular carcinoma[J]. *Hepatol Int*, 2015,9(3):438–446.
12. Rhodes D R, Yu J, Shanker K, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform[J]. *Neoplasia*, 2004,6(1):1–6.
13. Tomczak K, Czerwińska P, Wiznerowicz M. The Cancer Genome Atlas (TCGA): an immeasurable source of knowledge[J]. *Contemp Oncol (Pozn)*, 2015,19(1A):A68-A77.
14. Tang Z, Li C, Kang B, et al. GEPIA: a web server for cancer and normal gene expression profiling and interactive analyses[J]. *Nucleic Acids Res*, 2017,45(W1):W98-W102.
15. Chandrashekar D S, Bashel B, Balasubramanya S, et al. UALCAN: A Portal for Facilitating Tumor Subgroup Gene Expression and Survival Analyses[J]. *Neoplasia*, 2017,19(8):649–658.
16. Uhlén M, Fagerberg L, Hallström B M, et al. Proteomics. Tissue-based map of the human proteome[J]. *Science*, 2015,347(6220):1260419.
17. Nagy Á, Munkácsy G, Györfy B. Pancancer survival analysis of cancer hallmark genes[J]. *Sci Rep*, 2021,11(1):6047.
18. Györfy B, Surowiak P, Budczies J, et al. Online survival analysis software to assess the prognostic value of biomarkers using transcriptomic data in non-small-cell lung cancer[J]. *PLoS One*, 2013,8(12):e82241.
19. Cerami E, Gao J, Dogrusoz U, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data[J]. *Cancer Discov*, 2012,2(5):401–404.
20. Gao J, Aksoy B A, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal[J]. *Sci Signal*, 2013,6(269):l1.
21. Szklarczyk D, Gable A L, Lyon D, et al. STRING v11: protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets[J]. *Nucleic Acids Res*, 2019,47(D1):D607-D613.
22. Huang D W, Sherman B T, Lempicki R A. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources[J]. *Nat Protoc*, 2009,4(1):44–57.
23. Otasek D, Morris J H, Bouças J, et al. Cytoscape Automation: empowering workflow-based network analysis[J]. *Genome Biol*, 2019,20(1):185.
24. Li T, Fan J, Wang B, et al. TIMER: A Web Server for Comprehensive Analysis of Tumor-Infiltrating Immune Cells[J]. *Cancer Res*, 2017,77(21):e108-e110.
25. Li B, Severson E, Pignon J C, et al. Comprehensive analyses of tumor immunity: implications for cancer immunotherapy[J]. *Genome Biol*, 2016,17(1):174.
26. Roessler S, Jia H L, Budhu A, et al. A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients[J]. *Cancer Res*, 2010,70(24):10202–10212.
27. Wurmbach E, Chen Y B, Khitrov G, et al. Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma[J]. *Hepatology*, 2007,45(4):938–947.

28. Chen X, Cheung S T, So S, et al. Gene expression patterns in human liver cancers[J]. *Mol Biol Cell*, 2002,13(6):1929–1939.
29. Mas V R, Maluf D G, Archer K J, et al. Genes involved in viral carcinogenesis and tumor initiation in hepatitis C virus-induced hepatocellular carcinoma[J]. *Mol Med*, 2009,15(3–4):85–94.
30. Yesseyeva G, Aikemu B, Hong H, et al. Prefoldin subunits (PFDN1-6) serve as poor prognostic markers in gastric cancer[J]. *Biosci Rep*, 2020,40(2).
31. Chang K W, Yang P Y, Lai H Y, et al. Identification of a novel actin isoform in hepatocellular carcinoma[J]. *Hepatol Res*, 2006,36(1):33–39.
32. Tsao M L, Chao C H, Yeh C T. Interaction of hepatitis C virus F protein with prefoldin 2 perturbs tubulin cytoskeleton organization[J]. *Biochem Biophys Res Commun*, 2006,348(1):271–277.
33. Kim S Y, Kim J C, Kim J K, et al. Hepatitis B virus X protein enhances NFkappaB activity through cooperating with VBP1[J]. *BMB Rep*, 2008,41(2):158–163.
34. Collins C, Volik S, Kowbel D, et al. Comprehensive genome sequence analysis of a breast cancer amplicon[J]. *Genome Res*, 2001,11(6):1034–1042.
35. Miyoshi N, Ishii H, Mimori K, et al. Abnormal expression of PFDN4 in colorectal cancer: a novel marker for prognosis[J]. *Ann Surg Oncol*, 2010,17(11):3030–3036.
36. Dehghan-Nayeri N, Rezaei-Tavirani M, Omrani M D, et al. Identification of potential predictive markers of dexamethasone resistance in childhood acute lymphoblastic leukemia[J]. *J Cell Commun Signal*, 2017,11(2):137–145.

Figures

Figure 1

Flow chart of the entire study design.

Figure 2

mRNA expression levels of PFDNs in various types of cancers (Oncomine database).

Figure 3

mRNA expression levels of PFDNs in LIHC samples and normal liver samples. (A, B) The transcriptional levels of PFDNs in LIHC samples and normal liver samples (GEPIA2). (C) A matched comparison of 50 samples between LIHC tissues and paracancerous tissues (XIAN TAO platform).

Figure 5

Immunohistochemistry (IHC) and prognostic values of PFDNs in LIHC patients(HPA, Kaplan-Meier Plotter). (A)The IHC images about the expressions of PFDNs in LIHC tissues and normal liver tissues(HPA). (B)The prognostic values of PFDNs in OS, RFS, PFS, and DSS for LIHC patients (Kaplan-Meier Plotter).

Figure 6

Genetic mutations and co-expression of PFDNs in LIHC patients and Protein-Protein Interaction (PPI) network for PFDNs and their 50 frequently altered neighboring genes in LIHC(cBioPortal, STRING, Cytoscape). (A, B) Genetic mutations and expression heatmap of PFDNs in LIHC patients (cBioPortal). (C) The correlations among different expressions of the 6 PFDNs family proteins (cBioPortal). (D)PPI network among the 6 PFDNs family proteins(STRING). (E)PPI network for PFDNs and their 50 frequently

neighboring genes(STRING). (F)Nine hub genes includingRPL24, RPL37, RPL37A, RPS15, ZNF317, MYS6B, LSM7, PNAJC7, and ATP5Q2 were presented in PPI network (Cytoscape).

A

B

C

D

Figure 7
Functional and enrichment analysis for PFDNs and their 50 frequently altered neighboring genes in LIHC (DAVID 6.8). (A, B, C)GO enrichment analysis for PFDNs and their 50 frequently altered neighboring genes

(DAVID 6.8). (D)KEGG pathway enrichment analysis for PFDNs and their 50 frequently altered neighboring genes(DAVID 6.8).

Figure 8

The association between PFDNs expressions and six types of infiltrated immune cells(TIMER).

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTables.rar](#)