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Systematic summarization of the expression profiles and prognostic roles of the *dishevelled* gene family in hepatocellular carcinoma

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Abstract

Background: Dishevelled (DVL) family members are crucial to Wnt-induced signaling transduction, and their expression is highly correlated with the progression of multiple malignant cancers. However, the expression profiles and exact prognostic values of DVLs in hepatocellular carcinoma (HCC) have not been explored until now.

Methods: The expression of DVL isoforms was assessed using the Oncomine, HCCDB and UALCAN databases. The prognostic roles of DVLs were further evaluated using the GEPIA database. The relationship between the expression of DVLs and immune infiltration of HCC was investigated using the Timer and ImmuCellAI tools. Furthermore, protein–protein interaction (PPI) networks were built and enrichment analyses were conducted.

Results: We found that the expression levels of DVL2 (OMIM accession number: 602151) and DVL3 (OMIM accession number: 601368) were upregulated in HCC tissues as revealed by the Oncomine and HCCDB databases. Additionally, the expression of DVLs tended to be associated with advanced clinical features in the UALCAN database. Prognostic analysis revealed that the expression levels of DVL1 (OMIM accession number: 601365) and DVL3 were remarkably associated with a poor prognosis in HCC patients. The results also revealed that the DVL expression level was correlated with the infiltration levels of multiple immune cells. By constructing the PPI network and enrichment analyses, the *DVL1-3* gene was identified to interact with 20 key genes and participate in several pathways.

Conclusion: In summary, DVL2 and DVL3 are highly expressed in HCC, and DVL1 and DVL3 are related to a poor prognosis, which might be used as candidate targets for targeted therapy and reliable prognostic biomarkers in HCC.

KEYWORDS
bioinformatics, dishevelled, gene expression, hepatocellular carcinoma

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is a severe global health problem that has a high cancer-associated mortality. Epidemiological statistics issued by the American Cancer Society (ACS) suggests that more than 30,000 deaths will occur due to HCC in 2019 in the United States (Siegel, Miller, & Jemal, 2019). Despite the combination of various treatment methods for HCC, including surgery, chemotherapy, and radiotherapy, the prognosis of this disease is still poorer among all solid tumors (Tai et al., 2017) because most patients are initially diagnosed at advanced stage. Thus, it is urgent to investigate novel biomarkers associated with the diagnosis and prognosis of HCC. Although accumulating studies are emerging that focus on biomarkers of HCC and significant advances have achieved already (Liu, Zeng, Zhang, & Xu, 2019; Shimoda et al., 2018), potential mechanisms in HCC carcinogenesis and development and distinct biomarkers need to be further explored.

The *dishevelled* (*DVL*) gene family comprises three isoforms: *DVL1*, *DVL2*, and *DVL3*. Normally, all the three proteins, located in the cytoplasm, are implicated in phosphorylation and mediate the downstream signal transduction of various Wnt proteins (Sharma, Castro-Piedras, Simmons, & Pruitt, 2018). DVL1 has been reported to be the main signal transduction molecule of the classical Wnt (Wnt/β-catenin) pathway, which is significantly associated with the progression of multiple malignant cancers, including breast cancer and lung cancer (Zeng et al., 2018; Zhao et al., 2010). DVL2 is the regulatory molecule of the classical and nonclassical Wnt (Wnt/PCP) pathways, contributing to the metastasis and invasion of tumors (Zhang et al., 2017; Zhu et al., 2012). DVL3 could regulate the nonclassical Wnt pathway in lung cancer and promote malignant progression by activating the p38 protein and JNK pathways (Zhao et al., 2010). However, the expression profiles and distinct prognostic values of the DVLs in HCC are not well identified.

In the present study, the expression levels of the DVLs were evaluated in HCC using the Oncomine, HCCDB and UALCAN databases to determine the expression pattern of distinct DVL family members in HCC tissues and their associations with clinical patterns. Additionally, the exact prognostic values of HCC were assessed in HCC using the GEPIA database. The correlation between DVL expression and immune cell infiltration was investigated using the Timer and ImmuCellAI tools. Moreover, the protein–protein interaction (PPI) network of DVLs was constructed to reveal the potential roles of DVLs and their cooperators. Consequently, our research preliminarily and systematically summarizes the expression profiles of DVLs and discusses their potential roles in HCC.

2 | MATERIALS AND METHODS

2.1 | Oncomine database mining

The cancer-related public database Oncomine (https://www.oncomine.org/) was used to assess the mRNA expression level of DVLs in tumor and normal tissues (Rhodes et al., 2004). In the Oncomine database, all members of the *DVL* family were retrieved and differential gene analysis combined with the mRNA data type was chosen. Regarding the differential analysis of DVLs between HCC and normal samples, the thresholds were set as follows: analysis type: cancer versus normal; cancer type: liver cancer; gene rank: top 10%; *p* value: .05; fold-change: all; data type: mRNA.

2.2 | HCCDB database analysis

The HCCDB database (http://lifeome.net/database/hccdb/home.html) is a gene expression atlas of HCC, containing fifteen public HCC transcriptional expression datasets, with 3,917 samples (Lian et al., 2018). HCCDB offers visualization of the findings from multiple bioinformatics analyses, such as differential expression analysis and tissue-specific and tumor-specific expression analyses. We used HCCDB to analyze the expression of DVLs in tumor and normal tissues to explore the expression patterns of DVLs in HCC.

2.3 | UALCAN database mining

UALCAN (http://ualcan.path.uab.edu/) is an open-access platform based on level 3 RNA-seq and pathological files from the TCGA database (Chandrashekar et al., 2017). It can be used to compare the relative transcriptional levels of candidate genes between tumor and para-cancerous tissues, as well as the correlation of the gene mRNA levels with pathological features. In this study, UALCAN was employed to compare the association between the transcriptional levels of DVLs and pathological features.

2.4 | GEPIA database mining

GEPIA (http://gepia.cancer-pku.cn/), an interactive website based on the Cancer Genome Atlas (TCGA) database, was used for RNA sequencing and RNA expression analyses (Tang et al., 2017). In the present study, the GEPIA website was used to explore the expression levels of DVLs in HCC and adjacent normal liver tissue samples. Additionally, the analysis of the prognostic values of DVL members in patients with HCC was performed using the browser. The cutoff *p* value of the differential levels of DVLs was defined as .05.

2.5 | Timer database analysis

Gene expression and immune infiltration analysis across different cancer types can be performed using the Timer database (https://cistrome.shinyapps.io/timer/; Li et al., 2017). The screening conditions for the immune infiltration of the submitted DVLs in HCC were as follows: 1. Gene Symbol: *DVL1*, *DVL2*, *DVL3*, respectively; 2. Cancer Types: Hepatocellular carcinoma; 3. Immune Infiltrates: B Cell, $CD8^+$ T Cell, $CD4^+$ T Cell, Macrophage, Neutrophil, Dendritic.

2.6 | ImmuCellAI analysis

ImmuCellAI (http://bioinfo.life.hust.edu.cn/web/ImmuCellAI/) is an emerging tool to estimate the abundance of 24 immune cells based on a gene expression data set (Miao et al., 2020). The infiltrating data of TIICs corresponding to TCGA-HCC samples were downloaded from the ImmuCellAI website. Next, the correlations between DVL expression and immune cell abundance were examined by Pearson's test and visualized by heat map using R language.

2.7 | Protein-protein interaction network construction

GeneMANIA (http://www.genemania.org/) is an interactive and visual online PPI prediction tool, which provides a customizable function of the detection of genes with similar functions (Franz et al., 2018; Mostafavi, Ray, Warde-Farley, Grouios, & Morris, 2008). GeneMANIA constructed PPI networks in terms of physical interaction, coexpression, predicted, colocalization, common pathway, genetic interaction, and shared protein domains. In this research, GeneMANIA was used for the PPI analysis of DVL family members.

2.8 | Gene function annotation and pathway enrichment analysis

DAVID (https://david.ncifcrf.gov/) is a widely used gene functional annotation website (Dennis et al., 2003). In this study, DAVID was applied to perform Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) analyses of DVLs and their most relevant cooperators. The human genome (homo sapiens) was selected as the background variables. Enrichment terms were considered statistically significant when the Benjamini-adjusted p values were $<.05$ and the top 5 terms of each analysis were retained.

2.9 | Statistical analysis

All statistical analyses were performed online using the relevant bioinformatics websites. T test was used to check the differential expression of DVLs, Pearson's test was used for the correlation analysis of gene expression and immune cell infiltration, and the log-rank test was used for survival analysis. For all analyses, differences were considered statistically significant if the p values were $<.05$.

3 | RESULTS

3.1 | Differential expression of DVLs in HCC and normal liver tissues

We first explored the expression of DVLs in HCC and normal liver tissues using the Oncomine database. By analyzing expression data from the Oncomine database, 10 analyses met the thresholds for DVLs (Figure 1). To further determine the expression levels of *DVL1*, *DVL2*, and *DVL3* genes in HCC, the data corresponding to the four genes regarding the HCC tissue number, normal tissue number, fold-change, t test T, and p value are summarized in Table 1. Among the DVL family members, DVL1 was shown to be expressed at a low level in liver cell dysplasia samples (Wurmbach et al., 2007); thus, this result was excluded in this study. Four results from three studies indicated that DVL2 is remarkably overexpressed in HCC tissues (Chen et al., 2002; Roessler et al., 2010; Wurmbach et al., 2007). Although five results met the thresholds for DVL3, the expression data of DVL3 in 13 cirrhosis samples were also included. The remaining findings all suggested that DVL3 is overexpressed in HCC tissues (Chen et al., 2002; Roessler et al., 2010; Wurmbach et al., 2007).

We further validated the differential expression of DVLs in the HCCDB database. The data suggested that DVL1 expression was not obviously dysregulated in HCC, a finding that was inconsistent in several studies (Figure 2a). However, DVL2 and DVL3 were remarkably overexpressed in HCC samples compared with normal liver samples (Figure 2b,c).

3.2 | Association of the expression of DVLs with the clinical characteristics of patients with HCC

After the high expression of DVLs was confirmed in HCC, we speculated that the overexpression of DVLs may correlate with advanced clinical characteristics of patients with HCC. Next, we analyzed the association between the mRNA expression of *DVLs* with the clinical characteristics of patients with HCC using UALCAN, including

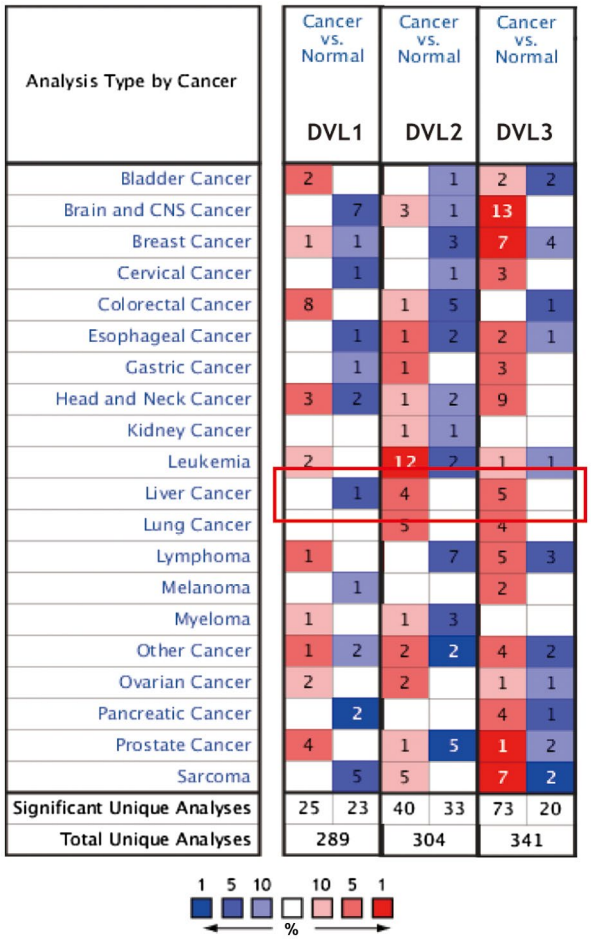

FIGURE 1 Transcript levels of dishevelled (DVLs) in different types of cancer. Dysregulation of *DVL* mRNA was observed in various cancers. Threshold setting: *p* value: 0.05; fold change: all; gene rank: top 10%. Red represents upregulation, and blue represents downregulation. The numbers in the colored cells represent the numbers of dataset meeting the threshold

the patients' clinical stages and tumor grades. As shown in Figure 3, excluding *DVL1*, the mRNA expression levels of *DVLs* were correlated with advanced clinical stages—namely, patients who were with advanced clinical stages tended to express higher *DVL* mRNA (Figure 3a–c). The highest mRNA expression levels of *DVL2* and *DVL3* were found in Stage 3. The mRNA expression levels of *DVLs* in Stage 3 seemed to be higher than those in Stage 4 because of the limited number of Stage 4 patients (only six patients with HCC were at Stage 4).

Similarly, the mRNA expression levels of *DVLs* were positively related to tumor grades. The highest mRNA expression levels of *DVLs* were found in Grade 4 (Figure 3d–f). Overall, the findings above imply that the mRNA levels of *DVLs* are markedly correlated with the clinical characteristics in patients with HCC and may serve as a potential biomarker for advanced HCC stages or HCC with poor differentiation.

TABLE 1 Transcriptional levels of dishevelled (DVLs) between hepatocellular carcinoma (HCC) and normal tissues

DVLs	HCC samples	Normal samples	Fold-change	*t* test	*P* value
DVL1	17[a]	10	−1.254	−2.937	.004
DVL2	35	10	1.688	6.400	5.35E−07
	103	76	1.554	5.303	1.83E−07
	22	21	1.344	4.637	2.83E−05
	225	220	1.498	13.444	3.72E−33
DVL3	4	76	1.458	4.659	0.003
	104	76	1.530	6.425	6.12E−10
	22	21	1.980	6.349	2.38E−07
	35	10	2.253	6.017	1.23E−06
	13[b]	10	1.425	2.914	5.00E−03

[a]Seventeen liver cell dysplasia samples, excluded in this research.

[b]Thirteen cirrhosis samples, excluded in this research.

3.3 | Prognostic values of DVLs in patients with HCC

Overexpressed genes in tumors tend to serve as oncogenes and contribute to the progression and aggressiveness of this disease. Additionally, high expression levels of these genes are always associated with poor survival in patients with cancers. In the present study, we next evaluated the relationship between the expression levels of DVLs and the prognosis of patients with HCC. As shown in Figure 4, the results demonstrated that the overexpression of DVL1 (OS: $p = .005$; DFS: $p = .002$, Figure 4a,e) and DVL3 (OS: $p = .007$; DFS: $p = .003$, Figure 4c,g) were associated with shorter overall survival (OS) and disease-free survival (DFS). However, no statistically significant difference was found in the predictive value of the DVL2 expression level for both OS ($p = .085$, Figure 4b) and DFS ($p = .250$, Figure 4f) in patients with HCC. Furthermore, the three-DVL signature showed excellent value in evaluating the prognosis. The high signature group (OS: $p = .003$; DFS: $p < .001$, Figure 4d,h) was markedly associated with a shorter OS and DFS. In summary, DVLs might be promising biomarkers to evaluate HCC prognosis.

3.4 | Relationship between the expression of DVLs and immune infiltration

Tumor-infiltrating lymphocytes are independent predictors of the sentinel lymph node status and cancer survival. Therefore, our study further evaluated the correlation between DVL expression and immune invasion in HCC using the Timer database. The results showed that the expression of DVL1 was negatively correlated with the infiltration levels of B cells,

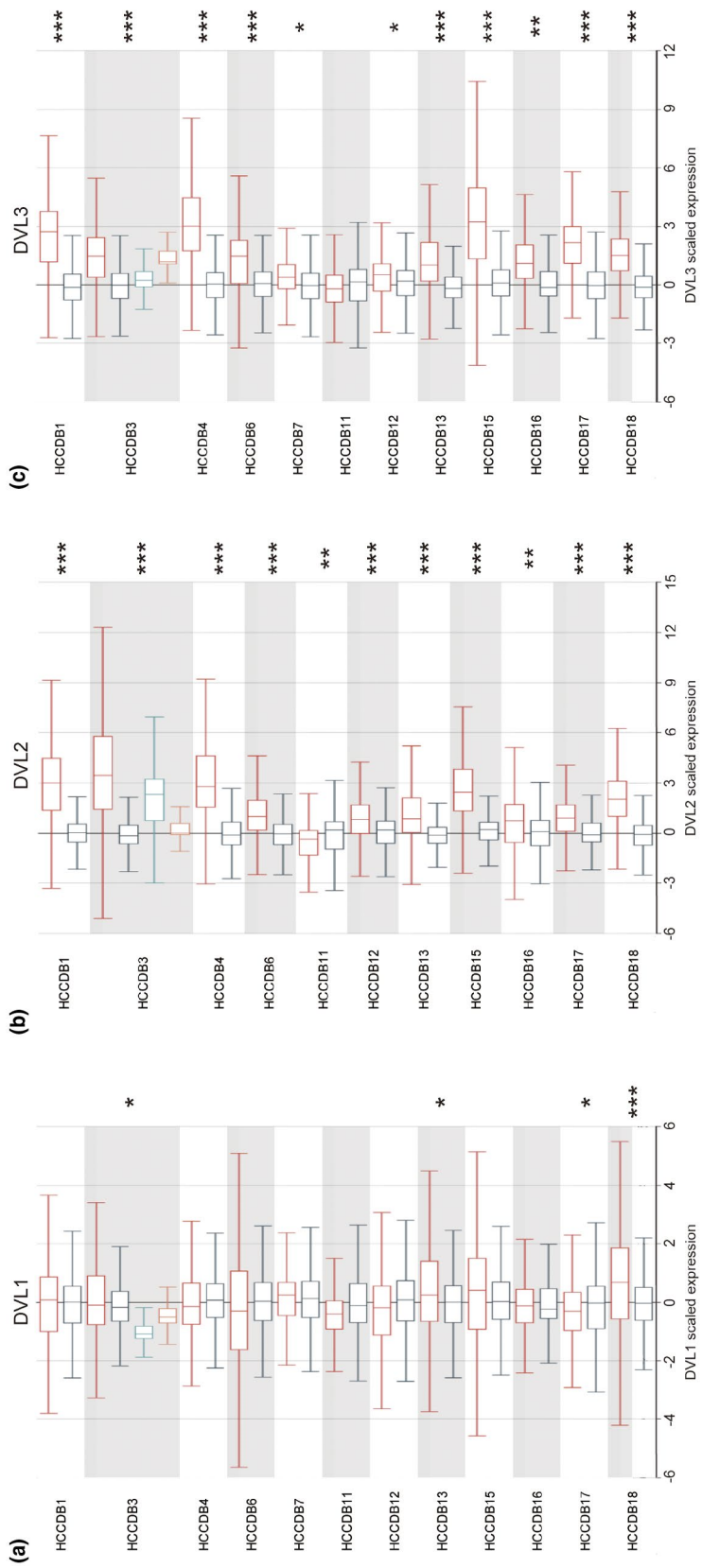

FIGURE 2 Expression of dishevelled (DVLs) in hepatocellular carcinoma (HCC) and normal tissues in the HCCDB database. Translational expression of three dishevelled (DVLs) (a) DVL1, (b) DVL2, and (c) DVL3 in HCC and normal tissues. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

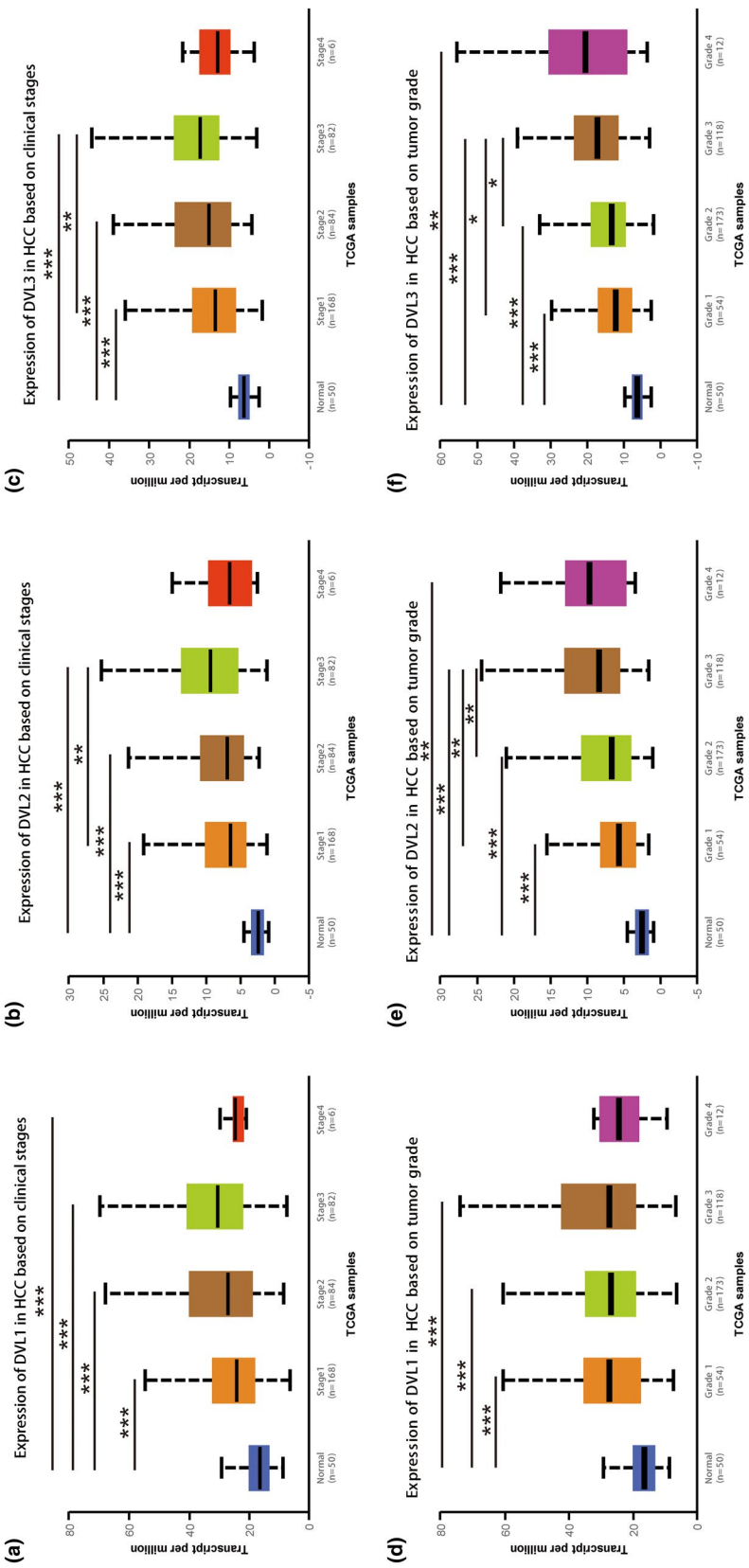

FIGURE 3 Relationship between the expression of dishevelled (DVLs) and clinical stage or tumor grade in hepatocellular carcinoma (HCC). The translational expression of three DVLs was remarkably correlated with the patients' clinical stage and tumor grade, as well as patients who were in advanced stages, and poor differentiation tended to express higher mRNA expression of *DVLs*. (a–c) The highest mRNA expressions of *DVLs* were found in Stage 3. (d–f) The highest mRNA expressions of *DVLs* were found in tumor Grade 4. $^{*}p < .05$, $^{**}p < .01$, $^{***}p < .001$

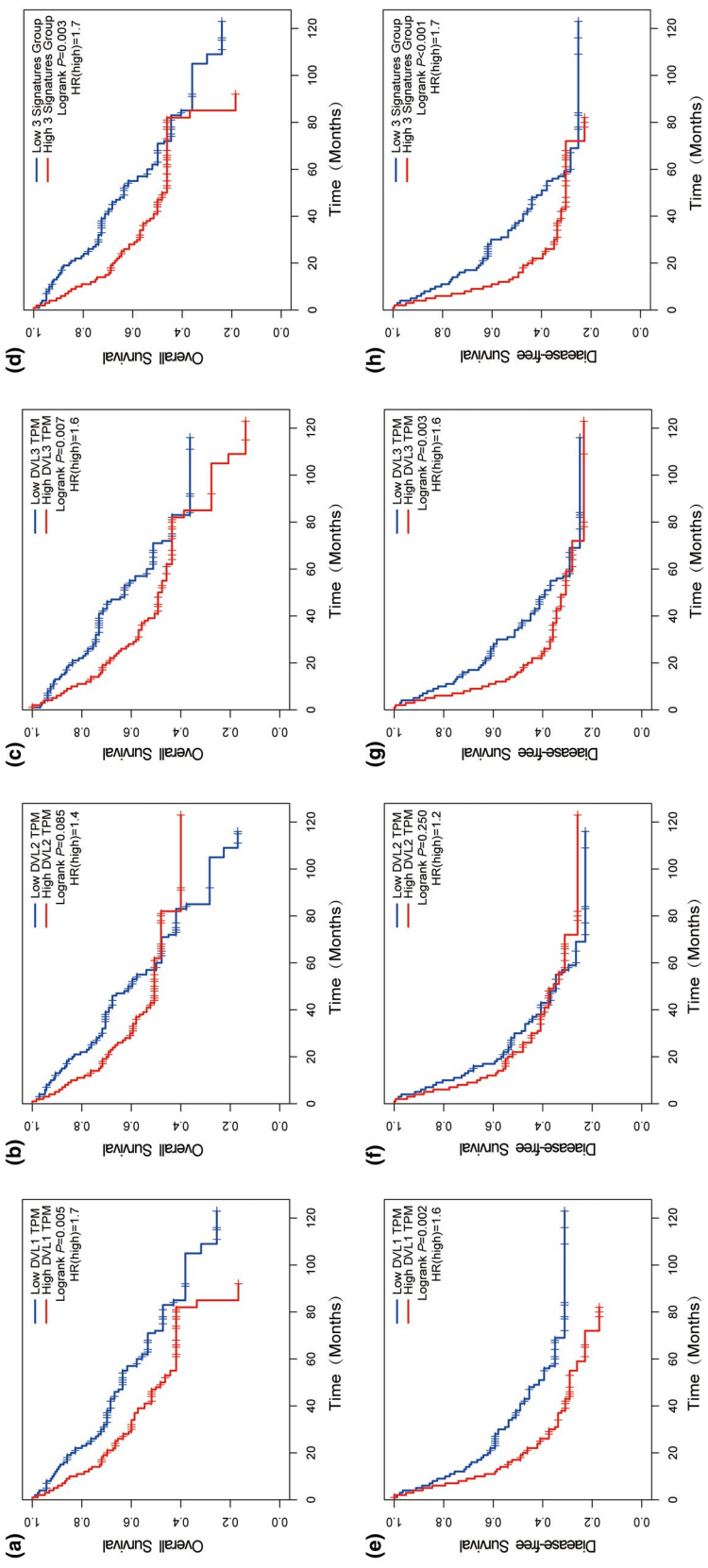

FIGURE 4 The prognostic values of dishevelled (DVLs) expression in hepatocellular carcinoma (HCC) patients. Kaplan–Meier plots show the association between the expression of DVLs and OS and DFS in HCC patients, respectively. OS curves of (a) DVL1, (b) DVL2, (c) DVL3, and (d) DVLs signature in HCC patients. DFS curves of (e) DVL1, (f) DVL2, (g) and (h) DVLs signature in HCC patients

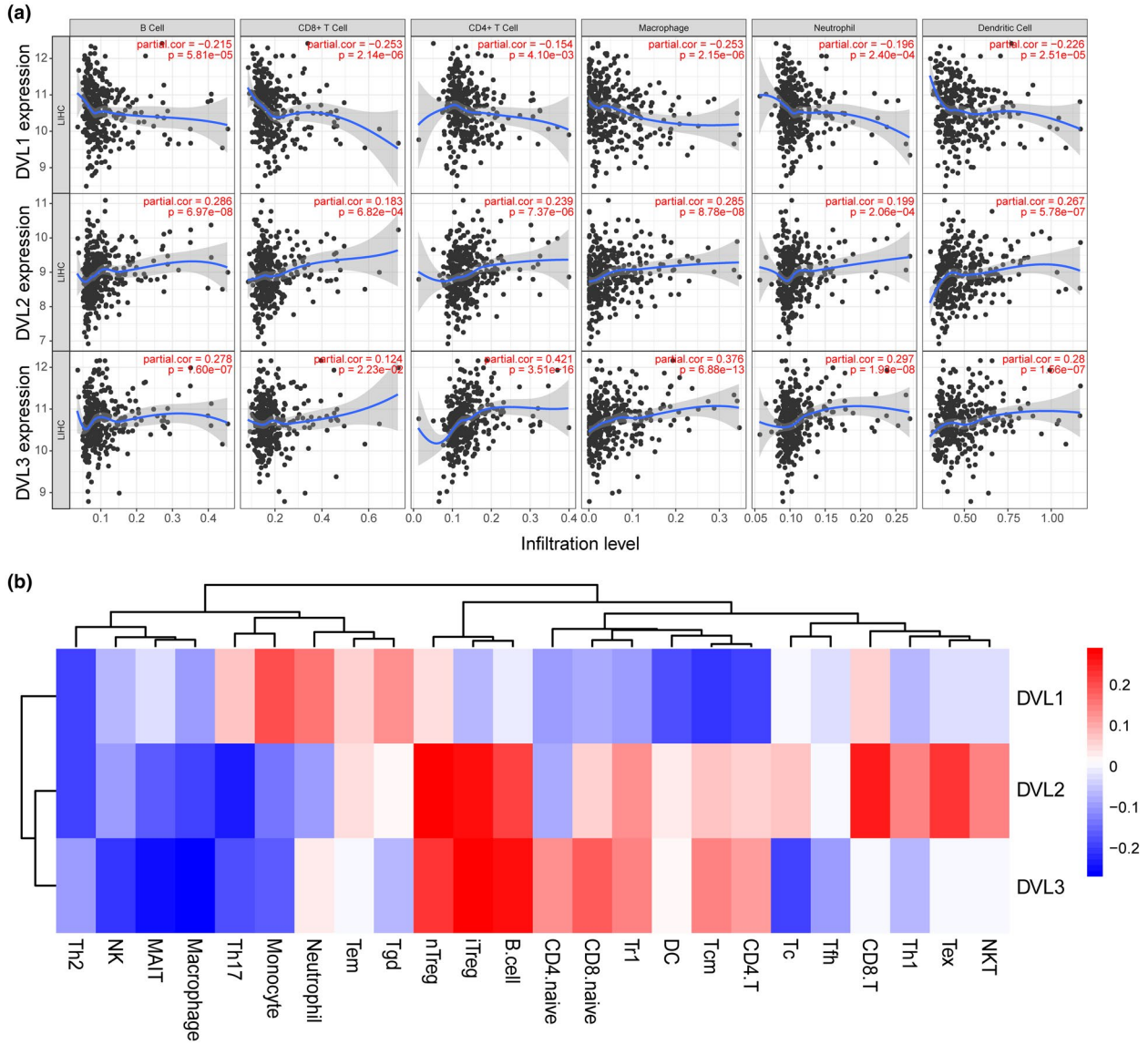

FIGURE 5 Correlation of the dishevelled (DVL) expression with the immune infiltration level in hepatocellular carcinoma (HCC). (a) DVL1 expression has negative correlations, while DVL2 and DVL3 expression have positive correlations with infiltrating levels of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells in HCC. (b) Correlation between DVL expression and immune cell infiltration revealed by ImmuCellAI

$CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells in HCC ($p < .05$, Figure 5a). DVL2 was positively correlated with the infiltration levels of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells in HCC ($p < .05$, Figure 5a). Additionally, DVL3 had a positive correlation with the infiltrating levels of B cells, $CD8^+$ T cells, $CD4^+$ T cells, macrophages, neutrophils, and dendritic cells in HCC ($p < .05$, Figure 5a).

To further support our findings, we used ImmuCellAI with a higher resolution of the immune cell landscape to estimate the abundance of 24 immune cells. Next, the correlations between DVLs and immune cell infiltration were explored. DVL1-3 was related to several specific immune cells (Figure 5b). Additionally, the correlations of immune infiltration with DVL2 and DVL3 were similar, but those with DVL1 and DVL2/3 were inconsistent, findings that agreed with the result of Timer analysis. However, the correlations between DVL expression and infiltration of several immune cell types contrasted between Timer and ImmuCellAI, such as that between DVL2/3 and macrophages, we speculated that different algorithms using different immune cell molecular markers may lead to this difference.

3.5 | The PPI network of DVLs and functional analysis

The present results indicated that high expression of DVLs in HCC predicted a poor prognosis, suggesting DVLs

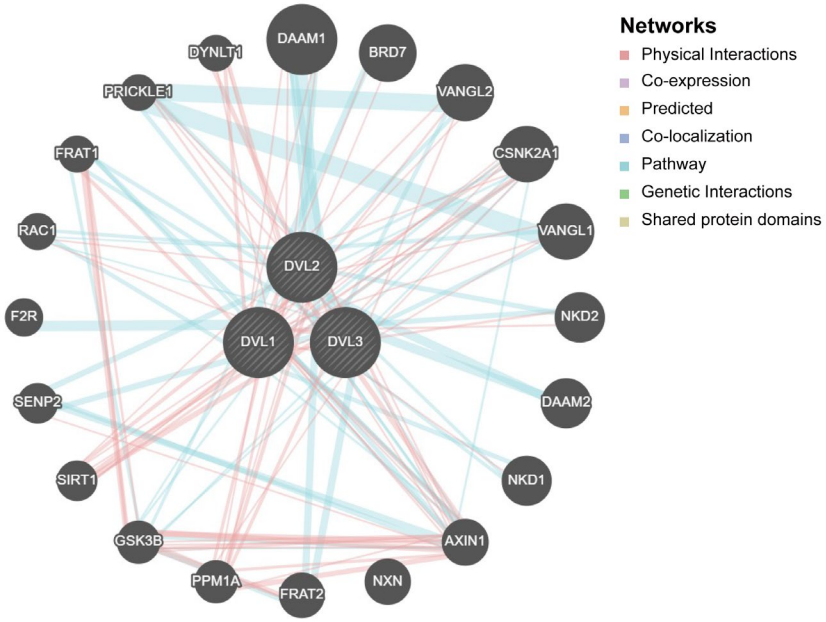

FIGURE 6 Construction of protein-protein interaction (PPI) network of dishevelled (DVLs). The PPI network for DVLs was constructed using the GeneMANIA website. The interconnections among proteins were explored in terms of physical interaction, coexpression, predicted, colocalization, common pathway, genetic interaction, and shared protein domains

TABLE 2 List of 20 critical interacting genes of dishevelled (DVLs) uncovered by GeneMANIA

Gene	Ensembl ID	Gene description
DAAM1	ENSG00000100592.15	Dishevelled associated activator of morphogenesis 1
BRD7	ENSG00000166164.15	Bromodomain containing 7
VANGL2	ENSG00000162738.5	VANGL planar cell polarity protein 2
CSNK2A1	ENSG00000101266.16	Casein kinase 2, alpha 1 polypeptide
VANGL1	ENSG00000173218.14	VANGL planar cell polarity protein 1
NKD2	ENSG00000145506.13	Naked cuticle homolog 2
DAAM2	ENSG00000146122.16	Dishevelled associated activator of morphogenesis 2
NKD1	ENSG00000140807.5	Naked cuticle homolog 1
AXIN1	ENSG00000103126.14	Axin 1
NXN	ENSG00000167693.16	Nucleoredoxin
FRAT2	ENSG00000181274.6	Frequently rearranged in advanced T-cell lymphomas 2
PPM1A	ENSG00000100614.17	Protein phosphatase, Mg^{2+}/Mn^{2+} dependent, 1A
GSK3B	ENSG00000082701.14	Glycogen synthase kinase 3 beta
SIRT1	ENSG00000096717.11	Sirtuin 1
SENP2	ENSG00000163904.12	SUMO1/sentrin/SMT3 specific peptidase 2
F2R	ENSG00000181104.6	Coagulation factor II (thrombin) receptor
RAC1	ENSG00000136238.17	Ras-related C3 botulinum toxin substrate 1
FRAT1	ENSG00000165879.8	Frequently rearranged in advanced T-cell lymphomas 1
PRICKLE1	ENSG00000139174.10	Prickle homolog 1
DYNLT1	ENSG00000146425.10	Dynein, light chain, Tctex-type 1

serve as oncogenes in HCC. Next, we explored the key genes that interact with DVLs to determine the possible mechanism in HCC. The GeneMANIA website was used to predict the PPI network of DVLs, and the results revealed 20 critical interacting molecules (Figure 6, Table 2), including the DVL-associated activator of morphogenesis 1 (DAAM1), which showed the strongest interaction with the DVL family.

Subsequently, we carried out GO analysis, including biological process (BP), cellular component (CC), and molecular function (MF) analyses, as well as KEGG analysis, on DVLs and their interacting genes using the

TABLE 3 Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analyses

Category	Term	Count	Ratio (%)	Adjusted *p* value	Genes
BP	GO: 0016055 ~ Wnt signaling pathway	12	52.17	4.90E-14	DVL2, SENP2, DVL3, NKD1, NKD2, CSNK2A1, NXN, GSK3B, PPM1A, BRD7, AXIN1, DVL1
BP	GO: 1904886 ~ beta-catenin destruction complex disassembly	7	30.43	3.89E-11	DVL2, DVL3, GSK3B, FRAT1, FRAT2, AXIN1, DVL1
BP	GO: 0090090 ~ negative regulation of canonical Wnt signaling pathway	8	34.78	1.58E-07	DVL2, DVL3, NKD1, NKD2, PRICKLE1, GSK3B, AXIN1, DVL1
BP	GO:0060071 ~ Wnt signaling pathway, planar cell polarity pathway	7	30.43	1.76E-07	DVL2, DVL3, VANGL1, PRICKLE1, RAC1, DAAM1, DVL1
BP	GO: 0001934 ~ positive regulation of protein phosphorylation	7	30.43	9.90E-07	DVL2, SENP2, DVL3, RAC1, SIRT1, AXIN1, DVL1
CC	GO: 0016328 ~ lateral plasma membrane	6	26.09	3.91E-07	DVL2, NKD2, VANGL1, VANGL2, AXIN1, DVL1
CC	GO: 1990909 ~ Wnt signalosome	3	13.04	3.41E-03	DVL3, GSK3B, DVL1
CC	GO: 0031410 ~ cytoplasmic vesicle	5	21.74	3.69E-03	DVL2, SENP2, NKD2, AXIN1, DVL1
CC	GO: 0005829 ~ cytosol	13	56.52	4.05E-03	DVL2, DVL3, PPM1A, DAAM1, DVL1, CSNK2A1, PRICKLE1, NXN, GSK3B, RAC1, FRAT1, FRAT2, AXIN1
CC	GO: 0016023 ~ cytoplasmic, membrane-bounded vesicle	4	17.39	1.05E-02	NKD2, RAC1, AXIN1, DVL1
MF	GO: 0005515 ~ protein binding	20	86.96	9.22E-03	DVL2, DVL3, NKD1, NKD2, VANGL1, VANGL2, PPM1A, DYNLT1, DAAM1, SIRT1, DVL1, SENP2, CSNK2A1, PRICKLE1, GSK3B, RAC1, FRAT1, BRD7, AXIN1, F2R
MF	GO: 0008013 ~ beta-catenin binding	4	17.39	1.17E-02	DVL3, GSK3B, AXIN1, DVL1
MF	GO: 0048365 ~ Rac GTPase binding	3	13.04	1.88E-02	DVL2, DVL3, DVL1
MF	GO: 0019901 ~ protein kinase binding	5	21.74	2.26E-02	DVL2, GSK3B, RAC1, AXIN1, DVL1
MF	GO: 0005109 ~ frizzled binding	3	13.04	2.53E-02	DVL2, DVL3, DVL1
KEGG	hsa04310: Wnt signaling pathway	17	73.91	1.85E-23	DVL2, DVL3, NKD1, NKD2, VANGL1, VANGL2, DAAM1, DAAM2, DVL1, SENP2, CSNK2A1, PRICKLE1, GSK3B, RAC1, FRAT1, FRAT2, AXIN1
KEGG	hsa05217: Basal cell carcinoma	5	21.74	4.28E-04	DVL2, DVL3, GSK3B, AXIN1, DVL1
KEGG	hsa04550: Signaling pathways regulating pluripotency of stem cells	5	21.74	8.88E-03	DVL2, DVL3, GSK3B, AXIN1, DVL1
KEGG	hsa04390: Hippo signaling pathway	5	21.74	9.45E-03	DVL2, DVL3, GSK3B, AXIN1, DVL1
KEGG	hsa05200: Pathways in cancer	7	30.43	1.13E-02	DVL2, DVL3, GSK3B, RAC1, AXIN1, F2R, DVL1

DAVID platform. As shown in Table 3, the most critical genes were located in the cytosol, shared protein-binding functions and were enriched in Wnt pathway signaling. Taken together, GO and KEGG analyses revealed the potential molecular mechanisms of DVLs and their key interactions in HCC.

4 | DISCUSSION

With the deeper acknowledgment of HCC oncogenesis, increasing numbers of risk factors, including hepatitis virus and alcoholism, have been uncovered and controlled, contributing to the declining incidence of HCC in the past four decades (Siegel et al., 2019). Presently, due to the wide application of tumor markers such as AFP, early ultrasound and comprehensive treatment, the diagnosis and treatment of HCC have been improved to a certain extent. However, the overall prognosis of patients with HCC remains poor because of its high degree of malignancy, easy recurrence, and high invasiveness (Rich, Yopp, & Singal, 2017). Therefore, further exploration of abnormally expressed genes with potential clinical correlation is needed that will be beneficial to the individual diagnosis, treatment, and assessment of prognosis.

As downstream signal transduction molecules of various Wnt proteins, DVLs are involved in the regulation of multiple cellular behaviors, including cell proliferation, migration, and invasion. The classical mechanism in Wnt signal transduction in which DVLs participate is as follows: The Wnt signals specifically bind to the membrane Frizzled receptors (Fzds). Next, Fzds recruit DVLs and form complexes to regulate classical and nonclassical Wnt pathways by inhibiting the Axin-GSK3-APC pathway, upregulating the expression of β-catenin and activating the Wnt/PCP pathway, respectively (Gao & Chen, 2010; Sharma et al., 2018; Xu et al., 2018). A growing number of studies on the involvement of DVLs in the carcinogenesis and progression of malignant tumors have been reported. However, most studies have focused on the mechanism of regulating the malignant process of tumors in the Wnt signal pathway. For example, in breast cancer, DVL1 could accelerate tumor growth by regulating the Wnt/β-catenin pathway (Zeng et al., 2018). Similarly, Zhu et al. found that DVL2 could activate the DAAM1/RhoA pathway and mediate Wnt5a-induced migration and invasion of breast cancer cells (Zhu et al., 2012). In HCC, several studies have elucidated that the downregulated expression of DVL1 could sensitize HepG2 cells to the chemotherapeutic drug fluorouracil (Xu et al., 2018). However, systematic summarizations of the associations between DVL expression and the prognosis of patients with HCC have not been reported previously.

In the current study, we found that the expression levels of *DVL2* and *DVL3* mRNA were upregulated in HCC, and the overexpression of DVL1 and DVL3 was closely related to advanced clinical features and poor OS and DFS in patients with HCC. The findings of our study are consistent with expression analysis of DVL2 in HCC (Zhang et al., 2017). Furthermore, PPI network analysis revealed 20 key interaction molecules, and DAAM1 has the strongest interaction with DVLs. DAAM1 is the downstream regulatory molecule of DVL2 responding to Wnt signals. Several studies have uncovered the role of DAAM1 in cancers. DAAM1 was found to be overexpressed in breast cancer and promoted the invasion and migration of breast cancer, ovarian cancer, and glioma by recruiting and activating RhoA (G. Liu et al., 2018; Mei, Huang, et al., 2019; Mei, Xu, et al., 2019; Zhu et al., 2012). Additionally, GO and KEGG analyses revealed the potential molecular mechanism of DVLs and their key interacting molecules in cells, laying the foundations for future research.

In summary, our study systematically summarized the expression profiles and prognostic values of DVL family members in HCC. The high expression of *DVL1* and *DVL3* mRNA was related to the poor prognosis of patients with HCC. Therefore, detection of the expression levels of DVLs in HCC tissues might be used as a novel strategy to predict the prognosis of patients with HCC. However, our study also has some shortcomings. The expression data from the public database concerned gene expression at the transcriptional level, which may not fully reflect DVL protein levels or their activity at the phosphorylation level. Therefore, in the future, further basic research is needed to explore the exact molecular mechanism of its involvement in the oncogenesis and development of HCC.

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTION

CL and HW conceived the study and participated in the study design, performance, coordination and manuscript writing. JM, XY, DX, WZ, and GD carried out the assays and analysis. CL, HW, and JM wrote and revised the manuscript. All authors reviewed and approved the final manuscript. JM and XY contributed equally to this work.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the relative bioinformatics databases.

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