

## Overexpression of *DDx49* promotes cell proliferation and associated with poor prognosis in liver cancer: *In silico* analysis

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Liver hepatocellular carcinoma is a common cancer type with an increasing mortality rate. Identifying the potential biomarker will increase the accuracy of the diagnosis and prognosis. The aim of the study is to comprehend the *DDx49* association with the liver tumoring various bioinformatic tools. STRING and ShinyGO web-based tools were utilized to study the interacting partners and their associated pathways. The UALCAN database was used to study the expression of different demographic variables. GEPIA was performed to analyze the expression, correlation and survival of the patients. The results show that *DDx49* is up-regulated in liver cancers ( $P < 0.0001$ ). *DDx49* is a DEAD-box helicase that is involved in RNA metabolism and the ribosomal biogenesis pathway. The alteration in the *DDx49* expression may be associated with dysfunction of the pathway and promote proliferation. Additionally, the Spearman correlation revealed that *DDx49* is significantly correlated with the cell cycle and apoptotic genes. In TCGA datasets, *DDx49* is mostly altered by amplification in liver cancer patients. Survival analysis showed that overexpression of *DDx49* leads to a poor prognosis ( $P = 0.0038$ ) in hepatocellular carcinoma patients. In conclusion, this study suggests *DDx49* may act as a prognostic or/and diagnostic biomarker in a patient with liver cancer.

**Keywords:** Apoptosis, Biomarker, *DDx49*, Liver cancer, TCGA

Hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) are the primary types of liver cancers caused by metabolic dysfunctions, infections, heritability and lifestyle factors<sup>1</sup>. HCC is the sixth most common cancer type worldwide<sup>2</sup>. According to the WHO, mortality due to liver cancer will be one million in 2030<sup>3</sup>. The main manifestation of primary liver cancer is HCC (90%), which leads to poor prognosis and low survival<sup>4</sup>. CCA is a rare and deadly epithelial cell cancer that can develop anywhere in the biliary tree as well as in the hepatic parenchyma<sup>5</sup>. It is the second most frequent primary hepatic neoplasia, with an increased incidence in the past three decades<sup>6</sup>. CCA is commonly classified as intrahepatic, extrahepatic, or hilar tumors, depending on their location in the body<sup>7</sup>. Many types of epithelial cancers are grouped together by CCA and are indicated by a late diagnosis and poor prognosis<sup>8</sup>.

The RNA DEAD/H-box helicase superfamily contains 44 DEAD-box and 15 DEAH-box helicases. Alterations in the regulation of functionally distinct DEAD/H-box helicases may be unfavourable to cellular homeostasis and promote the development and progression of cancer<sup>9</sup>. The protein sequence contains conserved Asp-Glu-X-Asp motifs in the helicases. This RNA helicase is involved in RNA biogenesis, pre-mRNA slicing and regulation of the cell cycle<sup>10</sup>. DEAD-Box Helicase 49 (*DDx49*) plays a crucial role in the exportation of Poly(A)<sup>+</sup> mRNA and the regulation of translation. Overexpression of the *DDx49* protein affects apoptosis and proliferation in cancer cells<sup>11</sup>. Similarly, *DDx49* higher expression is associated with cell proliferation and poor survival in prostate cancer<sup>12</sup>. Therefore, identifying the role of this gene in liver cancer is crucial.

In this study, we analyzed the expression level of *DDx49* in liver hepatocellular carcinoma and cholangiocarcinoma using TCGA patient datasets. The protein-protein and gene-gene interactions and the pathways related to *DDx49* were analyzed. Further, the correlation between the apoptosis related

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genes and *DDx49*, as well as the prognosis of cancer patients were studied.

## Materials and Methods

### Gene expression analysis

GEPIA (<http://gepia.cancer-pku.cn/index.html>) and UALCAN (<https://ualcan.path.uab.edu/analysis.html>) are the online tools based on the TCGA dataset<sup>13,14</sup> and we evaluated the expression of *DDx49* with different clinical parameters (Suppl. Table 1). TCGA provides the TCGA-LIHC (liver hepatocellular carcinoma) dataset, which contains 50 normal and 371 tumor tissues. The TCGA-CHOL (cholangiocarcinoma) dataset contains 9 normal and 36 tumor tissues, which are explored on the GEPIA and UALCAN web-based tools. The parameter was set as Log<sub>2</sub>FC cutoff 1 and the p value cutoff as 0.01.

### Protein-protein interaction and functional enrichment analysis

The STRING (<https://string-db.org/>) database was used to predict protein-protein interaction between two groups and also to identify the proteins correlated with dead-box helicase to study the biological functions, KEGG pathway and gene expression<sup>15</sup>. The search was conducted using the protein name “*DDx49*” and the organism “*Homo sapiens*” with the default options and confidence set to 0.900. ShinyGO 0.77 (<http://bioinformatics.sdstate.edu/go/>) is a web-based tool and database to analyze and visualize the list of genes in the context of biological pathways, gene ontology, and other functional annotations<sup>16</sup>. GO and KEGG pathway gene enrichment were analyzed with a 0.05 FDR cutoff and default options. The top twenty GO biological function and top KEGG pathway terms were visualized and listed by “lollipop” plot.

### Genetic interaction analysis

GeneMANIA (<https://genemania.org>) is an online bioinformatic tool that provides physical interaction, co-expression, co-localization, genetic interactions, *etc*<sup>17</sup>. Using GeneMANIA, we identified the interacting genes with *DDx49*. The parameter was set as the organism “*Homo sapiens*”, the gene name “*DDx49*” and the automatically selected weighting method.

### Analysis of genetic alterations

cBioPortal (<https://www.cbioportal.org>) is an online database for cancer genomics<sup>18</sup>. For the genetic alteration analysis, we used cholangiocarcinoma (TCGA, Pan-Cancer Atlas) and liver hepatocellular carcinoma

(TCGA, Pan-Cancer Atlas) datasets containing 36 and 372 samples, respectively, with the default options.

### Correlation analysis with apoptosis genes

Using the GEPIA online tool, the correlation between *DDx49* expression and apoptosis-related genes in the TCGA-LIHC cohort (HCC) dataset (50 normal and 371 tumor tissues) and the TCGA-CHOL cohort (CCA) dataset (9 normal and 36 tumor tissues) was studied. The Pearson correlation coefficient method was performed and a p value less than 0.05 was considered statistical significance<sup>13</sup>.

### Survival analysis

The TCGA dataset was used to identify the overexpression of *DDx49* and its correlation with the patient’s prognosis in liver HCA (TCGA-LIHC) and CCA (TCGA-CHOL) using the Kaplan-Meier curve in the GEPIA online tool<sup>13</sup>. Expression was grouped by median value and the y axis plotted in months.

## Results

### Analysis of *DDx49* expression in liver cancers

The expression level of *DDx49* was evaluated in various transcriptome profiles using the GEPIA database. The two types of cancer tissue (liver HCA and CCA) and normal tissue were compared in the TCGA-LIHC and TCGA-CHOL cohorts. The results show the significantly up-regulated expression ( $P < 0.0001$ ) of *DDx49* in both cholangiocarcinoma and liver hepatocellular carcinoma patients (Fig. 1). Additionally, we evaluated the expression of *DDx49* with different clinical characteristics of the patients (age, tumor Stage, tumor grade, nodal metastasis status). In CCA, the upregulation was found in age of 41-60 and 81-100 year old patients. Similarly, the expression was gradually increasing with a higher tumor grade in liver HCA (Suppl. Fig. 1).

### Identification of *DDx49* interacting partners and functional enrichment analysis

The investigation in the string database showed the interactive proteins to the *DDx49* with the highest confidence of 0.900. The interactive genes are from the active interaction sources of co-expression, text-mining, databases, experiments, gene fusion, co-occurrence and neighborhood. The protein-protein interaction enrichment p-value cut-off was set at  $< 0.05$ . The study revealed that *DDx49* strongly interacts with the ABT1, UTP3 and NOL6 proteins. Additionally, it interacts with BYSL, DHX37, EMG1, WDR46, UTP25, DDX52 and PDCD11 proteins (Fig. 2A).

The GO and KEGG enrichment pathways of *DDx49* interacting proteins were analysed using the ShinyGo database. In GO enrichment analysis, top 20 functions were identified and the *DDx49* interacting partners were associated with rRNA processing, rRNA metabolic process, NcRNA processing, ribonucleoprotein complex biogenesis, NcRNA metabolic process, RNA processing, ribosomal small subunit biogenesis and maturation of SSU-rRNA processes (Fig. 2B). The KEGG enrichment pathway

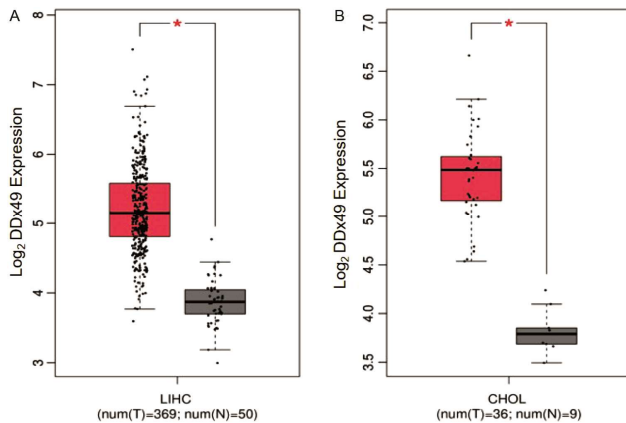


Fig. 1 — Box Plot shows relative *DDx49* gene expression. (A) Liver hepatocellular carcinoma (LIHC) and normal cohort; and (B) Cholangiocarcinoma and the normal cohort ( $P < 0.0001$ )

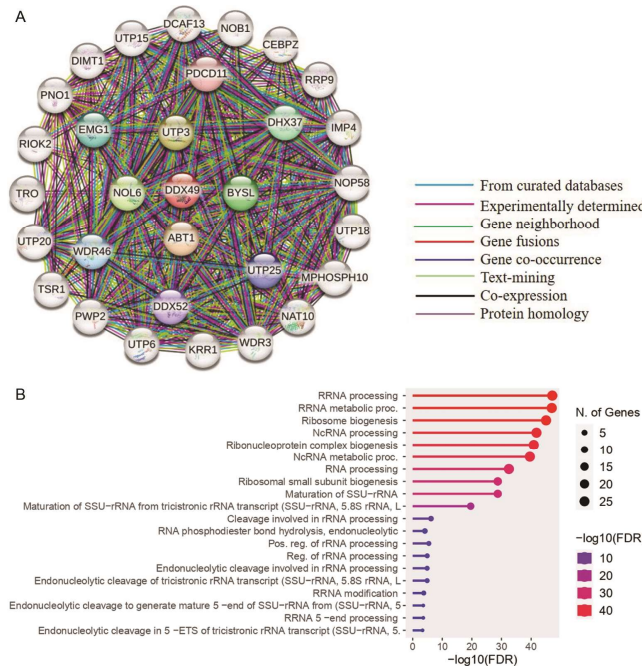


Fig. 2 — (A) Protein-Protein interaction network and DEGs of *DDx49* gene from STRING database; and (B) Lollipop plot for functional enrichment analysis of Differential Expressing Genes in GO Biological pathway

analysis revealed that *DDx49* is significantly associated with ribosome biogenesis in eukaryotes. The comprehensive data for the GO and KEGG pathways are given in (Suppl. Table 2).

**Genetic interaction analysis**

We analyzed the interaction of *DDx49* using GeneMANIA and 20 genes had significant interactions which are signal sequence receptor subunit 2, interleukin 13, cell division cycle 23, triggering receptors expressed on myeloid cells 2, large tumor suppressor kinase 1, NOTCH regulated ankyrin repeat protein, Wnt family member 8B, interferon induced transmembrane protein 1, family with sequence similarity 234 member B, keratin 79, myosin IC, growth arrest specific 2 like 2, EMI domain containing, retrotransposon Gag like 3, Euchromatic Histone Lysine Methyltransferase 2, Hydroxysteroid 17-Beta Dehydrogenase 8, Immediate Early Response 3, Olfactory Receptor Family 2 Subfamily B Member 3, Translocase of Inner Mitochondrial Membrane 22, Transmembrane Protein 179 (Fig. 3 & Suppl. Table 3).

**Analysis of genetic alterations in *DDx49***

Nine (2.4%) out of 372 patients possessed genetic alteration of *DDx49* in liver HCA of which 7 had amplifications, 1 deep deletion and 1 missense mutation (Fig. 4A). In CCA patients, amplification variation was found in 1 (2.8 %) out of 36 patients (Fig. 4B).

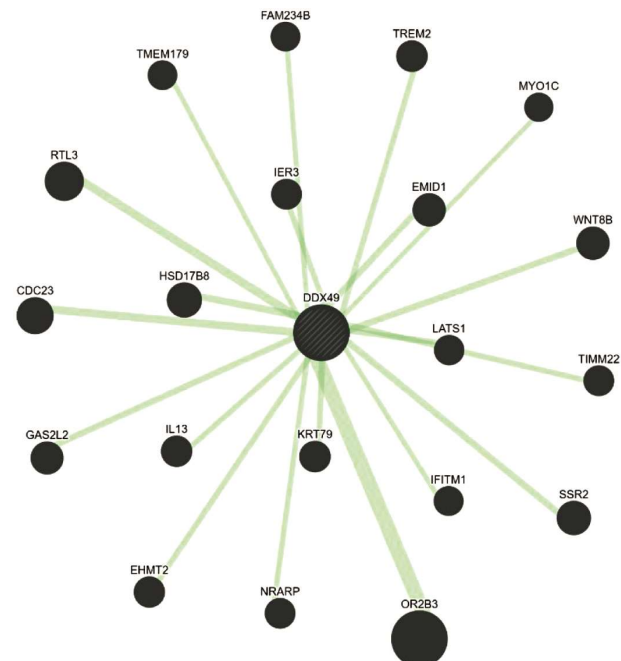


Fig. 3 — Gene-Gene Interaction of *DDx49* in GeneMania

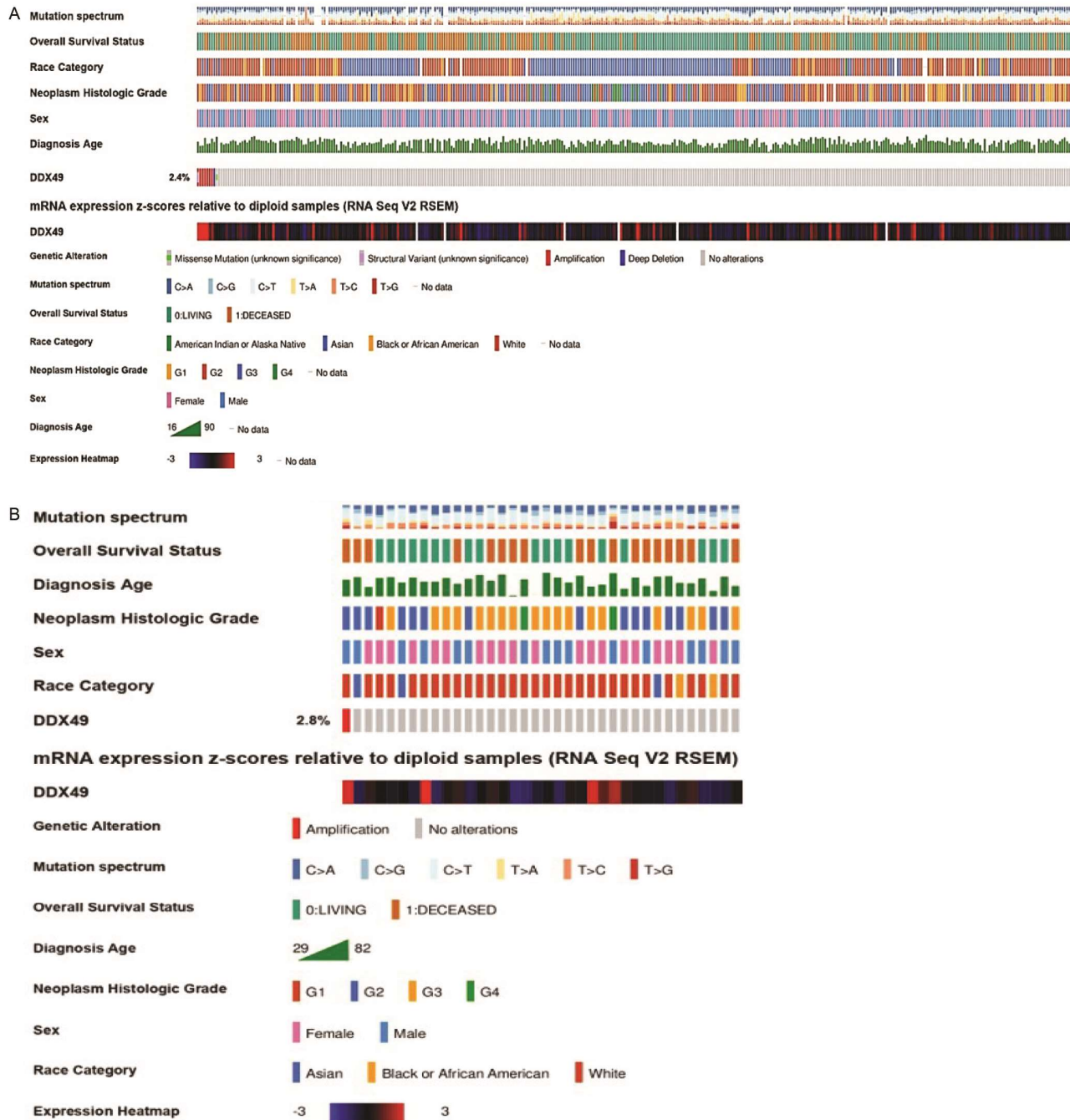


Fig. 4 — Genetic alteration of *DDx49* gene, characteristics of patients and gene expression in cBioPortal. (A) Liver Hepatocellular Carcinoma is 9 (2.4 %) of 372 patients; and (B) Cholangiocarcinoma is 1 (2.8 %) of 36 patients

**Correlation analysis of *DDx49* with apoptosis genes**

The correlation of *DDx49* mRNA expression with apoptosis-related genes was studied using the TCGA-LIHC and TCGA-CHOL cohorts in GEPIA. In liver HCA, mRNA expression showed significant correlation with HSPB1 ( $P < 0.001$ ,  $r=0.61$ ), TP53 ( $P = 0.0023$ ,  $r=0.15$ ), CASP3 ( $P < 0.001$ ,  $r=0.3$ ) and CASP7 ( $P = 0.028$ ,  $r=0.11$ ) (Fig. 5A). In CCA,

mRNA expression showed significant correlation with HSPB1 ( $P < 0.001$ ,  $r=0.69$ ), TP53 ( $P = 0.028$ ,  $r=0.33$ ), CASP3 ( $p=0.056$ ,  $r=0.29$ ) and BCL2 ( $P = 0.0024$ ,  $r=0.44$ ) (Fig. 5B).

**Survival analysis**

The Kaplan-Meier curve was used to study the prognostic significance in liver HCA and CCA patients.

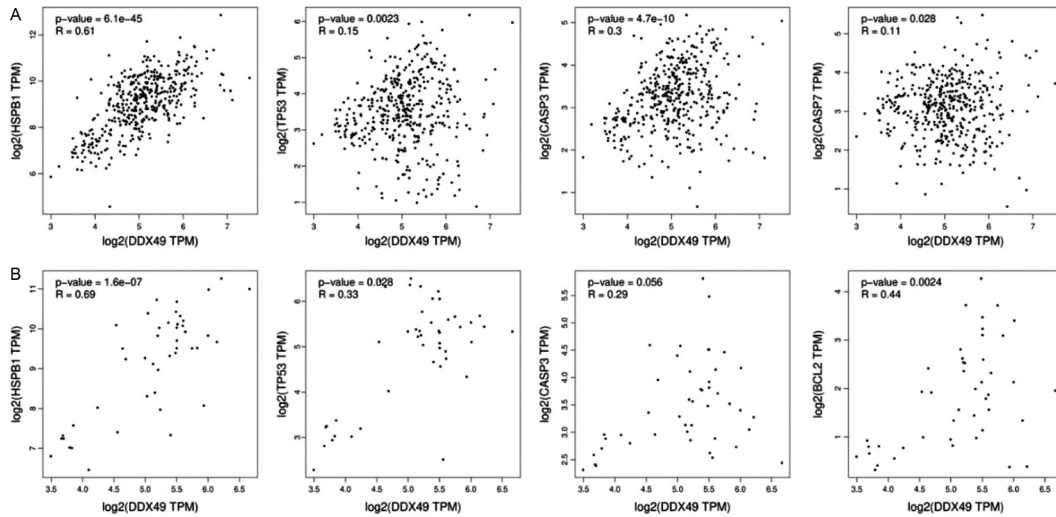


Fig. 5 — Correlation of mRNA expression of *DDX49* gene and apoptosis related genes. (A) correlation of *DDX49* expression between Liver hepatocellular carcinoma and HSPB1, P53, CASP3 and CASP7; and (B) correlation of *DDX49* expression between Cholangiocarcinoma and HSPB1, P53, CASP3 and BCL2

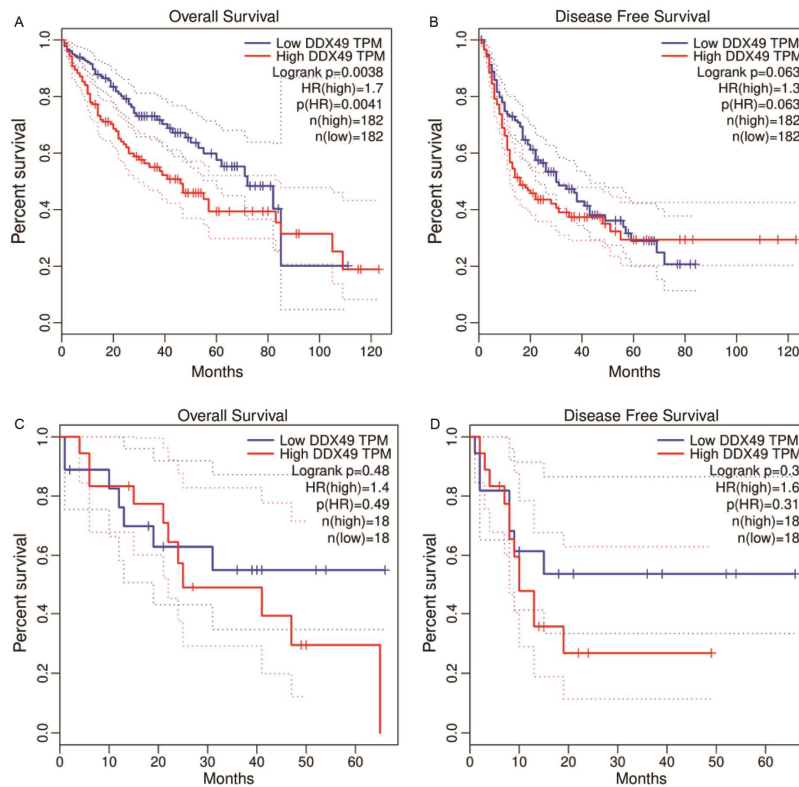


Fig. 6 — Kaplan-Meier survival curve of Disease-free survival and Overall survival based on GIPEA dataset. (A & B) are overall survival (OS) and disease-free survival (DFS) of Liver hepatocellular carcinoma (TCGA-LIHC) cohort, respectively; and (C & D) are overall survival (OS) and disease-free survival (DFS) of Cholangiocarcinoma (TCGA-CHOL) cohort, respectively

The K-M curve shows that the over expression of *DDX49* is significantly correlated with poor overall survival ( $P = 0.0038$ ,  $HR=1.7$ ) and poor disease-free survival ( $P = 0.063$ ,  $HR=1.3$ ) in liver

HCA patients (Fig. 6A & B). In the CCA patients as well, there is low overall survival with a hazard ratio of 1.4 ( $P = 0.48$ ) and low disease-free survival ( $P = 0.3$ ,  $HR=1.6$ ) (Fig. 6C & D). The hazard

ratio of more than one indicates the patients have a shorter survival.

### Discussion

Hepatocellular carcinoma (HCC) is a highly malignant tumor because of its poor prognosis and it is a threat to human society<sup>1</sup>. Identification of the potential biomarker for liver cancer is crucial for the patient's outcome.

In this study, the gene expression profiles of patients were found to carry liver HCA and CCA from the TCGA cohorts using the GEPIA online database. *DDx49* expression was significantly higher in both the TCGA-LIHC cohort and cholangiocarcinoma patients. Hence, overexpression of *DDX49* might be promoting tumor growth and metastasis and it has been shown that knocking it down inhibits these mechanisms in HCC tumors<sup>19</sup>. The pathways of *DDx49* overexpression in tumor environments have not been fully validated to date. Overexpression of *DDx49* expression may upregulate the MAPK pathway (phosphorylated) in hepatocellular carcinoma cells<sup>20</sup>.

The *DDx49* protein is closely associated with ABT1, UTP3, NOL6, BYSL, DHX37, EMG1, WDR46, UTP25, DDx52, PDCD11 and KRR1 proteins. Enrichment analysis of *DDx49* interacting proteins shows *DDx49* protein were highly associated with various RNA processing processes, including slicing, transport and messenger RNA delay. Alteration in the *DDx49* expression may lead to RNA processing dysregulation and drive tumor progression<sup>21</sup>. Moreover, *DDx49* interacts with cell cycle-associated genes, tumor suppressor genes, receptor proteins, transmembrane proteins and regulatory proteins. In liver HCA, about 2.4% and 2.8% of CCA patients had mutations in *DDx49* with a higher frequency of amplification. Which may increase the gene expression level in the tumor microenvironment. As in cancer, the generally accepted view that gene amplification is associated with increased expression is still a probable assumption but has not yet been conclusively demonstrated to be true<sup>22</sup>.

*DDx49* expression was significantly correlated with apoptotic related genes. *DDx49* expression was more significantly correlated with HSPB1, TP53, CASP3 and HSPB1, TP53 and BCL2 in the LIHC and CHOL cohorts, respectively. These genes have a crucial role in the regulation of the cell cycle, apoptosis, tumor suppression and anti-apoptosis<sup>23</sup>. Hence, overexpression of *DDx49* may be involved

in the alteration of these apoptosis-related gene expressions.

The K-M curve demonstrates the survival rate of the TCGA-LIHC and TCGA-CHOL datasets, which show significantly poor prognosis in liver hepatocellular carcinoma and cholangiocarcinoma. Up-regulated *DDx49* expression in liver hepatocellular carcinoma significantly contributes to a poor overall survival rate. According to these results, the expression level of *DDx49* may be sufficient to predict a patient's prognosis. KEGG pathway analysis reveals that *DDx49* is involved in the ribosomal biogenesis pathway and the alteration of the pathway leads to cell growth and proliferation in the cancer<sup>24</sup>. Therefore, overexpression of *DDx49* may lead to dysfunction of the ribosomal biogenesis pathway and promote proliferation in liver cancer. In conclusion, this study suggests that *DDx49* might be useful as a prognostic or/and diagnostic biomarker for liver cancer.

### Conclusion

This study identified the expression of *DDx49* in different liver cancer datasets using a bioinformatic approach. Variations in *DDx49* may influence alterations in apoptosis due to its association with the cell cycle and apoptotic genes. Overexpression of *DDx49* leads to poor overall survival among liver cancer patients. This investigation could further aid in the development of potential biomarkers to comprehend the in-depth mechanism between *DDx49* and liver cancer. Further studies can be used to validate this finding with more patient datasets and *in vitro* analysis.

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### Conflict of interest

The author declares no conflicts of interest.

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