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Bioinformatic Analysis of the Possible Mechanism of *TOP2A* Expression in the Development of Liver Cancer

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FY conceived and designed the study. MW analyzed the date and wrote the manuscript.

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ABSTRACT

The expression and significance of topoisomerase IIA in liver hepatocelluar carcinoma (LIHC) were analyzed to explore the possible mechanism of topoisomerase IIA in the development of liver cancer based on bioinformatics analysis. GEPIA, UALCAN, cbioportal and other tools were used to analyze the correlation between *TOP2A* gene expression and methylation, prognosis and immune cell infiltration in databases. The expressions of *TOP2A* gene and protein in tumor tissues were higher than those in normal tissues, and the differences were statistically significant (P<0.05); *TOP2A* gene expression was positively correlated with immune cells infiltration (P<0.05); The overall survival, disease-free survival and survival probability of patients with high expression of *TOP2A* gene were lower than those with low expression of *TOP2A* gene (P<0.05). In clinical specimens, the expression of *TOP2A* in cancer tissues were significantly higher than that in the normal tissues, and were related to Edmondson grade. To conclude, the methylation of *TOP2A* gene and the infiltration of tumor-associated immune cells may play an important role in the pathogenesis of liver cancer, and the expression of *TOP2A* gene can be used as a prognostic indicator of liver cancer.

INTRODUCTION

Liver cancer ranked sixth in morbidity and fourth in mortality in the world (Bray *et al*., 2018). The new cases of liver cancer in China accounted for more than half of the world (Shi *et al*., 2021). Liver cancer was characterized by strong heterogeneity and easy to relapse and metastasis. Although surgical treatment, individualized diagnosis and treatment have achieved certain results, liver cancer was still a major disease threatening life and health. Topoisomerase IIA (TOP2A) gene was located in 17q12-21 and consists of two subunits. It played an important role in DNA recombination and repair. Studies have shown that TOP2A plays an important role in the proliferation and invasion of tumor cells, such as breast cancer (Zhong *et al*., 2020), prostate cancer (de Resende *et al*., 2013), lung adenocarcinoma (Guo *et al*., 2020) and bladder cancer (Zeng *et al*., 2019), etc. In this study, the correlation between the expression of *TOP2A* gene and the prognosis of liver cancer was analyzed based on bioinformatics techniques, providing a theoretical basis for the early diagnosis and treatment of liver cancer and the identification of prognostic indicators.

MATERIALS AND METHODS

In this study GEDIA, TIMER, Ualcan, eBioportal and STRING were used to analyze the expression of *TOP2A* gene in liver cancer tissue and normal tissues. GEPIA is an online tool for analyzing gene expression and survival analysis in The Cancer Genome Atlas (TCGA) database. The Box Plot module in Expression DIY analyzed the expression of *TOP2A* gene in tumor tissue and normal tissue by Student's t-test. The correlation between *TOP2A* gene expression and clinical staging was analyzed by Box Plot module by Pearson correlation or spearman correlation. Survival Analysis module analyzed overall survival (OS) and disease-free survival (DFS) in the group with high and low expression of *TOP2A* gene by Kaplan-Meier Plotter.

Abbreviations
LIHC, liver hepatocelluar carcinoma; TOP2A, topoisomerase IIA; TCGA, The Cancer Genome Atlas; DSS, disease-specific survival; OS, overall survival; DFS, disease-free survival; PFI, progression-free interval.

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Ualcan is an effective online analysis and mining of cancer data based on the TCGA database. Select TCGA Analysis module, enter the gene name, and select LIHC to analyze the expression and methylation of *TOP2A* gene in tumor tissue and normal tissue by Student's t-test.

TIMER is a tool for online analysis of immune cell and tumor-associated fibroblast infiltration. TIMER analysis tool Gene module, input TOP2A and LIHC for immune cell infiltration analysis; Diff-exp module, input TOP2A and LIHC to analyze the gene expression in liver cancer tumor tissue and normal tissue.

The cBioportal network analysis tool was used to analyze *TOP2A* gene mutations. In the Query module, enter LIHC for Query, select the database containing LIHC, and enter TOP2A for mutation analysis.

Enter TOP2A for the protein name, and choose Organism for the Organism of *Homo sapiens*. Analyze the proteins that interact with TOP2A, construct the protein interaction map, and perform the enrichment analysis of gene function by GO and KEGG.

Western-blot was used to detect the expression of TOP2A protein in 53 cases of liver cancer and 15 cases of normal liver tissue. All the tissue samples were confirmed by pathology without radiotherapy and chemotherapy. In accordance with the Declaration of Helsinki, the present study was approved by the Ethics Committee of Taihe Hospital (Shiyan, China), and written informed consent was obtained from all patients or their families.

Statistical calculations were performed using SPSS software version 16.0, and $P<0.05$ was considered to indicate a statistically significant difference. The measured data were expressed as the mean ± standard deviation. A Student's t-test was compared using the two-sided data log-rank method.

RESULTS

The various bioinformatic tools used in this study showed that the expression level of TOP2A was significantly higher in tumor tissues than in normal tissues (Fig. 1A, B, C). The relationship between gene expression and clinical stage was also analyzed, showing that *TOP2A* gene expression increased with tumor progression of stage (Fig. 1D). Methylation analysis showed that the methylation level of *TOP2A* gene in tumor tissue was significantly lower than that in normal tissue (Fig. 1E).

A total of 1072 samples from 4 studies in the database were selected, 9 cases of mutations were detected, and the mutation rate was 0.84%. Including 2 cases of nonsense mutations at R450* and 2 cases of missense mutations at R877W*, as shown in Table I.

In this study, TIMER tool was used to analyze the correlation between *TOP2A* gene expression and tumor immune cells (B Cell, CD8+ T Cell, CD4+ T Cell, macrophage, neutrophil, dendritic cell). The expression of *TOP2A* gene was positively correlated with the infiltration of 6 kinds of immune cells. The K-M survival curve showed that the survival rate of patients with high levels of TOP2A was lower than that of patients with low levels (Fig. 2). At the same time, the multivariate Cox proportional risk model was constructed (Table II). The prognostic factors include expression of CD4_Tcell, Macrophage, Dendritic and TOP2A. Further analysis of the correlation between TOP2A enpression and the surface markers of immunoinfiltrating cells in liver cancer by TIMER and GEPIA, it was found that TOP2A was correlated with most of the surface markers of macrophage and dendritic (Fig. 3).

The relationship between the prognostic indicators (OS, DFS and survival rate) of patients with high and low *TOP2A* expression was analyzed by the bioinformatics tool. It was shown that the OS DFS and survival rate of patients with high gene expression were lower than those with low gene expression, and the difference was statistically significant (Fig. 4).

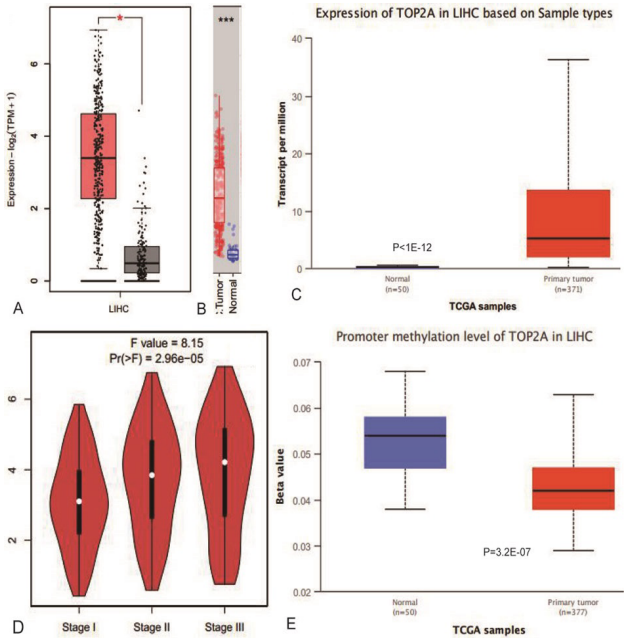

Fig. 1. The correlation between gene expression and clinical stages, gene methylation levels, and expression levels of *TOP2A* genes analyzed by different tools in tumor and normal tissues. A: GEPIA2; B: TIMER; C: Ualcan; D: The correlation between gene expression and clinical stages; E: methylation level.

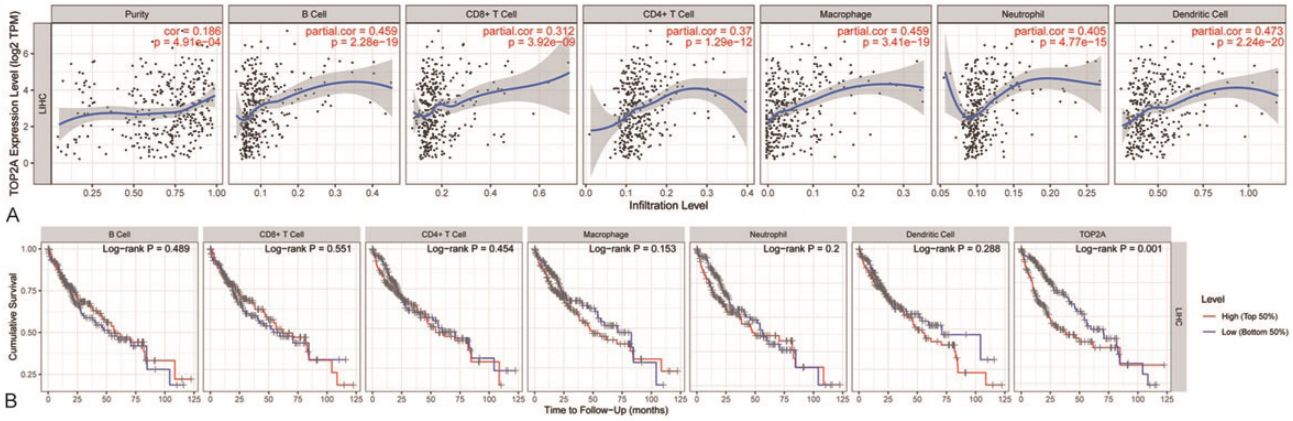

Fig. 2. Analysis of the correlation between immune cell infiltration and *TOP2A* expression (2A), the relationship between immune cell infiltration with different gene expression levels and prognosis (2B).

Table I. Mutation analysis of *TOP2A* gene in liver cancer.

Sample ID	Type	Protein change	Mutation type	Allele freq	Mut in sample
TCGA-WQ-A9G7-01	LIHC	R450*	Nonsense_Mutation	0.19	365
TCGA-WQ-A9G7-01	LIHC	R450*	Nonsense_Mutation	0.19	452
TCGA-DD-AACT-01	LIHC	R877W	Missense_Mutation	0.16	179
TCGA-DD-AACT-01	LIHC	R877W	Missense_Mutation	0.17	159
TCGA-G3-A3CG-01	LIHC	I70T	Missense_Mutation	0.05	305
TCGA-G3-A3CJ-01	LIHC	K1199Rfs*25	Frame_Shift_Del	0.08	1290
H060515	LIHC	N555H	Missense_Mutation	0.32	72
TCGA-2Y-A9HA-01	LIHC	T1315K	Missense_Mutation	0.38	140
TCGA-DD-A73E-01	LIHC	T689N	Missense_Mutation	0.54	118

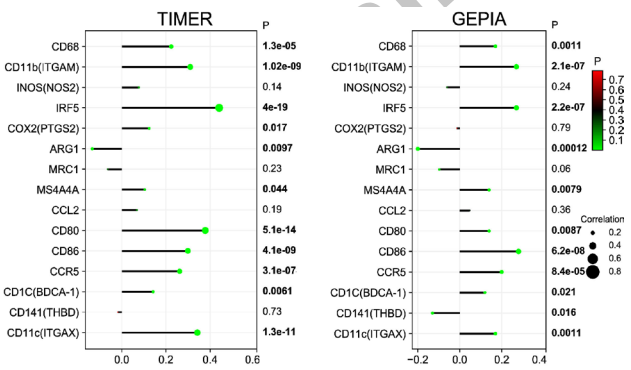

Fig. 3. TIMER and GEPIA analyzed the correlation between gene expression and surface markers of immune cell.

For univariate and multivariate analysis of prognosis in patients with liver cancer clinical data of liver cancer were downloaded from TCGT database, and the influencing factors of different prognostic indexes OS, DSS (disease-specific survival) and PFI (progression-free interval) of liver cancer patients were verified by Univariate and Multivariate analysis, as shown in Figure 5. TOP2A expression was a prognostic factors of HCC patients.

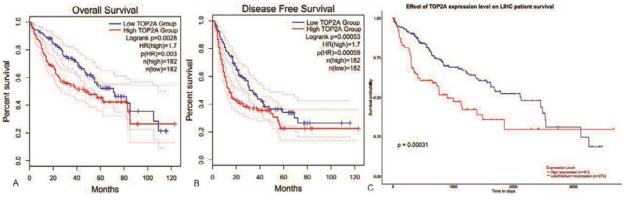

Fig. 4. Analysis of the correlation between *TOP2A* expression and clinical prognostic. A: OS; B: DFS; C: Survival probability.

The STRING database was used to analyze the experimentally verified proteins related to TOP2A, and the TOP2A protein interaction network was constructed and obtained, as shown in Figure 6. At the same time, the

possible functions and participating signal pathways are analyzed through enrichment, as shown in Table III.

Table II. Multivariate Cox proportional risk model for liver cancer.

	HR	95%CI_l	95%CI_u	P value	sig
Purity	1.51	0.46	4.92	0.49	
B_cell	0.001	0	1.015	0.05	
CD8_Tcell	0.009	0	1.406	0.068	
CD4_Tcell	0	0	0.484	0.031	*
Macrophage	5613.91	21.21	1486464.46	0.002	**
Neutrophil	0.007	0	798.86	0.401	
Dendritic	71.54	1.49	3430.30	0.031	*
TOP2A	1.23	1.04	1.46	0.014	*

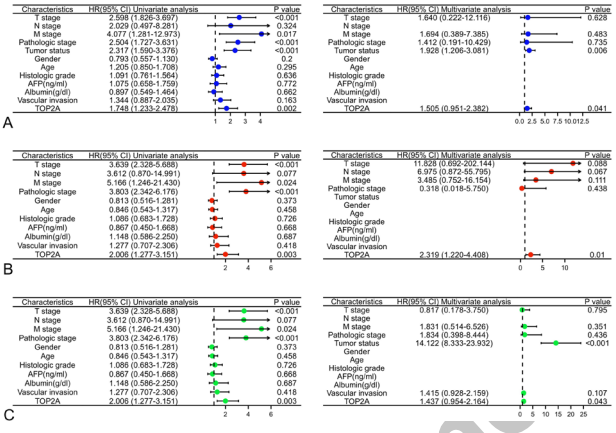

Fig. 5. Univariate and Multivariate analysis of prognosis in patients with liver cancer. A: OS; B: DSS; C: PFI.

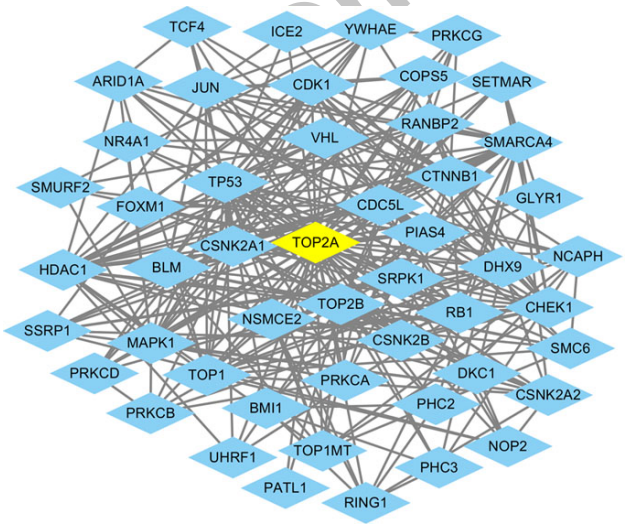

Fig. 6. Protein-protein Interaction network of TOP2A related proteins.

The expression of TOP2A in HCC tissues (0.54±0.25) was higher than that in normal tissues (0.18±0.12), and the difference was statistically significant (T =4.98, P<0.001). The expression of TOP2A increased with the increase of Edmondson grade, and was independent of the pathological factors such as tumor size, age, embolus and envelope, as shown in Figure 7 and Table IV.

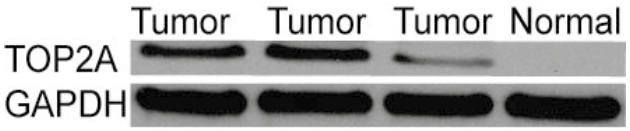

Fig. 7. Expression of TOP2A protein in liver cancer tissue and normal tissue.

DISCUSSION

Liver cancer is one of the most common malignant tumors, and its morbidity and mortality are on the rise in recent years (Anwanwan *et al*., 2020). As a topoisomerase, *TOP2A* mainly regulates DNA topological structure by participating in DNA division, replication and chromosome separation and concentration (Järvinen and Liu, 2003). More and more attention has been paid to the analysis of tumor pathogenesis and prognosis by bioinformatics technology. We used bioinformatics technology to analyze the value of *TOP2A* in the pathogenesis and prognosis of liver cancer in this study.

In this study, different tools were used to analyze the expression of *TOP2A* in liver cancer tissues, showing that *TOP2A* is highly expressed in tumor tissues, which was consistent with the expression in breast cancer (Nuncia-Cantarero *et al*., 2018), gastric cancer (Terashima *et al*., 2017), cervical cancer and other solid cancers (Wang *et al*., 2020), suggesting that the high expression of TOP2A may play a certain role in the pathogenesis of liver cancer. The methylation level of *TOP2A* promoter in liver cancer tissues was also predicted, which showed that the methylation level in liver cancer tissues was hypomethylated. In addition, we analyzed the *TOP2A* gene mutation in liver cancer tissues and found that the mutation rate was only 0.84%, indicating that *TOP2A* gene mutation may not be the main mechanism of liver cancer. This also indicated from another aspect that the high expression caused by the hypomethylation of *TOP2A* gene might be a potential pathogenic mechanism of liver cancer, and provided a theoretical basis for whether TOP2A could be an indicator for the early diagnosis of liver cancer.

The microenvironment of liver cancer is mainly composed of tumor-related immune cells, tumor-related fibroblasts and extracellular matrix. This microenvironment

Table III. Enrichment analysis of GO and KEGG.

Term ID	Category	term description	Strength	FDR	matching proteins in network
GO:0006265	BP	DNA topological change	2.23	7.17E-07	TOP1MT, TOP1, TOP2B, TOP2A
GO:0030263	BP	apoptotic chromosome condensation	2.19	0.0012	DFFB, TOP2A
GO:0040016	BP	embryonic cleavage	2.04	0.0018	TOP1, TOP2A
GO:0009330	CC	DNA topoisomerase complex (ATP-hydrolyzing)	2.58	0.0003	TOP1, TOP2A
GO:0003918	MF	DNA topoisomerase type II (double strand cut, ATP-hydrolyzing) activity	2.41	0.00032	TOP2B, TOP2A
GO:0003916	MF	DNA topoisomerase activity	2.34	3.54E-07	TOP1MT, TOP1, TOP2B, TOP2A
GO:0008094	MF	DNA-dependent ATPase activity	1.46	1.11E-05	BLM, DHX9, SMARCA4, TOP2B, TOP2A
hsa01524	KEGG	Platinum drug resistance	1.34	0.00019	MAPK1, TP53, TOP2B, TOP2A

Table IV. Association between *TOP2A* expression and clinicopathologic features of liver cancer.

	n	Expression	t	P
Age				
≥50y	30	0.58±0.29	1.1	0.31
<50y	23	0.52±0.27		
Size				
≥5cm	20	0.53±0.22	0.65	0.44
<5cm	33	0.57±0.27		
Edmondson				
I ~II	28	0.65±0.23	2.6	0.02
III~IV	25	0.45±0.26		
Envelope				
Yes	37	0.55±0.27	0.31	0.49
No	16	0.59±0.29		
Embolus				
Yes	19	0.52±0.25	0.64	0.41
No	34	0.58±0.26		

plays an important role in the occurrence, development and immune escape of liver cancer. In this study, tumor-related immune cell infiltration was found to be positively correlated with the expression of *TOP2A*, and a multivariate Cox proportional risk model was established to show that patients with high levels of TOP2A had a worse prognosis (OS, DFS, and survival rate were lower than those with low expression of *TOP2A*). The level of CD4+ in patients with HBV-infected was significantly higher than that in patients without HBV-infected in liver cancer (Li and Wang, 2016; Cui *et al*., 2013). In the pathogenesis of liver cancer, especially HBV-infected and HCV-infected, the induction and interaction of inflammatory cells and viruses lead to the complex microenvironment of liver cancer patients, which can induce immune escape of liver cancer, and then affect the therapeutic effect and prognosis (Zhu *et al*., 2019).

Not only immune cell infiltration affected the prognosis of patients with liver cancer, but also we found that the expression of *TOP2A* was closely related to the prognosis of patients with liver cancer through bioinformatics and clinical data downloaded from TCGA database for verification, especially patients with low expression of *TOP2A* have a better prognosis. However, the relationship between the expression of *TOP2A* and immune cell infiltration needs to be further verified by subsequent experiments.

Further through protein interaction and functional enrichment, it was found that the proteins related to TOP2A were mainly enriched in DNA topology changes, chromosome apoptotic condensation, oocyte meiosis, DNA topoisomerase inactivation, and DNA-dependent ATPase Inactivation and so on. These were related to the involvement of TOP2A in DNA division, recombination, repair, and chromosome separation and concentration to regulate the topological structure of DNA. In addition, studies have shown that high expression of *TOP2A* in pancreatic cancer can activate the Wnt-β-catenin pathway, induce epithelial-mesenchymal transition, and then participate in the invasion and distant metastasis of pancreatic cancer. This mechanism also existed in liver cancer needs further research (Pei *et al*., 2018).

In summary, we analyzed the role of *TOP2A* expression in the pathogenesis and prognosis of liver cancer through bioinformatics, and found that the high expression of *TOP2A* caused by hypomethylation may

be a potential mechanism of liver cancer, and this high expression was related to the prognosis of liver cancer. High gene expression and immune cell infiltration may be the main factors affecting the prognosis of patients.

Statement of conflict of interest

The authors have declared no conflict of interest.

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