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Topoisomerase Ⅱα Gene as a Marker for Prognostic Prediction of Hepatocellular Carcinoma: A Bioinformatics Analysis

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ABSTRACT

Objective To investigate the expression of *topoisomerase Ⅱα* (*TOP2α*) in hepatocellular carcinoma (HCC) and its role in predicting prognosis of HCC patients.

Methods We used HCC-related datasets in UALCAN, HCCDB, and cBioPortal databases to analyze the expression and mutation of *TOP2α* and its co-expressed genes in HCC tissues. GO function and KEGG pathway enrichment of *TOP2α* and its co-expressed genes were identified. The TIMER database was used to analyze infiltration levels of immune cells in HCC. The impacts of *TOP2α* and its co-expression genes and the infiltrated immune cells on the survival of HCC patients were assayed by *Kaplan-Meier* plotter analysis.

Results *TOP2α* and its co-expression genes were highly expressed in HCC ($P < 0.001$) and detrimental to overall survival of HCC patients ($P < 0.001$). *TOP2α* and its co-expression genes were mainly involved in cell mitosis and proliferation, and cell cycle pathway (ID: hsa04110, $P = 0.001945$). *TOP2α* and its co-expression genes were mutated in HCC and the mutations were significantly detrimental to overall survival ($P = 0.0247$) and disease-free survival ($P = 0.0265$) of HCC patients. High *TOP2α* expression was positively correlated with the infiltration of B cell ($r = 0.459$, $P < 0.01$), $CD8^+$ T cell ($r = 0.312$, $P < 0.01$), $CD4^+$ T cell ($r = 0.370$, $P < 0.01$), macrophage ($r = 0.459$, $P < 0.01$), neutrophil ($r = 0.405$, $P < 0.01$), and dendritic cell ($r = 0.473$, $P < 0.01$) in HCC. The $CD8^+$ T cell infiltration significantly prolonged the 3- and 5-year survival of HCC patients (all $P < 0.05$), and $CD4^+$ T cell infiltration significantly shortened the 3-, 5-, and 10-year survival of HCC patients (all $P < 0.05$)

Conclusion *TOP2α* may be an oncogene, which was associated with poor prognosis of HCC patients and could be used as a biomarker for the prognostic prediction of HCC.

Key words: *topoisomerase Ⅱα*; disease-free survival; overall survival; hepatocellular carcinoma; bioinformatics analysis

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of common malignant tumors of the liver and its main risk factors include chronic infection with hepatitis B virus, liver cirrhosis, alcoholic liver disease, mildew food and gene mutation[1]. Its 5-year survival rate and disease-free survival (DFS) are less than 40%[2]. China has become one of countries with high incidence and mortality rates of HCC[3]. Surgical treatment can improve the overall survival (OS) and prognosis in less than 30% HCC patients. Because of elevated postoperative complications and recurrence rates, it is difficult to

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accurately determine the prognosis of HCC[4]. Extensive researches have discovered a variety of HCC associated oncogenes[5] that may be associated with the prognosis of HCC patients.

Topoisomerase Ⅱ α (TOP2α) is a nuclear matrix protein of 170 kD, whose coding gene is located on chromosome 17q12-21, and functions in replication, transcription, and injury repair of DNA and regulates topological structure of DNA[6]. Its abnormal expression is considered to be related to tumor occurrence, recurrence, invasion, metastasis, and chemotherapy resistance[7]. Currently, *TOP2α* gene has been widely studied in breast cancer and ovarian cancer[8], but few studies in HCC are available. *TOP2α* has been identified as a key gene responsible for OS and prognosis of patients in early stage HCC by big data analysis[9], indicating the potential to predict OS and prognosis of HCC. Therefore, we carried out the bioinformatic study to investigate the expression and mutations of *TOP2α* gene in HCC and its role in predicting clinical prognosis of HCC patients.

MATERIALS AND METHODS

UALCAN database analysis

The UALCAN (http://ualcan.path.uab.edu/analysis.html) database is a portal website established based on the Cancer Genome Atlas (TCGA) database. We used UALCAN database to analyze the expression of the target genes in normal liver and HCC tissues, and its impact on OS of HCC patients.

HCCDB database analysis

The HCCDB (http://lifeome.net/database/hccdb/search.html) database was constructed based on 15 public liver cancer expression datasets and about 4,000 clinical samples from HCC patients. In addition, HCCDB combines TCGA with Genetype-Tissue expression (GTEx) data. The co-expression genes of *TOP2α* in HCC were analyzed using the HCCDB database. Spearman correlation analysis was used to assess the associations of *TOP2α* with the co-expression genes.

Gene Ontology and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis

The Enrichr (http://amp.pharm.mssm.edu/Enrichr/) database is a website dedicated to analyzing gene function and pathway enrichment. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment of *TOP2α* and its co-expression genes were analyzed using the Enrichr database.

cBioPortal database analysis

The cBioPortal (http://www.cbioportal.org) for Cancer Genomics is a repository for cancer genomics datasets. The mutation of *TOP2α* and its co-expression genes, and their effects on prognosis of HCC were analyzed using the HCC-related dataset [Liver Hepatocellular Carcinoma (TCGA, PanCancer Atlas)] in the cBioPortal database.

Kaplan-Meier plotter analysis

Kaplan-Meier plotter (http://kmplot.com/analysis/) is a tool that can be used to evaluate the correlation between the target gene and survival of patients with tumors, and its data comes from Gene Expression Omnibus (GEO), European Genome-Phenome Archive (EGA), and TCGA. We used Kaplan-Meier plotter to analyze the influence of *TOP2α* and co-expression genes on prognosis of HCC patients.

TIMER database analysis

The TIMER (https://cistrome.shinyapps.io/timer/) database was used to analyze immune cell infiltration in tumors and to estimate the abundance of six immune cells (B cells, $CD4^+$ T cells, $CD8^+$ T cells, neutrophils, macrophages, and dendritic cells).

Kaplan-Meier plotter was used to explore the relationship between patients' survival and the abundance of infiltrated immune cells.

RESULTS

Effect of high *TOP2α* expression of on OS of HCC

Compared to the normal live tissues, *TOP2α* gene was highly expressed in HCC tissues ($P < 0.001$, **Fig. 1A**). The high expression of *TOP2α* gene in HCC significantly reduced OS of HCC patients ($P = 0.00031$, **Fig. 1B**).

Genes co-expressed with *TOP2α* in HCC

HCCDB database analysis showed that a total of 19 genes, including *NUSAP1*, *PRC1*, *MELK*, *KIFC1*, *CCNB2*, *HJURP*, *TPX2*, *KIF4A*, *KIF20A*, *TTK*, *DLGAP5*, *KIF18B*, *BUB1B*, *CKAP2L*, *NCAPG*, *BUB1*, *ANLN*, *KIF11*, and *GINS1*, were significantly co-expressed with *TOP2α* in HCC (**Fig. 2**). Spearman correlation analysis showed a strong association between *TOP2α* gene and its co-expression genes (all $P < 0.001$, **Table 1**).

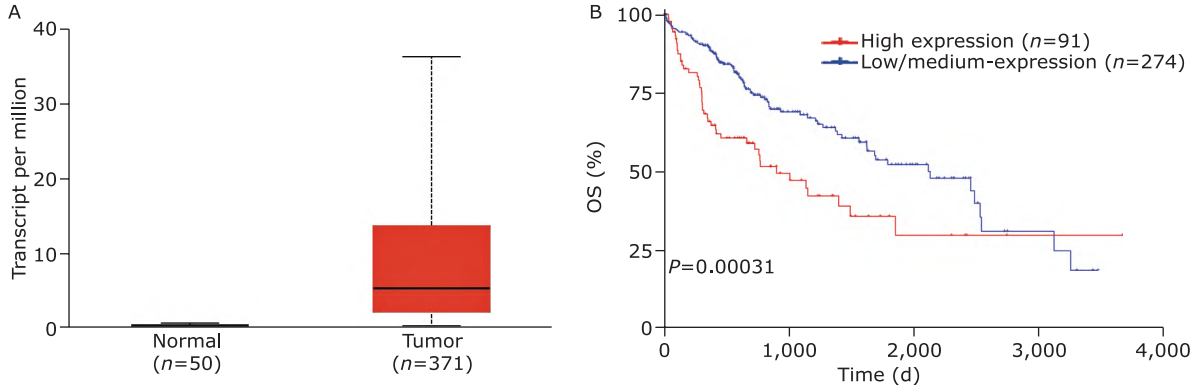

Figure 1. Expression of *TOP2α* in HCC and its effect on survival of HCC patients.
(A) Comparison of expression of *TOP2α* gene in HCC and normal liver tissues based on the Cancer Genome Atlas (TCGA) database ($P < 0.001$). (B) Effect of *TOP2α* high expression on OS of HCC patients.
TOP2α: *topoisomerase Ⅱ α*; HCC: hepatocellular carcinoma; OS: overall survival.

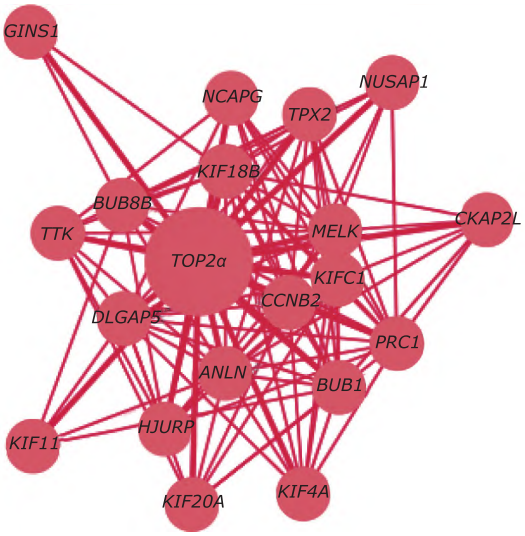

Figure 2. Schematic diagram of the genes co-expressed with *TOP2α* gene in HCC.

Table 1. Correlation between *TOP2α* and co-expression genes in HCC

Co-expression genes	*TOP2α* coeffcient		
	Log_2OR	*P* value	Spearman correlation
KIF18B	>3	<0.001	0.95
BUB1B	>3	<0.001	0.94
CKAP2L	>3	<0.001	0.93
ANLN	>3	<0.001	0.93
TPX2	>3	<0.001	0.93
BUB1	>3	<0.001	0.92
PRC1	>3	<0.001	0.92
NUSAP1	>3	<0.001	0.92
KIF11	>3	<0.001	0.92
GINS1	>3	<0.001	0.91
HJURP	>3	<0.001	0.91
MELK	>3	<0.001	0.90
TTK	>3	<0.001	0.90
DLGAP5	>3	<0.001	0.90
KIFC1	>3	<0.001	0.90
KIF4A	>3	<0.001	0.90
NCAPG	>3	<0.001	0.90
KIF20A	>3	<0.001	0.90
CCNB2	>3	<0.001	0.87

OR: odds ratio.

Functional annotation and KEGG pathway enrichment of co-expression genes

Functional enrichment analyses revealed that *TOP2α* and the co-expression genes were mainly related to biological functions of mitotic spindle organization, mitotic sister chromatid segregation, spindle, microtubule cytoskeleton, microtubule motor activity, and motor activity (**Table 2**). KEGG analysis revealed that *TOP2α* and the co-expression genes were enriched in cell cycle pathway (ID: hsa04110, $P = 0.001945$, **Table 2**).

Associations of mutations of *TOP2α* and its co-expression genes with prognosis of HCC patients

The various mutations, such as amplification, mRNA high, and deep deletion etc., were found in *TOP2α* gene and its co-expression genes of HCC (**Fig. 3**). Gene mutations in *TOP2α* gene and its co-expression genes significantly reduced the OS ($P = 0.0247$) and DFS of HCC patients ($P = 0.0265$, **Fig. 4**).

Association of *the* co-expression genes with OS of HCC

The 19 co-expression genes were highly expressed in

Table 2. GO and KEGG pathway enrichment of *TOP2α* and the co-expression genes

Category	Biological function	GO term ID	Adjusted *P* value
Biological process	Mitotic spindle organization	GO:0007052	4.30E-07
	Mitotic sister chromatid segregation	GO:0000070	4.01E-07
	Mitotic nuclear division	GO:0140014	1.63E-05
	Sister chromatid segregation	GO:0000819	3.72E-05
	Microtubule cytoskeleton organization involved in mitosis	GO:1902850	1.08E-04
	Mitotic spindle assembly	GO:0090307	1.07E-04
	Mitotic cytokinesis	GO:0000281	2.49E-04
	Mitotic spindle assembly checkpoint	GO:0007094	3.68E-04
	Mitotic spindle checkpoint	GO:0071174	3.27E-04
	Spindle assembly checkpoint	GO:0071173	2.94E-04
	Cytoskeleton-dependent cytokinesis	GO:0061640	3.14E-04
	Chromosome condensation	GO:0030261	6.36E-04
	Centromeric sister chromatid cohesion	GO:0070601	0.005580
Cellular component	Spindle	GO:0005819	3.04E-13
	Microtubule cytoskeleton	GO:0015630	2.39E-10
	Mitotic spindle	GO:0072686	2.71E-08
	Microtubule	GO:0005874	1.01E-07
	Kinesin complex	GO:0005871	1.18E-07
	Spindle microtubule	GO:0005876	1.21E-05
	Condensed chromosome kinetochore	GO:0000777	5.23E-05
	Polymeric cytoskeletal fiber	GO:0099513	1.22E-04
	Condensed chromosome, centromeric region	GO:0000779	1.22E-04
	Condensed nuclear chromosome kinetochore	GO:0000778	0.002317
	Condensed nuclear chromosome, centromeric region	GO:0000780	0.002527
	Microtubule-organizing center	GO:0005815	0.004354
	Spindle pole	GO:0000922	0.005601
Molecular function	Microtubule motor activity	GO:0003777	3.85E-06
	Motor activity	GO:0003774	1.11E-05
	Microtubule-binding	GO:0008017	1.09E-05
	Tubulin-binding	GO:0015631	3.98E-05
	ATPase activity	GO:0016887	3.31E-04
	ATP-dependent microtubule motor activity, plus-end-directed	GO:0008574	7.99E-04
	ATP-dependent microtubule motor activity	GO:1990939	0.00251
	Nucleoside-triphosphatase activity	GO:0017111	0.00725
KEGG	Cell cycle pathway	hsa04110	0.001945

GO: Gene Ontology; KEGG: Kyoto Encyclopedia of Genes and Genomes.

HCC compared to the normal tissues (all $P < 0.001$, **Table 2**). Kaplan-Meier survival analysis showed that the 19 co-expression genes in HCC tissues were significantly associated with poor OS in HCC patients (all $P < 0.001$, **Table 3**).

Correlation of *TOP2α* with infiltration levels of immune cells in HCC

High amplication of *TOP2α* gene significantly reduced the infiltration levels of all types of immune cells in HCC compared with other mutation types (all $P < 0.01$, **Fig. 5**). *TOP2α* gene expression level was positively correlated with the purity of tumor cell ($r = 0.186$, $P < 0.01$) and the infiltration levels of various immune cells such as B cell ($r = 0.459$, $P < 0.01$), $CD8^+$ T cell ($r = 0.312$, $P < 0.01$), $CD4^+$ T cell ($r = 0.370$, $P < 0.01$), macrophage ($r = 0.459$, $P < 0.01$), neutrophil ($r = 0.405$, $P < 0.01$), and dendritic cell ($r = 0.473$, $P < 0.01$) in

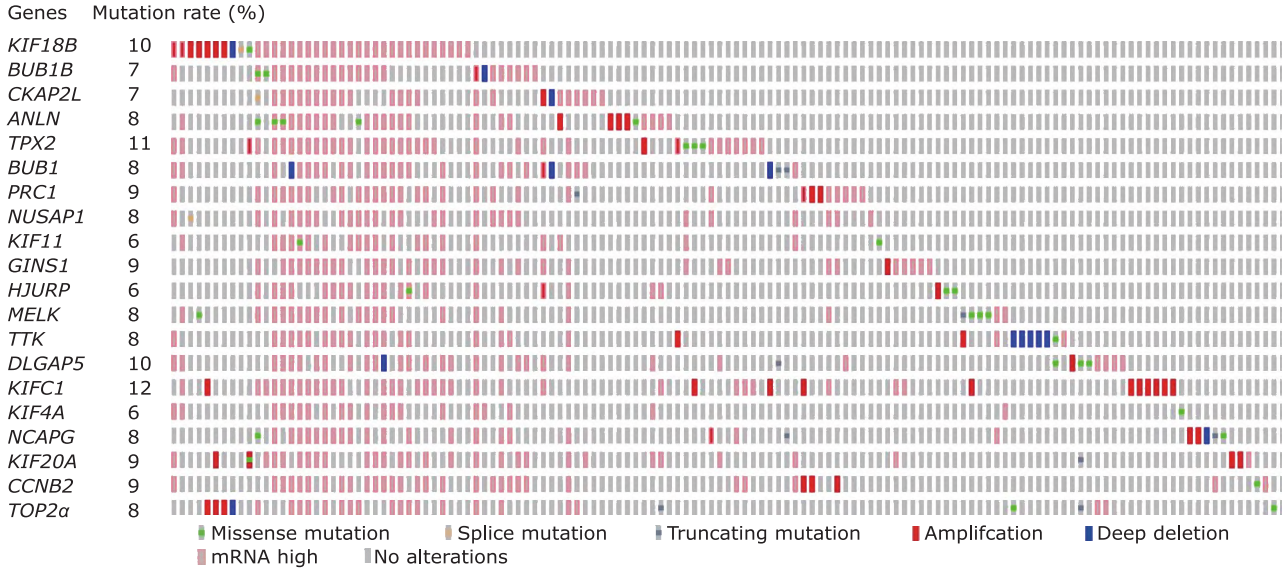

Figure 3. Genetic mutations occurred in *TOP2α* and 19 co-expression genes analysed with the cBioPortal database.

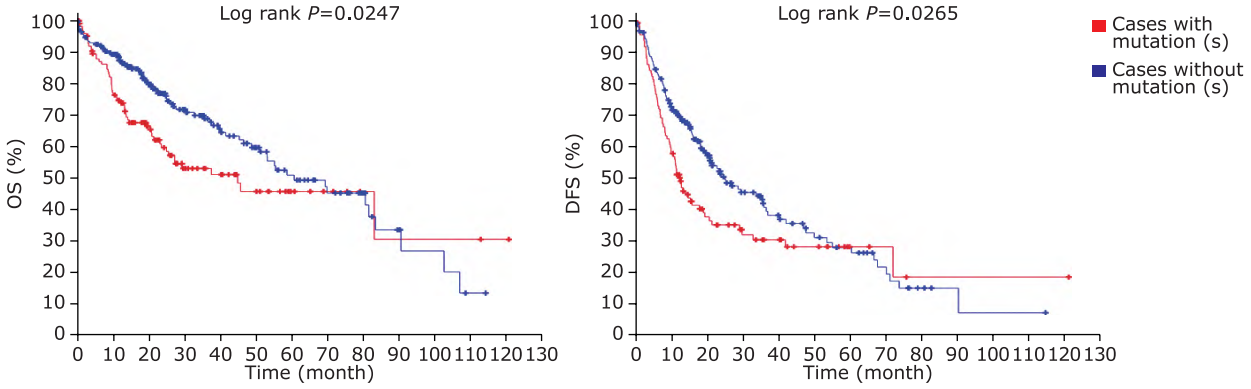

Figure 4. Effect of mutations of *TOP2α* and the co-expression genes on the OS (A) and DFS (B) of HCC patients analyzed with Kaplan-Meier potter.
DFS: disease free survival.

the HCC immune microenvironment (**Fig. 6**).

Effects of levels of *TOP2α* expression and infiltrated immune cells in HCC on prognosis of HCC patients

The high expression of *TOP2α* gene was associated with poor 1-year (P = 0.021), 3-year (P < 0.001), 5-year (P < 0.001), and 10-year (P < 0.001) OS of HCC patients (**Fig. 7**). However, high infiltration of $CD8^+$ T cell significantly prolonged 3-year (P = 0.047) and 5-year (P = 0.034) survival of HCC patients (**Fig. 7**), which was beneficial to OS of HCC patients. The high infiltration of $CD4^+$ T cell significantly shortened 3-year (P = 0.029), 5-year (P = 0.005), and 10-year (P = 0.019) survival of HCC patients (**Fig. 7**), which was unfavorable for OS of HCC patients.

DISCUSSION

The prognosis of HCC has been proved to be associated with a variety of genetic factors such as oncogenes[10,11]. Late diagnosis makes it difficult to accurately predict the survival and prognosis of HCC patients[12]. Therefore, it is imperative to find the potential biomarkers among numerous oncogenes for efficient and early diagnosis and treatment, and determining the survival and prognosis of HCC patients. The combination of genomics and biological information is a new strategy to investigate tumor oncogenes. Through giant data analysis of tumor cells, the expression, mutation, co-expression networks of the key oncogenes in tumors, and the their relationships with survival and

Table 3. Comparisons of expression of the 19 *TOP2α* co-expression genes between HCC tissues and normal liver tissues and their associations with OS of HCC

Genes	Expression level			OS		
	Normal liver	HCC	*P* value	*HR*	95%*CI*	*P* value
ANLN	Low	High	<0.001	2.38	1.68-3.38	5.90E-07
BUB1	Low	High	<0.001	2.11	1.49-2.99	1.60E-05
BUB1B	Low	High	<0.001	2.10	1.46-3.03	4.30E-05
CCNB2	Low	High	< 0.001	1.89	1.31-2.71	0.00048
CKAP2L	Low	High	<0.001	2.03	1.43-2.88	4.70E-05
DLGAP5	Low	High	<0.001	2.28	1.61-3.22	1.50E-06
GINS1	Low	High	<0.001	2.36	1.64-2.37	1.50E-06
HJURP	Low	High	<0.001	2.09	1.48-2.95	2.00E-05
KIF11	Low	High	<0.001	2.35	1.64-3.36	1.70E-06
KIF18B	Low	High	<0.001	2.20	1.54-3.16	1.10E-05
KIF20A	Low	High	<0.001	2.46	1.72-3.51	3.30E-07
KIF4A	Low	High	<0.001	2.17	1.52-3.10	1.30E-05
KIFC1	Low	High	<0.001	2.53	1.78-3.58	7.10E-08
MELK	Low	High	<0.001	2.38	1.68-3.37	4.90E-07
NCAPG	Low	High	<0.001	2.20	1.53-3.17	1.20E-05
NUSAP1	Low	High	<0.001	1.77	1.25-2.50	0.0012
PRC1	Low	High	<0.001	1.91	1.35-2.70	0.00021
TPX2	Low	High	<0.001	2.32	1.64-3.27	9.30E-07
TTK	Low	High	<0.001	2.82	1.96-4.07	6.50E-09

HR: hazard ratio; 95%*CI*: confidence interval.

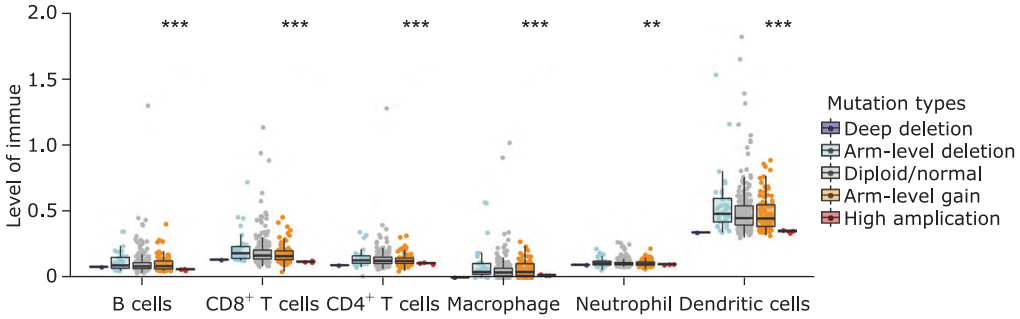

Figure 5. Effect of mutation type on infiltration of immune cells in HCC.
$^{**}P < 0.01$, $^{***}P < 0.001$, TIMER database analysis showed when HCC patients with high amplication of *TOP2α*, the infiltration level of immune cells in the HCC immune microenvironment was significantly lower than that of patients carrying other mutations.

prognosis of patients can be analyzed.

By using bioinformatics analysis, we found that *TOP2α* gene was significantly over-expressed in HCC, and was related to the poor OS of HCC patients. This result is consistent with Zhou *et al*'s findings[13]. Besides, we found there were various mutations in *TOP2α* and the co-expression genes. These mutations were associated with poor OS and DFS of HCC patients. In breast cancers high expression of *TOP2α* gene has been revealed to be negatively correlated with the survival and prognosis of patients, however, gene mutations of *TOP2α* significantly improve the sensitivity of breast cancer to tetracycline chemotherapeutic drugs[14], which makes breast cancer patients carrying *TOP2α* mutations have a higher pathological complete remission, thus prolonging their OS and DFS time[15].

It has been confirmed that the cell cycle regulation associated genes may contribute to tumorigenesis,

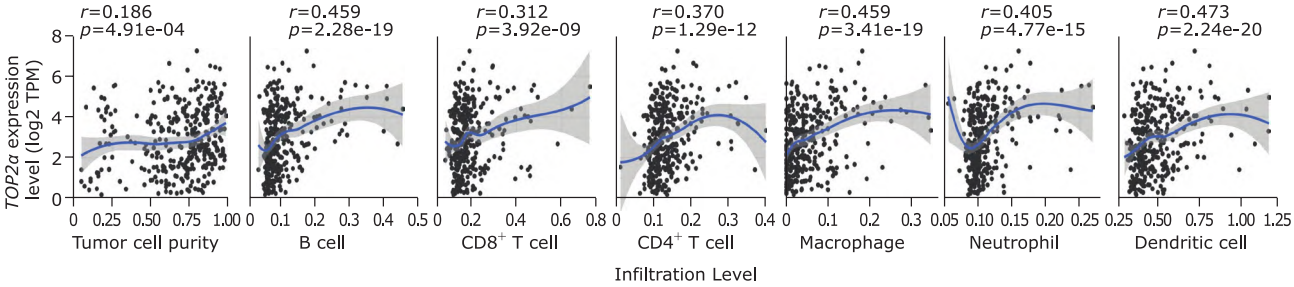

Figure 6. Correlations of *TOP2α* gene expression level with tumor cell purity and infiltration levels of immune cells in HCC immune microenvironment.

TPM: transcripts per kilobase million.

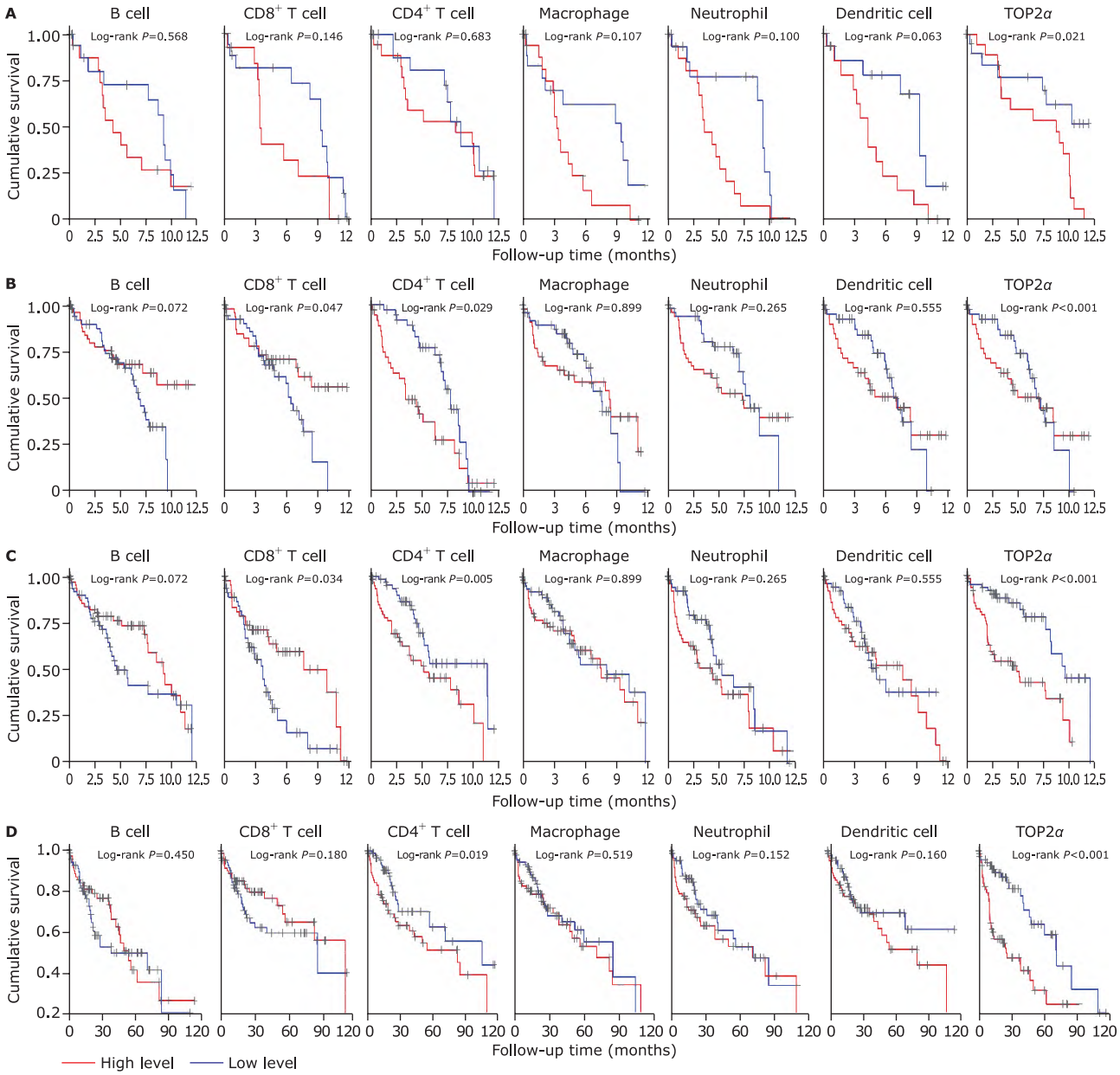

Figure 7. Effects of high expression level of *TOP2α* and infiltration level of immune cells on OS of HCC patients analyed by Kaplan-Meier potter.

(A) 1-year OS curve; (B) 3-year OS curve; (C) 5-year OS curve; (D) 10-year OS curve.

development, and metastasis[16,17]. Our study showed that *TOP2α* and its co-expression genes in HCC was mainly enriched in cell proliferation and mitosis, and cell cycle pathways. Additional, the mutated *TOP2α* and co-expression genes in HCC can affect the prognosis of patients. This was possibly due to mutations occurred in *TOP2α* and its co-expression genes which lead to disruption of cellular chromosomal stability to the extent that tumor cells proliferate abnormally, resulting in a poorer survival prognosis[18]. Therefore, we considered *TOP2α* gene may mediate essential functional activities such as tumor cell proliferation and be involved in the development and progression of HCC.

Moreover, we found that high expression of *TOP2α* gene was positively correlated with the purity of tumor cells and the infiltration of immune cells in the tumor immune microenvironment. This results indicated that HCC activated the immune system, which in turn generated the corresponding anti-tumor immune response. However, this anti-tumor effect produced by the immune cells only benefits the OS of HCC patients in the short term, while elevated infiltration of $CD4^+$ T cell indicates poor OS in the medium-and long-term. This phenomen may be associated with the fact that $CD4^+$ T cells as helper T cells, produce lymphocyte factor, enhance the function of $CD8^+$ T cells in killing tumor cells, and activate auxiliary cellular immunity and humoral immune response. $CD8^+$ T cells is inhibitory and cytotoxic T cells, and important effector cells[19]. The balance between $CD4^+$ T cells and $CD8^+$ T cells maintains the normal immune response. When the two types of cells are out of proportion, the immune function would be damaged[20,21], which ultimately affects the immune response in HCC patients.

In conclusion, *TOP2α* and the co-expression genes played an important role in the cell cycle of HCC, and the high expression of these genes was associated with poor OS of HCC patients. Besides, the high infiltration of $CD4^+$ T cells in HCC can be used to predict the OS of HCC patients with high *TOP2α* expression. These findings, however, require to be further verified through clinical studies to clarify the underlying molecular mechanisms involved in OS of HCC.

Conflict of interest

The authors declare no conflict of interest.

Authors' contributions

Lu J wrote the manuscript. Zhang HX and Yu P designed the study. An SG and Ma JJ made the diagrams. Yang Y, Zhang L and Chen YF researched literatures. Tao H analyzed experimental results. All authors approved the final version of the manuscript.

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