DNA topoisomerase 1 and 2A function as oncogenes in liver cancer and may be direct targets of nitidine chloride

LI-MIN LIU[1*], DAN-DAN XIONG[2*], PENG LIN[3], HONG YANG[3], YI-WU DANG[2] and GANG CHEN[2]

[1]Department of Toxicology, College of Pharmacy, Guangxi Medical University; [2]Department of Pathology; [3]Ultrasonics Division, Radiology Department, The First Affiliated Hospital of Guangxi Medical University, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China

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Abstract. The aim of the present study was to determine the role of topoisomerase 1 (TOP1) and topoisomerase 2A (TOP2A) in liver cancer (LC), and to investigate the inhibitory effect of nitidine chloride (NC) on these two topoisomerases. Immunohistochemistry (IHC) staining and microarray or RNA sequencing data mining showed markedly higher expression of TOP1 and TOP2A at the protein and mRNA levels in LC tissues compared with that in control non-tumor tissues. The prognostic values of TOP1 and TOP2A expression were also estimated based on data from The Cancer Genome Atlas. The elevated expression levels of TOP1 and TOP2A were closely associated with poorer overall survival and disease-free survival rates. When patients with LC were divided into high- and low-risk groups according to their prognostic index, TOP1 and TOP2A were highly expressed in the high-risk group. Bioinformatics analyses conducted on the co-expressed genes of TOP1 and TOP2A revealed that the topoisomerases were involved in several key cancer-related pathways, including the 'p53 pathway', 'pathway in cancer' and 'apoptosis signaling pathway'. Reverse transcription-quantitative polymerase chain reaction and IHC performed on triplicate tumor tissue samples from LC xenografts in control or NC-treated nude mice showed that NC treatment markedly reduced the protein and mRNA expression of TOP1 and TOP2A in LC tissues. Molecular docking studies further confirmed the direct binding of NC to TOP1 and TOP2A. In conclusion, the present findings indicate that TOP1 and TOP2A are oncogenes in LC and could serve as potential biomarkers for the prediction of the prognosis of patients with LC and for identification of high-risk cases, thereby optimizing individual treatment management. More importantly, the findings support TOP1 and TOP2A as potential drug targets of NC for the treatment of LC.

Correspondence to: Mr. Yi-Wu Dang or Professor Gang Chen, Department of Pathology, The First Affiliated Hospital of Guangxi Medical University, 6 Shuangyong Road, Nanning, Guangxi Zhuang Autonomous Region 530021, P.R. China
E-mail: dangyiwu@126.com
E-mail: chengang@gxmu.edu.cn

[*]Contributed equally

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Introduction

Liver cancer (LC) is the second main cause of cancer-associated mortality threatening global public health, with hepatocellular carcinoma (HCC) being the main histopathological subtype (1,2). In the United States, the American Cancer Society projects that >30,000 patients will succumb to LC in 2018 (3). Currently, patients with unresectable advanced LC are treated with chemotherapy as the main therapeutic strategy (4,5), and antitumor drug research has made considerable advancements in recent years. However, due to drug resistance, conventional pharmacotherapy often fails to produce satisfactory results for patients with LC (6-8). In addition, the majority of the chemotherapeutic agents currently available for LC treatment are highly cytotoxic and have adverse side effects (9,10). Thus, novel low-toxicity drugs that can provide a high response rate for LC treatment are urgently required.

One class of potentially promising drug targets for LC is the topoisomerases, which are important ribozymes that serve key roles in cell growth by breaking and reconnecting DNA strands to alter DNA topology (11,12). Two topoisomerases are recognized: Topoisomerase 1 (TOP1) and topoisomerase 2 (TOP2); TOP2A is the main isoform of TOP2. TOP1 and TOP2A are tumor drivers in a myriad of malignant tumors (13-15), making them attractive and effective targets for the development of antitumor medicines (16,17). Numerous TOP inhibitors, including etoposide, Adriamycin and camptothecin, are now widely used in the clinical setting (18-20). However, the conventional TOP inhibitors have severe side effects that offset their antitumor potential, necessitating a search for novel TOP inhibitors with fewer side effects.

Natural products are receiving increasing attention as antitumor drugs due to their low tissue toxicity and extensive biological activities. One such product, nitidine chloride (NC), a major active compound of the traditional Chinese herb *Zanthoxylum nitidum* (Roxb) DC, has proven effective at suppressing the growth of various malignant tumors (21-23), including LC (9,24,25). Previous studies have indicated that the cytotoxic target of NC could be the topoisomerases (26,27),

but the exact action mode of NC with respect to TOP1 and TOP2A activity has not yet been established.

The present study investigated the expression, clinical value and potential pathological role of TOP1 and TOP2A in LC using immunohistochemistry (IHC), data mining and bioinformatics analyses. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR), IHC staining and molecular docking were then used to determine the inhibitory effects of NC on TOP1 and TOP2A.

Materials and methods

In vivo LC xenografts in nude mice. A total of 32 BALB/c nude mice (16 males and 16 females; 6 weeks old) with an initial body weight of 18-20 g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. (Shanghai, China). The mice were maintained in specific pathogen-free conditions, using a 12 h light/12 h dark cycle at a temperature of 24˚C. All animal experiments were conducted according to the international ethics guidelines and the National Institutes of Health Guide Concerning the Care and Use of Laboratory Animals. The Ethics Committee of the First Affiliated Hospital of Guangxi Medical University (Nanning, China) approved the study. HepG2 cells ($1x10^{10}$ cells/l) were subcutaneously injected into the right armpit of each nude mouse. The drug administration began 7 days after xenograft implantation. Mice were randomized into four groups of 8 mice: The negative control group with a daily intraperitoneal injection of saline for 14 days, and the high-, medium- and low-NC groups, with a daily intraperitoneal injection of 10, 5 or 2.5 mg/kg NC, respectively, for 14 days. The tumor volume was determined by the following formula: Tumor volume = $\pi/6$ x length x width^2. The maximum tumor volume did not exceed 1,600 mm^3 in either group. At the end of the experiment, the mice were euthanized and then the tumors were excised and stored at -80˚C prior to reverse transcription-quantitative polymerase chain reaction (RT-qPCR) experiments. The tumor tissues were fixed in 10% formaldehyde at room temperature for 12 h and paraffin-embedded prior to use in further IHC experiments.

Immunohistochemistry staining. Two tissue microarrays containing a total of 261 HCC samples and 33 non-tumor tissues were purchased from Pantomics, Inc. (Richmond, CA, USA). All tissues were treated according to appropriate applicable laws to protect the privacy of individual patients. Three pairs of NC-treated and NC-untreated tumor tissues were also obtained from the aforementioned LC nude mouse xenografts. Immunohistochemistry (IHC) staining was performed on deparaffinized 4-μm thick sections according to the manufacturer's protocols. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide at room temperature for 10 min. Rabbit monoclonal anti-TOP1 antibody (1:100 dilution; catalog no. ab109374) and rabbit monoclonal anti-TOP2A + TOP2B antibody (1:100 dilution; catalog no. ab109524) (both from Abcam, Cambridge, MA, USA) were used for the tissue microarrays. Mouse monoclonal anti-TOP1 antibody (1:50 dilution; catalog no. sc32736) and monoclonal anti-TOP2A antibody (1:50 dilution; catalog no. sc365916) (both from Santa Cruz Biotechnology, Inc., Dallas, TX, USA) were used for the tumor tissues from the nude mouse LC xenografts. Two pathologists examined all immunostained tissues by microscopy and scored them independently according to the following two criteria: i) The staining intensity was determined as 0 (no staining), 1 (weak staining), 2 (moderate staining) and 3 (strong staining); ii) the staining percentage of tumor cells was scored as 0 (<5%), 1 (5-25%), 2 (26-50%), 3 (51-75%) and 4 (76-100%). The percentages of positively stained cell nuclei were calculated for >1,000 cells in five successive and representative high-power fields (x400 magnification). An immunoreactive score (IRS) was calculated to determine the final staining score by multiplying the intensity score by the percentage score. All samples were allocated to a positive (IRS≥6) or negative (IRS<6) group.

RT-qPCR. Total RNA was isolated and purified from three NC-treated LC tissues and three control groups. RT-qPCR was performed using SYBR-Green with an iCycler iQ RT-qPCR system (Bio-Rad, Inc., Hercules, CA, USA). GAPDH served as the endogenous control. The primer sequences synthesized by Takara Bio, Inc., (Otsu, Japan) were as follows: GAPDH forward, 5'-AAGAAGGTGGTGAAGCAGGC-3' and reverse, 5'-TCC ACCACCCTGTTGCTGTA-3'; TOP1 forward, 5'-GGTGAG AAGGACTGGCAGAAAT-3' and reverse, 5'-CTTGTCGATG AAGTACAGGGCTA-3'; and TOP2A forward, 5'-CGGAATG ACAAGCGAGAAGTAA-3' and reverse, 5'-GCATTGTAAAG ATGTATCGTGGAC-3'. All samples were run in triplicate, and the mean of the three assays was used as the quantitation cycle (Cq) value. The relative expression of TOP1 and TOP2A was determined with the $2^{-\Delta\Delta Cq}$ method (28).

Data mining. Relevant microarray and RNA sequencing (RNA-seq) datasets providing expression data for TOP1 and TOP2A were collected from the Gene Expression Omnibus (29), Oncomine (30), ArrayExpress (31) and The Cancer Genome Atlas (TCGA) (32) databases using the following search terms: (hepatocellular OR liver OR hepatic OR HCC) and (cancer OR carcinoma OR tumor OR neoplas* OR malignan*).

Two reviewers independently gathered the following essential information from all available datasets: First author, publication year, region, dataset, platform, sample size of cancer group and normal control group, and expression of TOP1 and TOP2A. Any disagreement was settled by discussion with a third investigator.

All expression data were log2-transformed. The expression levels of TOP1 and TOP2A in each dataset were compared using Student's t-test and presented as scatter plots. The results from individual microarrays were not consistent, necessitating a comprehensive analysis. The combined standard mean deviation (SMD) and its 95% confidence intervals (CIs) were calculated to investigate the expression levels of TOP1 and TOP2. A summary receiver operating characteristic (SROC) curve was generated, and the area under the curve (AUC) value was calculated to provide a further estimate of the capability of TOP1 and TOP2A to discriminate HCC patients from normal controls. The inter-study heterogeneity was evaluated by the χ^2-based Q test and I^2 statistic. A random-effects model was selected to pool the SMD if heterogeneity existed (I^2>50% or P<0.05); otherwise, a fixed-effects model was selected. Begg's and Egger's tests were applied to estimate the potential publication bias. All these analyses were performed using STATA 12.0 software (Stata Corporation, College Station, TX, USA). P<0.05 was considered statistically significant.

Table I. Essential information of the 22 included microarray and RNA-seq datasets.

First author (year)	Region	Dataset	Platform	Sample size (T/N), n	TOP1 expression (mean ± SD)		TOP2A expression (mean ± SD)		(Ref.)
					T	N	T	N	
Wurmbach *et al* (2007)	USA	GSE6764	GPL570	35/10	6.90±0.53	6.53±0.53	3.22±0.64	2.90±0.25	(40)
Mas *et al* (2009)	USA	GSE14323	GPL571	38/19	8.90±0.46	9.15±0.45	5.35±0.90	4.70±0.26	(41)
Satow *et al* (2010)	Japan	GSE12941	GPL5175	10/10	7.70±0.31	7.59±0.17	7.06±0.87	4.85±0.28	(42)
Roessler *et al* (2010)	USA	GSE14520	GPL3921	225/220	8.76±0.34	8.59±0.27	6.92±1.52	3.87±0.56	(43)
Lim *et al* (2012)	South Korea	GSE36376	GPL10558	240/193	6.91±0.22	6.76±0.17	8.61±1.23	6.07±0.38	(44)
Jeng *et al* (2013)	Taiwan	GSE46408	GPL4133	6/6	11.05±0.95	10.15±0.54	10.31±1.24	4.78±1.37	NA
Wang *et al* (2013)	China	GSE49713	GPL11269	5/5	6.70±0.66	7.04±0.66	8.38±0.87	7.65±0.67	NA
Neumann *et al* (2012)	Germany	GSE50579	GPL14550	67/10	9.45±0.55	8.96±0.83	6.69±2.21	2.36±0.56	(45)
Kim *et al* (2014)	USA	GSE39791	GPL10558	72/72	6.74±0.10	6.70±0.09	8.15±0.93	6.67±0.26	(46)
Zhang *et al* (2014)	USA	GSE22405	GPL10553	24/24	5.37±0.37	5.28±0.17	5.46±1.08	4.21±0.10	NA
Villa *et al* (2016)	Italy	GSE54236	GPL6480	81/80	9.28±0.54	9.23±0.52	6.76±1.88	4.80±1.22	(47)
Melis *et al* (2014)	USA	GSE55092	GPL570	49/91	9.12±0.49	8.67±0.36	4.05±0.58	3.46±0.19	(48)
Schulze *et al* (2015)	France	GSE62232	GPL570	81/10	6.52±0.52	5.80±0.62	2.70±0.11	2.53±0.16	(49)
Mah *et al* (2014)	Singapore	GSE57957	GPL10558	39/39	7.46±0.13	7.49±0.15	9.39±1.08	7.17±0.76	(50)
Wang *et al* (2014)	Taiwan	GSE60502	GPL96	18/18	10.78±0.32	10.71±0.31	9.51±1.09	5.33±1.47	(51)
Tao *et al* (2015)	China	GSE74656	GPL16043	5/5	10.02±0.27	9.63±0.09	8.88±1.30	5.99±1.34	NA
Makowska *et al* (2016)	Switzerland	GSE64041	GPL6244	60/65	9.68±0.37	9.61±0.33	7.20±1.14	5.63±0.54	(52)
Wijetunga *et al* (2017)	USA	GSE82177	GPL11154	6/16	6.97±0.31	7.02±0.30	5.68±1.32	4.40±1.52	(53)
Grinchuk *et al* (2018)	Singapore	GSE76427	GPL10558	115/52	6.76±0.11	6.72±0.14	9.80±1.67	7.92±0.99	(54)
Tu *et al* (2017)	China	GSE84005	GPL5175	38/38	7.90±1.27	8.65±0.52	7.77±1.03	4.13±0.74	NA
Nojima *et al* (2017)	Japan	E-MTAB-4171	None	15/15	10.78±0.26	10.70±0.27	8.88±1.43	5.21±1.46	NA
TCGA (2017)	USA	TCGA	None	374/50	11.31±0.85	11.06±0.58	3.50±0.11	3.46±0.08	NA

T, tumor; N, normal control; SD, standard deviation; TOP, topoisomerase; TCGA, The Cancer Genome Atlas; NA, not available.

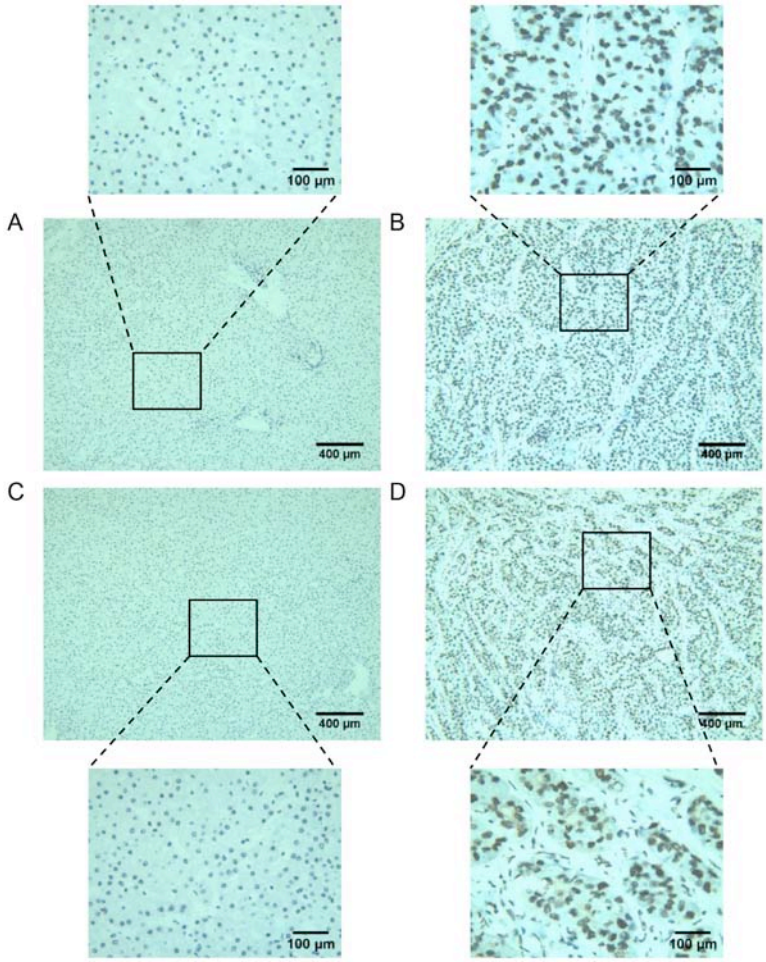

Figure 1. Immunohistochemical staining of TOP1 and TOP2A proteins in HCC and adjacent non-tumorous tissues. Nuclear staining was observed for TOP1 and TOP2A proteins. (A) A paracancerous liver tissue showing low TOP1 protein expression (magnification, x100 lower and x400 upper). (B) An HCC tissue showing high TOP1 protein expression (magnification, x100 lower and x400 upper). (C) A paracancerous liver tissue showing low TOP2A protein expression (magnification, x100 upper and x400 lower). (D) An HCC tissue showing high TOP2A protein expression. TOP, topoisomerase; HCC, hepatocellular carcinoma

The prognostic roles of TOP1 and TOP2A were also assessed using data from TCGA. The alterations in TOP1 and TOP2A expression were investigated with data from cBioPortal (33). The protein expression of TOP1 and TOP2A was also validated by data from The Human Protein Atlas (34).

Bioinformatics analysis. Genes that were co-expressed with TOP1 and TOP2A, and showed correlation coefficients r≥0.3 or r≤-0.3, were acquired from cBioPortal. Gene Ontology (GO) annotation, Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Panther pathway analyses were implemented using the Database for Annotation, Visualization and Integrated Discovery 6.7 (https://david-d.ncifcrf.gov/) (35). Disease Ontology (DO) analysis was performed with the R package 'DOSE' (http://www.bioconductor.org/packages/release/bioc/html/DOSE.html).

Molecular docking. Molecular docking studies were conducted using SYBYL 2.0 software (Tripos, Inc., St. Louis, MO, USA) to investigate the interactions between NC and TOP1/TOP2A. The crystal structures of the TOP1 and TOP2A proteins were obtained from the Protein Data Bank (36). The three-dimensional structure building and modeling were performed using the SYBYL 2.0 program package. Retrieved hits for docking studies were added to the hydrogen atoms and the charge was given by Gasteiger-Huckel calculations, which optimize the structure of compounds and predict the combination between compounds and a protein. Energy minimizations were performed using the Tripos force field with an energy optimization gradient convergence criterion provided by the system (37-39). A total score >6 indicated a favored affinity between NC and TOP1/TOP2A.

Statistical analysis. All statistical analyses were conducted with SPSS 22.0 (IBM Corp., Armonk, NY, USA). Continuity variable results were represented as the mean ± standard deviation. Student's t-test or one-way analysis of variance (ANOVA) was used for comparison between groups. A Least Significant Difference analysis was performed if the result of the ANOVA was statistically significant. P<0.05 was considered to indicate a statistically significant difference.

Results

TOP1 and TOP 2 are highly expressed in HCC tissues based on IHC staining. IHC staining revealed the expression of the TOP1 and TOP2A proteins in 261 HCC tissues and 33 non-tumor tissues. For TOP1, the positive rate was 25.7% (67/261) in tumor tissues and 3.0% (1/33) in non-tumor tissues (Fig. 1A and B). For TOP2A, the positive rate was 24.9% (65/261) in tumor tissues

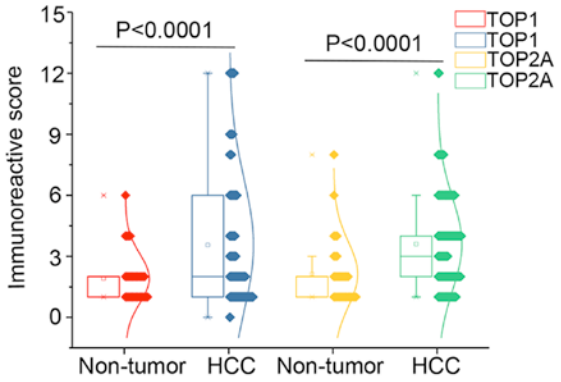

Figure 2. Box-scatter plot for expression of TOP1 and TOP2A proteins in HCC tissues and non-tumor tissues based on immunoreactive score. TOP, topoisomerase; HCC, hepatocellular carcinoma.

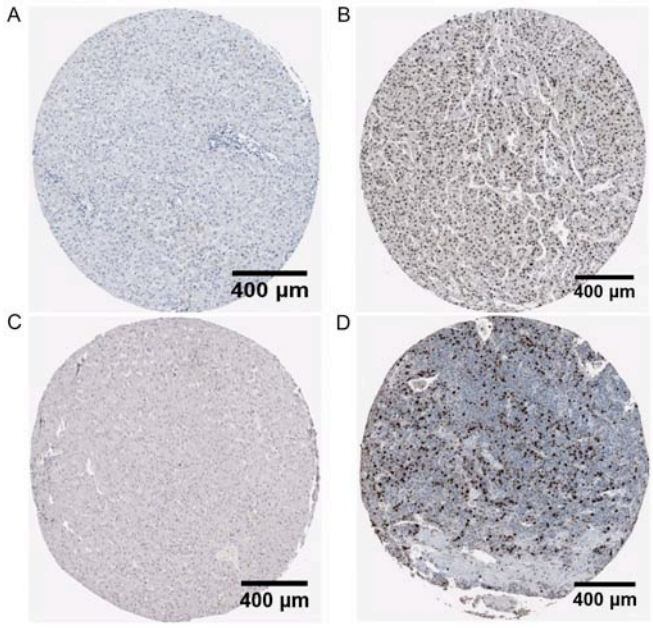

Figure 3. Validation of TOP1 and TOP2A proteins in HCC and adjacent non-tumorous tissues based on The Human Protein Atlas. (A) A normal liver tissue showing low expression of TOP1 protein. The image is available at https://www.proteinatlas.org/ENSG00000198900-TOP1/tissue/liver#img. (B) An HCC tissue showing high expression of TOP1 protein. The image is available at https://www.proteinatlas.org/ENSG00000198900-TOP1/pathology/tissue/liver+cancer#img. (C) A normal liver tissue showing low expression of TOP2A protein. The image is available at https://www.proteinatlas.org/ENSG00000131747-TOP2A/tissue/liver#img. (D) An HCC tissue showing high expression of TOP2A protein. The image is available at https://www.proteinatlas.org/ENSG00000131747-TOP2A/pathology/tissue/liver+cancer#img. Magnification, x100. TOP, topoisomerase; HCC, hepatocellular carcinoma.

and 6.1% (2/33) in non-tumor tissues (Fig. 1C and D). Assessment of the expression patterns of the TOP1 and TOP2A proteins in tumor tissues and corresponding non-cancerous tissues with Student's t-test and visualization in the form of scatter-box plots revealed a marked upregulation of TOP1 and TOP2A in HCC tissues (TOP1, P<0.0001; TOP2A, P<0.0001) (Fig. 2). The high expression of TOP1 and TOP2A proteins in HCC was corroborated by The Human Protein Atlas (Fig. 3).

Microarray and RNA-seq datasets indicate upregulation of TOP1 and TOP2A in HCC tissues. The 22 datasets (40-54) used

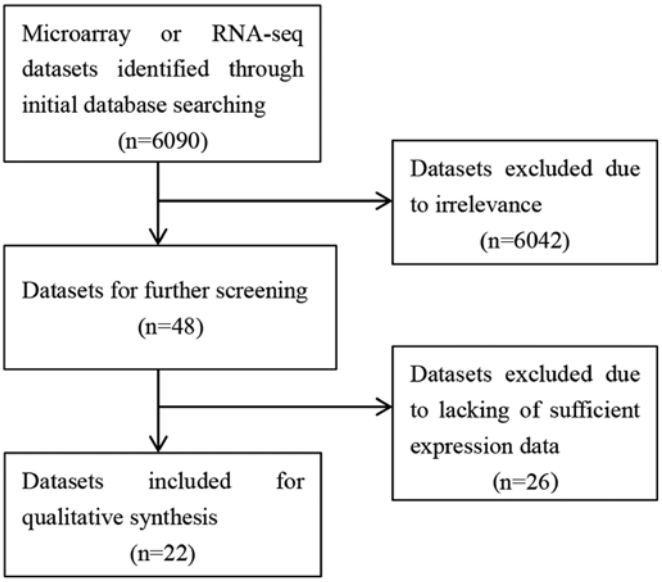

Figure 4. Flowchart illustrating the dataset collection procedure. RNA-seq, RNA sequencing.

in this study included 1,603 HCC samples and 1,048 non-tumor specimens, and provided expression data for the TOP1 and TOP2A genes, as shown in Fig. 4. The fundamental information for the 22 studies is listed in Table I.

The expression levels of TOP1 and TOP2A in each dataset were visualized in the form of scatter plots (Figs. 5 and 6). The results from individual microarrays were not consistent, so the 22 datasets were combined. The pooled SMD showed that TOP1 and TOP2A were markedly overexpressed in HCC tissues (TOP1: SMD, 0.31; 95% CI, 0.07-0.55; P=0.012; and TOP2A: SMD, 1.92; 95% CI, 1.51-2.32; P<0.0001) (Fig. 7). Recognizing that a significant heterogeneity arises from differences in RNA extraction, RNA detection and sample source (TOP1: I^2=84.6, P<0.0001; and TOP2A: I^2=92.6, P<0.0001) (Fig. 7), a random-effects model was selected to combine all the data. No significant publication bias existed in the present study according to the results of Begg's and Egger's tests (TOP1: Begg's P=0.91, Egger's P=0.36; and TOP2A: Begg's P=0.28, Egger's P=0.82; Fig. 8).

The ability of TOP1 and TOP2A to differentiate HCC patients from normal controls was further estimated with the SROC method. The AUC value for TOP1 was 0.69 (95% CI, 0.64-0.72), with a sensitivity of 0.58 and specificity of 0.73 (Fig. 9A). The AUC value for TOP2A was 0.96 (95% CI, 0.94-0.97), with a sensitivity of 0.87 and specificity of 0.92 (Fig. 9B).

The expression of TOP1 and TOP2A was also verified in the HCC cell line using the Cancer Cell Line Encyclopedia (55), as shown in Fig. 10.

TOP1 and TOP2 were biomarkers of unfavorable prognosis in LC. First, the prognostic value of TOP1 and TOP2A was assessed in LC using the Gene Expression Profiling Interactive Analysis database (56), an online database that contains and processes data from TCGA. Patients were divided into TOP1 or TOP2A high and low expression groups according to the median expression of the two genes. As shown in Fig. 11, the elevated expression of TOP1 and TOP2A was closely associated with

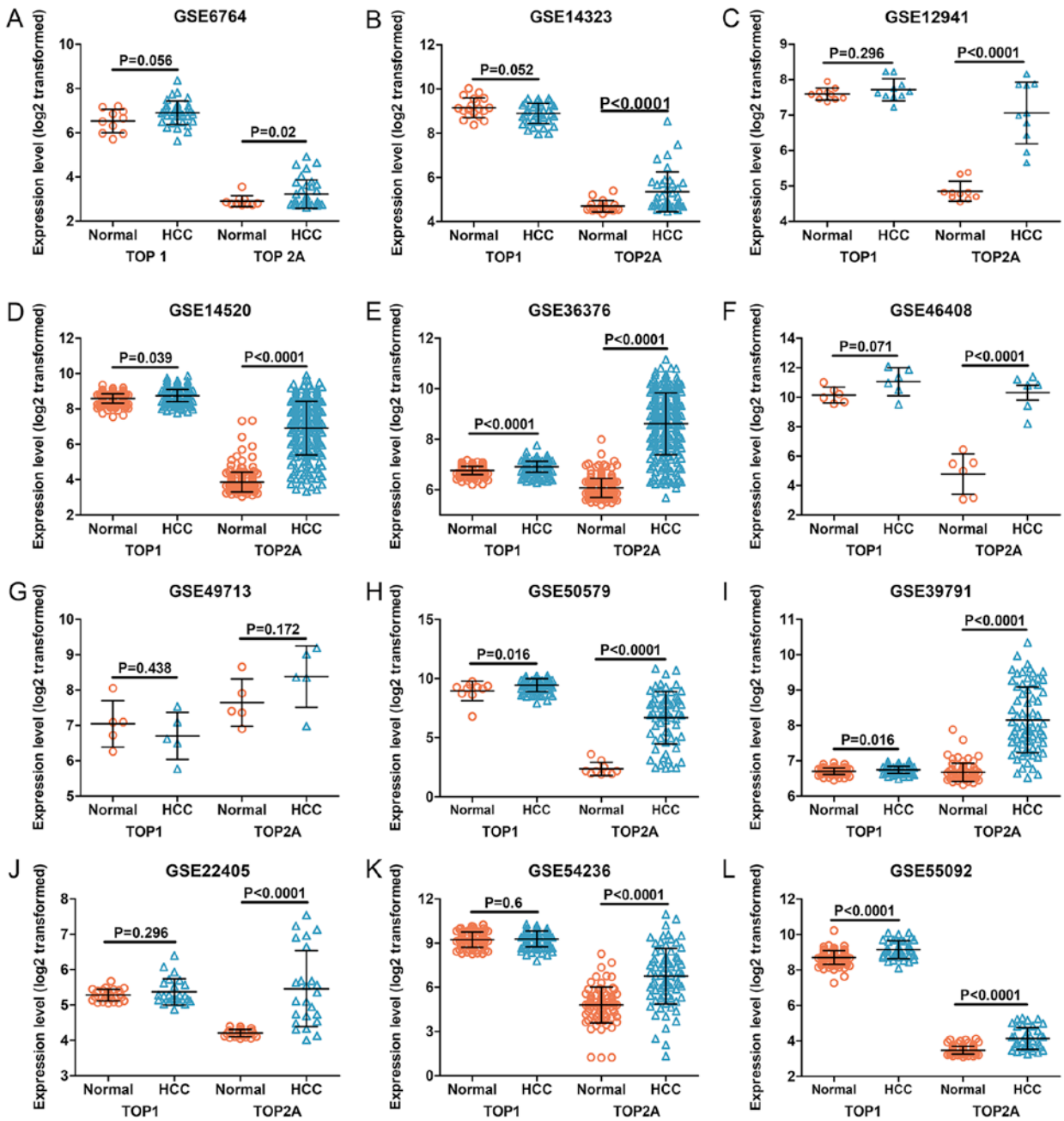

Figure 5. Scatter plot for mRNA expression of TOP1 and TOP2A in normal liver tissues and HCC tissues. (A) GSE6764; (B) GSE14323; (C) GSE12941; (D) GSE14520; (E) GSE36376; (F) GSE46408; (G) GSE49713; (H) GSE50579; (I) GSE39791; (J) GSE22405; (K) GSE54236; and (L) GSE55092. TOP, topoisomerase; HCC, hepatocellular carcinoma.

poor overall survival (OS) (TOP1, P=0.017; TOP2A, P=0.003) and disease-free survival (DFS) (TOP1, P=0.0007; TOP2A, P<0.0001). Further analysis of the prognostic role of TOP1 and TOP2A in different risk groups was then conducted using the SurvExpress online tool (57). A cohort of 361 patients with LC was divided into a high-risk (n=180) and a low-risk (n=181) group according to the prognostic index (Fig. 12A). TOP1 and TOP2A were highly expressed in the high-risk group (TOP1, P<0.0001; TOP2A, P<0.0001) (Fig. 12B andC). For TOP1, although not statistically significant, patients in the high-risk group tended to have a poorer prognosis [hazard ratio (HR), 1.25; 95% CI, 0.88-1.78; P=0.21) (Fig. 12D)]. For TOP2A, patients in the high-risk group showed a clearly poorer survival outcome (HR, 1.45; 95% CI, 1.02-2.05; P=0.04) (Fig. 12E).

The present study also examined the alterations in TOP1 and TOP2A expression in LC samples using the cBioPortal database. TOP1 was altered in 18 (4.1%) and TOP2A was altered in 30 (6.8%) of the 440 patients with LC (Fig. 13A). For TOP1, patients with alteration tended to have a worse survival (P=0.173) (Fig. 13B), while for TOP2A, the survival outcome was similar for patients with and without alteration (P=0.966) (Fig. 13C).

Functional annotation and pathway enrichment of TOP1 and TOP2A in LC. Based on cBioPortal data, a total of 3,959 genes were co-expressed with TOP1, including 2,448 positively associated and 1,511 negatively associated genes. For TOP2A, 2,485 genes were co-expressed, with 2,083 positively associated and 402 negatively associated genes. Examination of

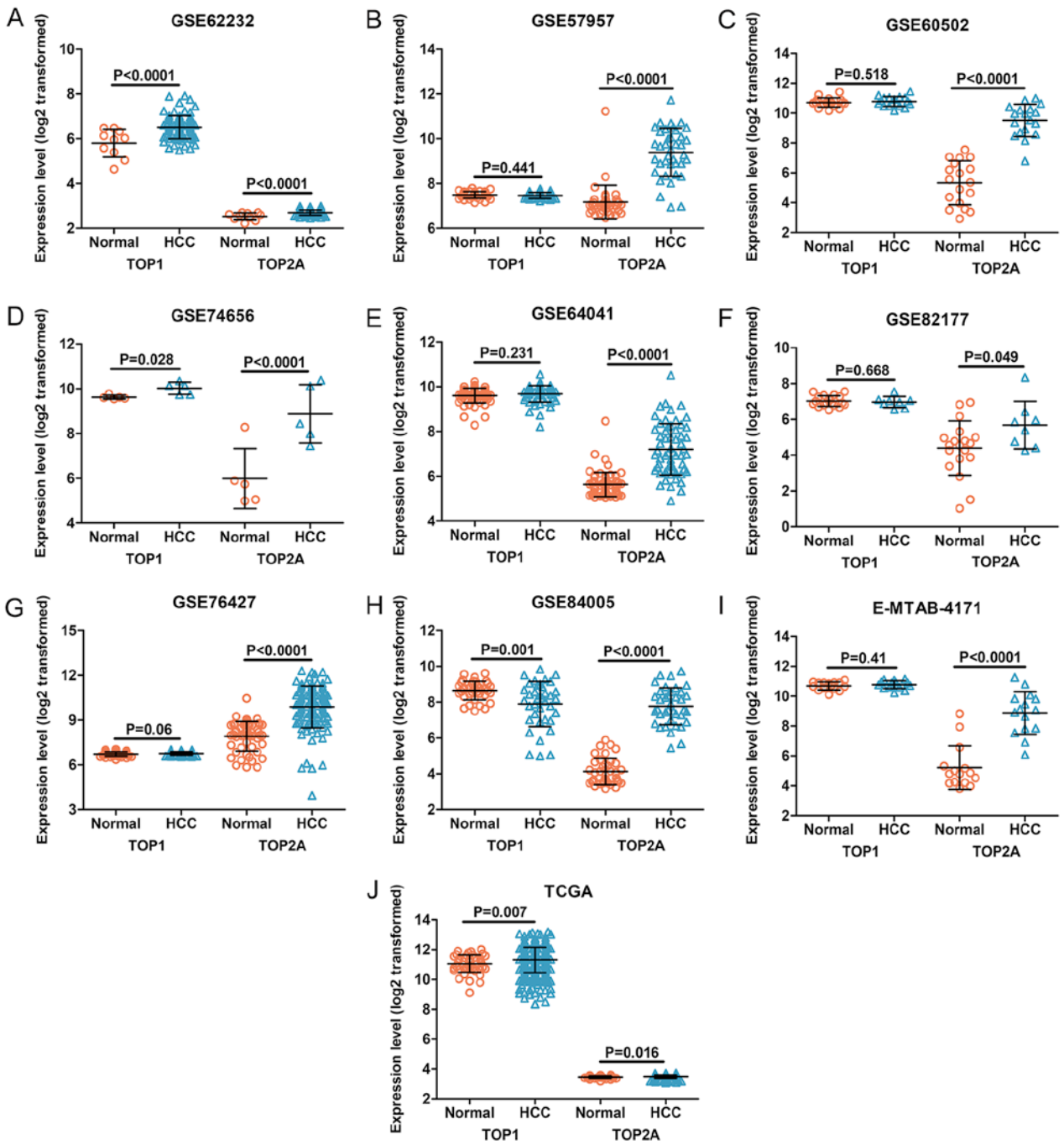

Figure 6. Scatter plot for mRNA expression of TOP1 and TOP2A in normal liver tissues and HCC tissues. (A) GSE62232; (B) GSE57957; (C) GSE60502; (D) GSE74656; (E) GSE64041; (F) GSE46408; (G) GSE82177; (H) GSE76427; (I) GSE84005; (J) E-MTAB-4171; (K) GSE54236; and (L) TCGA. TOP, topoisomerase; HCC, hepatocellular carcinoma; TCGA, The Cancer Genome Atlas.

the intersection between the genes co-expressed with TOP1 and with TOP2A revealed 1,209 overlapping genes, which were selected for further analysis. The top 10 GO terms are displayed in Fig. 14. The co-expressed genes were mainly enriched in nuclear genes and were involved in the regulation of transcription and the cell cycle.

KEGG and Panther pathway analyses revealed that these genes participated in several cancer-related pathways, such as the 'p53 pathway', 'pathway in cancer' and the 'apoptosis signaling pathway' (Fig. 15A and B). DO analysis, conducted to investigate the involvement of these genes in disease, revealed that these genes were associated with 'hereditary breast ovarian cancer', 'autosomal dominant disease', 'autosomal genetic disease', 'progressive multifocal leukoencephalopathy' and 'monogenic disease' (Fig. 15C).

NC inhibits the expression of TOP1 and TOP2A. NC exhibited an inhibitory effect on the growth of LC xenografts in the nude mice. When compared with that of the negative control group, the tumor volume in the NC group was significantly and dose-dependently reduced (Table II). For TOP1, the mean mRNA expression in the NC-treated and control groups was 0.073 and 0.236, respectively. For TOP2A, the mean mRNA expression in the NC-treated and control groups was 0.13 and 0.156,

Table II. Tumor volume of liver cancer xenografts in nude mice following NC treatment.

Treatment	Dosage mg/kg	TV, mm^3 Pre-treatment (mean ± SD)	TV, mm^3 Post-treatment (mean ± SD)	RTV	T/C, %
Saline	-	200.22±89.67	1474.4±109.34	7.36±1.21	100
NC	2.5	241.23±94.59	1313.77±242.41	5.44±1.47	73.95
NC	5.0	201.38±79.43	955.89±54.73	4.75±0.30[a]	64.55
NC	10	224.43±62.53	842.55±242.18	3.75±0.92[b]	51.07

[a]P<0.05 and [b]P<0.01 vs. saline group. TV, tumor volume; RTV, relative tumor volume; SD, standard deviation; NC, nitidine chloride; $RTV = TV_{pre\text{-}treatment}/TV_{post\text{-}treatment}$; $T/C\ (\%) = RTV_{NC}/RTV_{saline}$ x100.

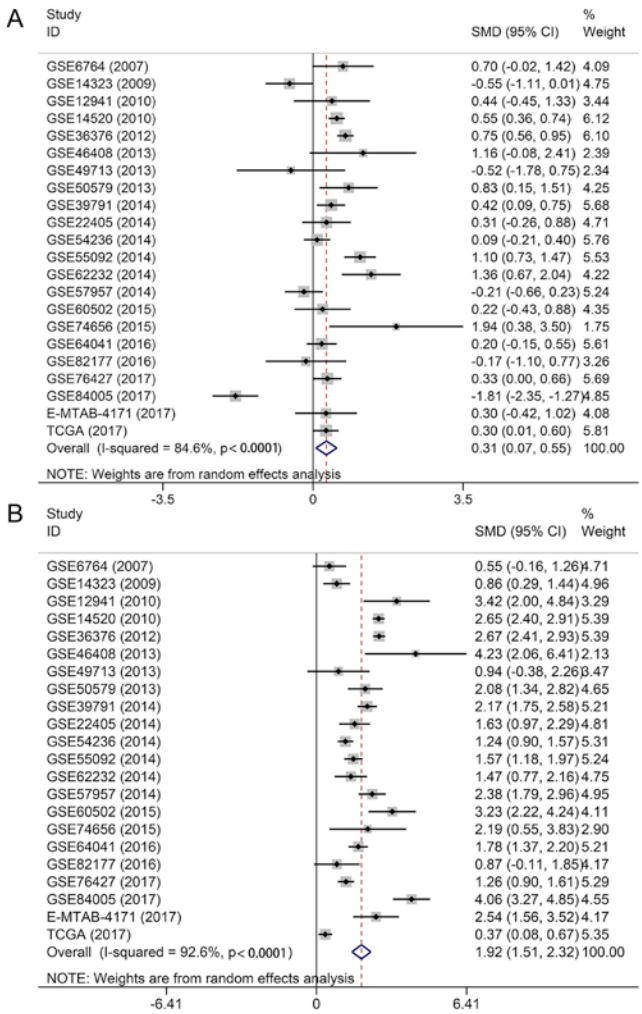

Figure 7. Forest plot of the 22 microarray and RNA-seq datasets evaluating TOP1 and TOP2A in HCC tissues. A pooled SMD>0 indicated that the expression of TOP1 and TOP2A was higher in HCC tissues than that in normal liver tissues. (A) TOP1 was upregulated in HCC (random-effects model); (B) TOP2A was upregulated in HCC (random-effects model). TOP, topoisomerase; HCC, hepatocellular carcinoma; RNA-seq, RNA sequencing; SMD, standard mean deviation; CI, confidence interval.

Table III. Results of the molecular docking calculations.

Protein	PDB ID	Total_Score	Crash	Polar	Cscore
TOP1	1k4T	8.2	-1.73	2.15	3
TOP2A	5btg	14.47	-2.02	1.22	5

TOP, topoisomerase; PDB ID, protein data bank identity.

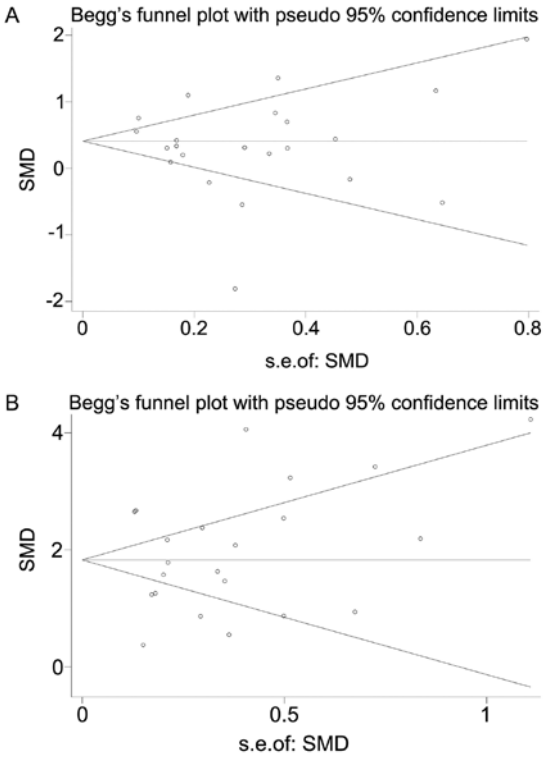

Figure 8. Potential publication bias among the included studies. (A) Funnel plot of the 22 included datasets for TOP1. (B) Funnel plot of the 22 included datasets for TOP2A. SMD, standard mean deviation; TOP, topoisomerase; s.e., standard error.

respectively. These findings suggested that NC could inhibit the expression of TOP1 and TOP2A at the mRNA level (P<0.05). Subsequent IHC staining confirmed a similar inhibition of TOP1 and TOP2A expression at the protein level by NC (Fig. 16).

Verification of the inhibitory effect of NC on TOP1 and TOP2A by molecular docking. The present study further investigated the binding modes and interactions between NC and TOP1/TOP2A with a molecular docking method. The results

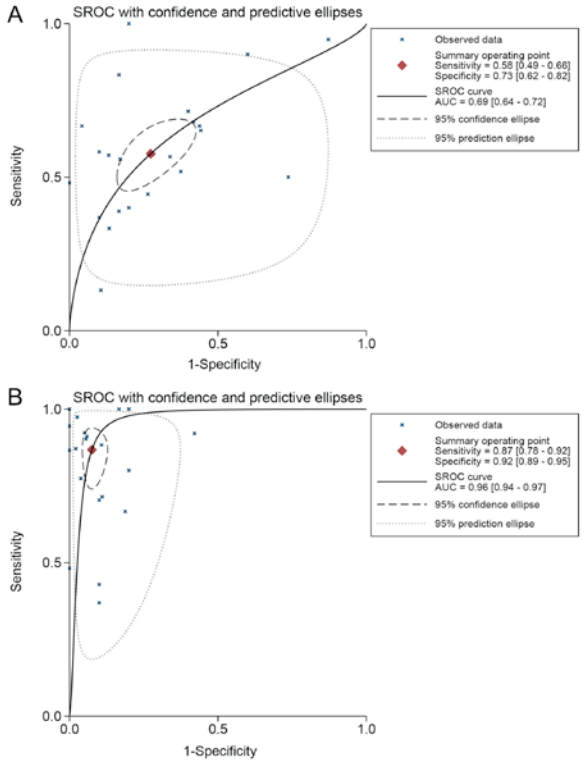

Figure 9. SROC curve for the discrimination of HCC patients from normal controls using TOP1 and TOP2A gene expression. (A) Ability of TOP1 to discriminate HCC patients from normal controls. (B) Ability of TOP2A to discriminate HCC patients from normal controls. SROC, summary receiver operating characteristic; HCC, hepatocellular carcinoma; TOP, topoisomerase; AUC, area under the curve.

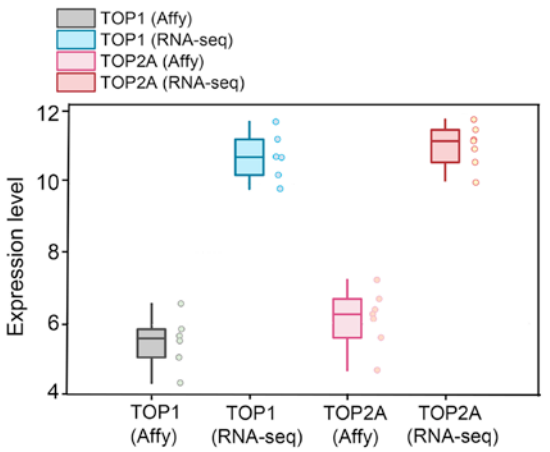

Figure 10. Expression of TOP1 and TOP2A in a liver cancer cell line using the Cancer Cell Line Encyclopedia online database. The expression (Affy and RNA-seq) of TOP1 and TOP2A mRNA is visualized as box plots. TOP, topoisomerase; RNA-seq, RNA sequencing.

of the molecular docking calculations are shown in Table III and Fig. 17. According to these results, the total docking scores of TOP1 and TOP2A were 8.2 and 14.47, respectively, indicating strong interactions between NC and TOP1 and TOP2A.

Discussion

DNA topoisomerases, which are well-known modulators of DNA topology, catalyze the alteration of DNA topological

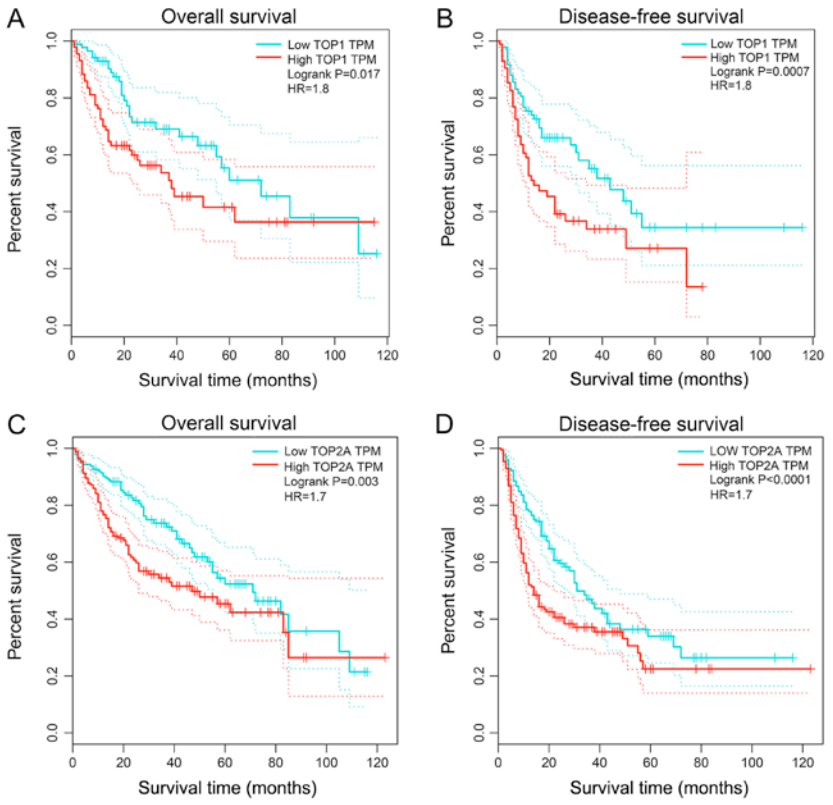

Figure 11. Kaplan-Meier survival curve showing the prognostic value of TOP1 and TOP2A in predicting liver cancer using the Gene Expression Profiling Interactive Analysis online tool. (A) High expression of TOP1 indicated poor OS. (B) High expression of TOP1 indicated poor DFS. (C) High expression of TOP2A indicated poor OS. (D) High expression of TOP2A indicated poor DFS. TOP, topoisomerase; OS, overall survival; DFS, disease-free survival; HR, hazard ratio; TPM, transcripts per million.

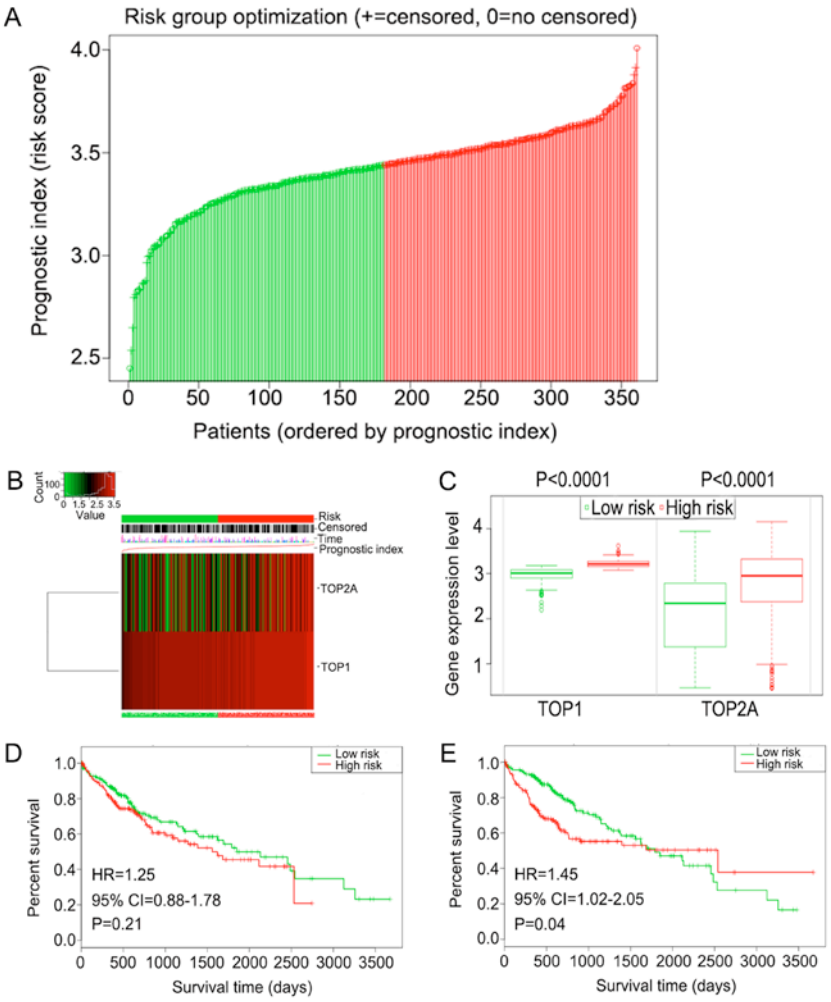

Figure 12. Ability of TOP1 and TOP2A to identify high- and low-risk patients with liver cancer using the SurvExpress online tool. Red: high-risk group; green: low-risk group. (A) The low- and high- risk group for the prognostic index (PI) in LC patients. (B) A heatmap indicating higher expression of TOP1 and TOP2A in the high-risk group than that in the low-risk group. (C) A box-plot confirming higher expression of TOP1 and TOP2A in the high-risk group than that in the low-risk group. (C) A Kaplan-Meier survival curve for TOP1 showing that patients in the high-risk group tended to a poorer prognosis. (D) A Kaplan-Meier survival curve for TOP2 showing that patients in the high-risk group had a poorer survival outcome. TOP, topoisomerase; HR, hazard ratio; CI, confidence interval.

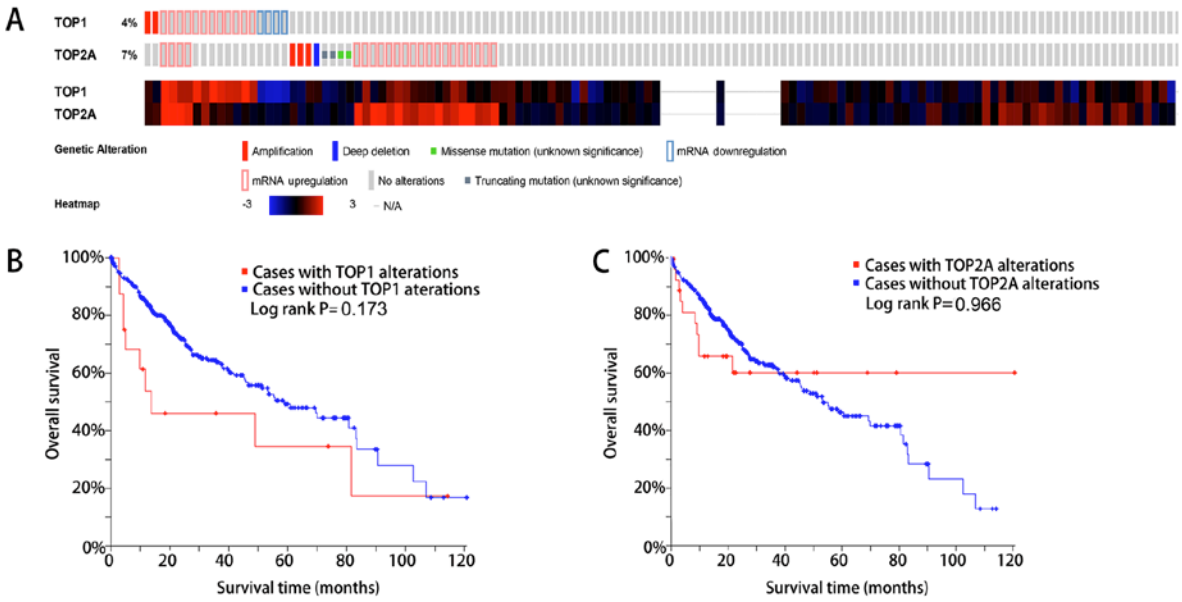

Figure 13. Genetic alterations of TOP1 and TOP2A in LC determined with the cBioPortal database. (A) The alterations in TOP1 and TOP2A expression in 440 patients with LC. The heatmap represents the mRNA expression of TOP1 and TOP2A. (B) Effects of TOP1 alterations on the OS of patients with LC. (C) Effects of TOP2A alterations on the OS of patients with LC. TOP, topoisomerase; LC, liver cancer; OS, overall survival.

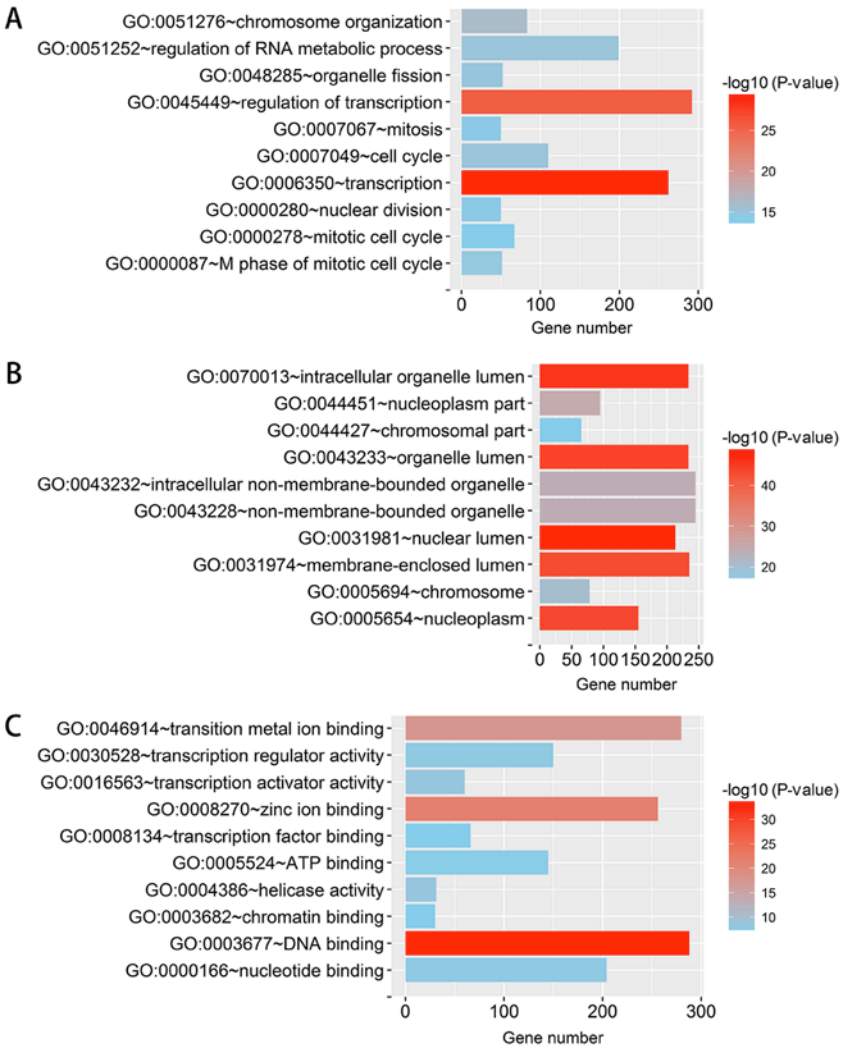

Figure 14. Top 10 GO terms for the genes co-expressed with TOP1 and TOP2A. (A) The top 10 GO biological processes. (B) The top 10 GO cellular components. (C) The top 10 GO molecular functions. GO, Gene Ontology; TOP, topoisomerase.

structures by cleaving and reconnecting single or double-stranded DNA (58,59). Depending on whether they make single- or double-stranded breaks, the topoisomerases are divided into TOP1 and TOP2 (60,61), with TOP2A being a subfamily of TOP2. During RNA transcription and DNA replication, TOP1 and TOP2A can relax positive and negative supercoils to regulate key cellular processes (62,63). TOP1 and TOP2A are tumor drivers in a myriad of human cancer types, including LC. The study by Ang *et al* (64), which employed a multiplatform profiling service method based on 350 LC samples, found upregulation of TOP1 and TOP2A in LC. Wong *et al* (65) detected TOP2A expression in LC cell lines and tissues. It was also determined that TOP2A was overexpressed in LC, and that the high expression of TOP2A was closely associated with microvascular invasion, advanced histological grading, an early age of occurrence of HCC and a poor survival outcome. Panvichian *et al* (66) also confirmed the high expression of TOP2A in LC.

In the present study, a high expression level of TOP1 and TOP2A protein was observed based on IHC staining. The combined use of microarrays and RNA-seq data mining also verified higher gene expression levels for TOP1 and TOP2A in HCC tissues than those in normal liver tissues. The gene expression level was much higher for TOP2A in HCC than that for TOP1 (TOP1: SMD, 0.31; 95% CI, 0.07-0.55; P=0.012; and TOP2A: SMD, 1.92; 95% CI, 1.51-2.32; P<0.0001); however, the protein expression levels were similar. Previous studies have demonstrated that TOP2A is regulated at the translational or transcriptional level (67,68), which may result in a discrepancy between protein expression and RNA expression.

Our data from the cBioPortal revealed genetic alterations of TOP1 and TOP2A in human hepatocellular carcinoma. More interestingly, patients with TOP1 alterations showed a lower OS compared to patients without TOP1 mutation. We assumed that the abnormal alterations may be responsible for the ontogenetic role of TOP1 and TOP2A in LC.

The data from TCGA was also used to investigate the prognostic value of TOP1 and TOP2A expression. High expression of TOP1 and TOP2A predicted unfavorable OS and poor DFS. Higher expression levels of TOP1 and TOP2A were also found in high-risk patients compared with those in low-risk patients. These findings indicated that TOP1 and TOP2A could be potential biomarkers for predicting the prognosis of patients with LC, and for identifying high-risk cases and

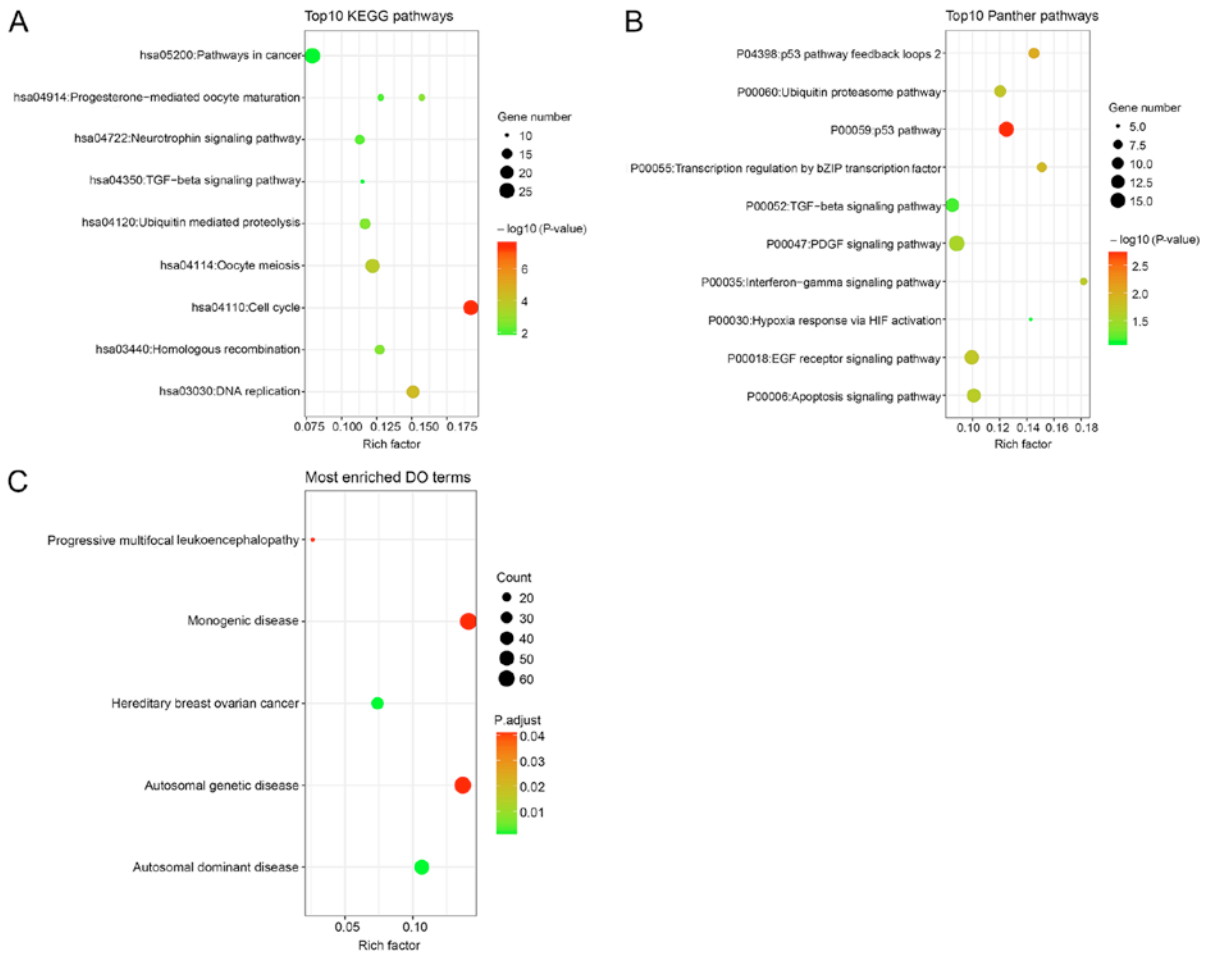

Figure 15. Top 10 KEGG and Panther pathways and the most enriched DO terms. (A) The top 10 KEGG pathways. (B) The top 10 Panther pathways. (C) The most enriched DO terms (P<0.05). KEGG, Kyoto Encyclopedia of Genes and Genomes; DO, Disease Ontology.

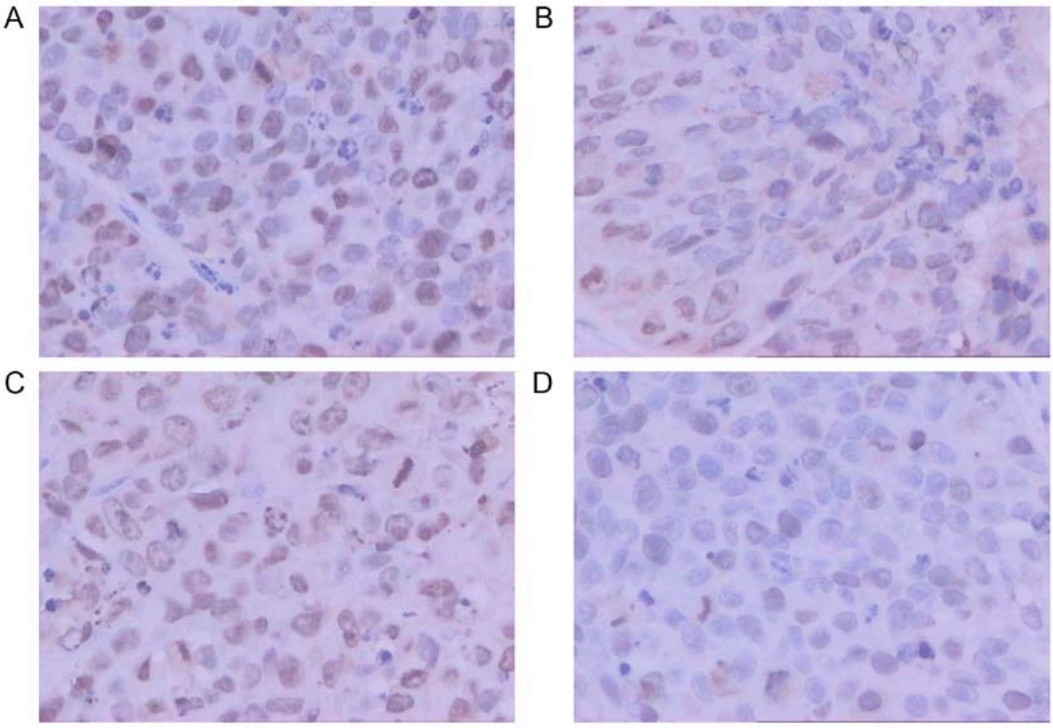

Figure 16. Immunohistochemical staining of TOP1 and TOP2A proteins in tumor tissues from liver cancer xenografts in nude mice (magnification, x400). (A) Expression of TOP1 in the control group. (B) Expression of TOP1 in the group treated with NC. (C) Expression of TOP2A in the control group. (D) Expression of TOP2A in the NC group. TOP, topoisomerase; NC, nitidine chloride.

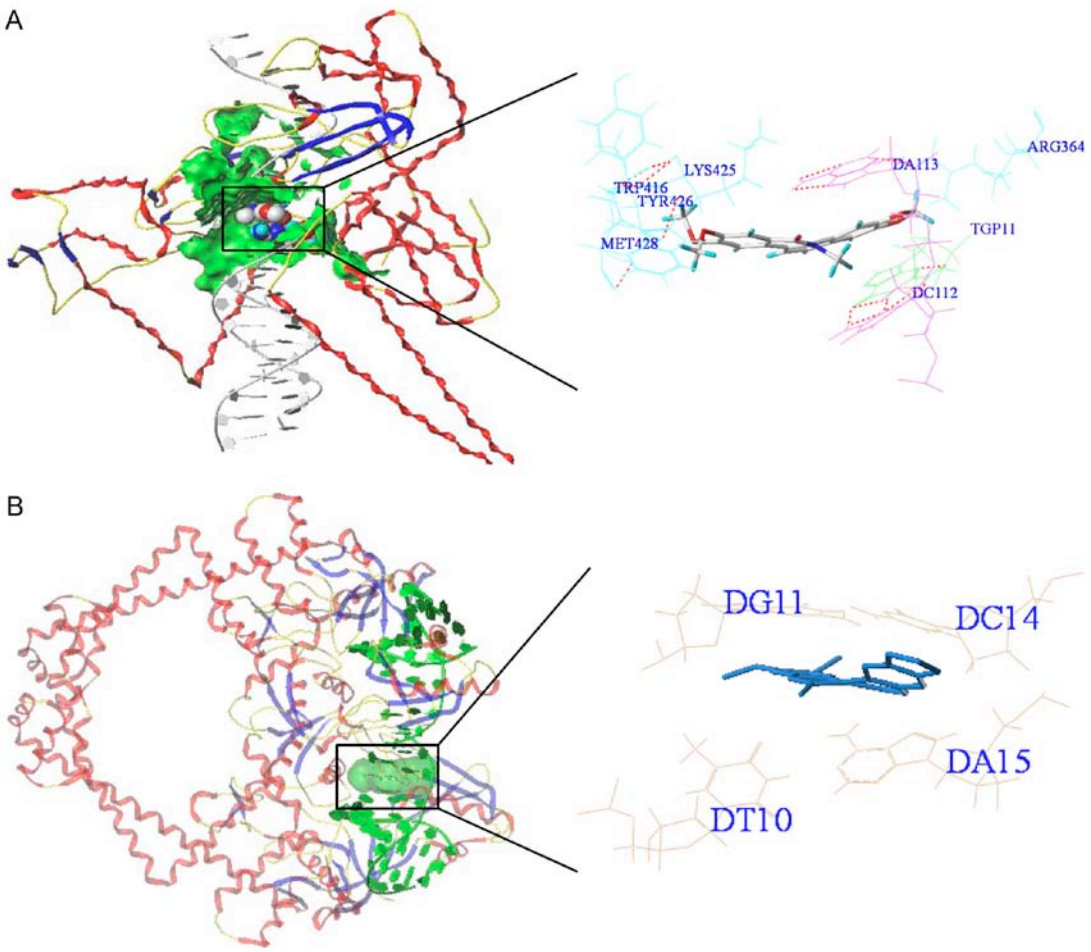

Figure 17. Docking of NC onto TOP1 and TOP2A proteins. (A) A strong hydrophobic interaction formed between NC and the amino acid residues TYR426, TRP416, LYS425, MET428, ARG364, DA113, DC112 and TGP11 of TOP1. (B) NC inlaid between the TOP2A base pairs of DG10-DA15 and DT11-DC14 by the base stacking force. TOP, topoisomerase; NC, nitidine chloride.

allowing optimization of individual treatment management. Bioinformatics analyses were also conducted to investigate the potential biological processes and signaling pathways in which TOP1 and TOP2A may be involved. Consistent with previous studies (69,70), it was found that TOP1 and TOP2A were located in the nucleus and regulated RNA transcription, chromosome organization, RNA metabolism and the cell cycle. Additionally, it was found that TOP1 and TOP2A were associated with several cancer-related pathways, including the 'p53 pathway', 'pathway in cancer' and the 'apoptosis signaling pathway'. The oncogenic role served by TOP1 and TOP2A in LC may therefore involve interactions with these signaling cascades. Further studies are required to verify this speculation.

TOP1 and TOP2A have essential roles in mammalian cells, making them valuable as drug targets for cancer pharmacotherapy (71,72). However, the poor selectivity of traditional TOP inhibitors for tumor cells often results in serious side effects in patients with cancer, so novel medicines with favorable targeting effects are urgently required. Natural products are receiving increasing attention in the treatment of cancer patients due to their low side effects and extensive biological activities (73,74).

NC is a natural bioactive alkaloid derived from a well-known Chinese herbal medicine, *Zanthoxylum nitidum* (Roxb) DC. This herbal ingredient is considered a promising chemotherapeutic agent for malignant tumors, including LC. Liao *et al* (9) conducted *in vivo* experiments and found that NC restrained LC cell growth by inhibiting the Janus kinase 1-signal transducer and activator of transcription 3 signaling pathway. Lin *et al* (25) also verified the inhibitory effect of NC on LC cell growth, and Ou *et al* (24) demonstrated that NC induced apoptosis in LC cells by regulation of a pathway that included p53, p21, apoptosis regulator Bax and B-cell lymphoma 2. Several studies have proposed that NC could be a TOP inhibitor (26,27), but the mechanism underlying NC action on topoisomerases required establishing further.

In the present study, IHC and RT-qPCR were used to compare the mRNA and protein expression of TOP1 and TOP2A in hepatic tumor tissues with or without NC treatment. The NC treatment reduced the expression of TOP1 and TOP2A at the mRNA and protein levels. Molecular docking studies also confirmed the direct binding of NC to TOP1 and TOP2A. Taken together, these results indicated that TOP1 and TOP2A could be direct targets of NC. However, further experiments are necessary to verify these findings.

In conclusion, the present study points to an oncogenic role for TOP1 and TOP2A in LC. TOP1 and TOP2A were upregulated in LC at the protein and mRNA levels, indicating their potential use as biomarkers to predict prognosis in patients with

LC and to identify high-risk cases, thereby optimizing individual treatment management. The present findings also increase our understanding of the antitumor effects of NC on LC and reveal its promise as a therapeutic agent for LC treatment.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

LML conducted the *in vivo* experiments, RT-qPCR, IHC and molecular docking, and contributed to the main writing of the manuscript. DDX collected and analyzed data from the GEO, Oncomine, ArrayExpress and TCGA, and contributed to the main writing of the manuscript. PL and HY conducted the bioinformatics analyses. YWD conducted the IHC of the tissue microarrays. GC guided the design of this study and the writing of the manuscript.

Ethics approval and consent to participate

All participants provided informed consent prior to sample collection. The Ethics Committee of the First Affiliated Hospital of Guangxi Medical University approved this investigation.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflicts of interest.

References

1. Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin DM, Forman D and Bray F: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136: E359-E386, 2015.
2. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, Kudo M, Johnson P, Wagner S, Orsini LS, *et al*: Global patterns of hepatocellular carcinoma management from diagnosis to death: The BRIDGE Study. Liver Int 35: 2155-2166, 2015.
3. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2018. CA Cancer J Clin 68: 7-30, 2018.
4. Yoshimoto T, Imura S, Morine Y, Ikemoto T, Arakawa Y, Iwahashi S, Saito YU, Takasu C, Ishikawa D, Teraoku H, *et al*: The outcome of sorafenib therapy on unresectable hepatocellular carcinoma: Experience of conversion and salvage hepatectomy. Anticancer Res 38: 501-507, 2018.
5. Neuzillet C, de Mestier L, Rousseau B, Mir O, Hebbar M, Kocher HM, Ruszniewski P and Tournigand C: Unravelling the pharmacologic opportunities and future directions for targeted therapies in gastro-intestinal cancers part 2: Neuroendocrine tumours, hepatocellular carcinoma, and gastro-intestinal stromal tumours. Pharmacol Ther 181: 49-75, 2018.
6. Yuan P, Cao W, Zang Q, Li G, Guo X and Fan J: The HIF-2α-MALAT1-miR-216b axis regulates multi-drug resistance of hepatocellular carcinoma cells via modulating autophagy. Biochem Biophys Res Commun 478: 1067-1073, 2016.
7. Li M, Zhang L, Ge C, Chen L, Fang T, Li H, Tian H, Liu J, Chen T, Jiang G, *et al*: An isocorydine derivative (d-ICD) inhibits drug resistance by downregulating IGF2BP3 expression in hepatocellular carcinoma. Oncotarget 6: 25149-25160, 2015.
8. Auyeung KK and Ko JK: Coptis chinensis inhibits hepatocellular carcinoma cell growth through nonsteroidal anti-inflammatory drug-activated gene activation. Int J Mol Med 24: 571-577, 2009.
9. Liao J, Xu T, Zheng JX, Lin JM, Cai QY, Yu DB and Peng J: Nitidine chloride inhibits hepatocellular carcinoma cell growth in vivo through the suppression of the JAK1/STAT3 signaling pathway. Int J Mol Med 32: 79-84, 2013.
10. Cazzamalli S, Corso AD and Neri D: Targeted delivery of cytotoxic drugs: Challenges, opportunities and new developments. Chimia (Aarau) 71: 712-715, 2017.
11. Inoue T, Kurimoto N, Furuya N, Handa H, Kida H, Nishine H, Ishida A, Nobuyama S, Mineshita M and Miyazawa T: New technique for endobronchial ultrasound-guided transbronchial needle aspiration to improve diagnostic yield. J Bronchology Interv Pulmonol 20: 28-32, 2013.
12. Moukharskaya J and Verschraegen C: Topoisomerase 1 inhibitors and cancer therapy. Hematol Oncol Clin North Am 26: 507-525, 2012.
13. Wang N, Zhu M, Tsao SW, Man K, Zhang Z and Feng Y: MiR-23a-mediated inhibition of topoisomerase 1 expression potentiates cell response to etoposide in human hepatocellular carcinoma. Mol Cancer 12: 119, 2013.
14. Both J, Wu T, Ten Asbroek AL, Baas F and Hulsebos TJ: Oncogenic properties of candidate oncogenes in chromosome region 17p11.2p12 in human osteosarcoma. Cytogenet Genome Res 150: 52-59, 2016.
15. Zhou Q, Abraham AD, Li L, Babalmorad A, Bagby S, Arcaroli JJ, Hansen RJ, Valeriote FA, Gustafson DL, Schaack J, *et al*: Topoisomerase IIα mediates TCF-dependent epithelial-mesenchymal transition in colon cancer. Oncogene 35: 4990-4999, 2016.
16. Delgado JL, Hsieh CM, Chan NL and Hiasa H: Topoisomerases as anticancer targets. Biochem J 475: 373-398, 2018.
17. Russo P, Del Bufalo A and Cesario A: Flavonoids acting on DNA topoisomerases: Recent advances and future perspectives in cancer therapy. Curr Med Chem 19: 5287-5293, 2012.
18. Kathiravan MK, Kale AN and Nilewar S: Discovery and development of topoisomerase inhibitors as anticancer agents. Mini Rev Med Chem 16: 1219-1229, 2016.
19. Wasim L and Chopra M: Synergistic anticancer effect of panobinostat and topoisomerase inhibitors through ROS generation and intrinsic apoptotic pathway induction in cervical cancer cells. Cell Oncol (Dordr) 41: 201-212, 2018.
20. Nukuzuma S, Nakamichi K, Kameoka M, Sugiura S, Nukuzuma C, Tasaki T and Takegami T: Suppressive effect of topoisomerase inhibitors on JC polyomavirus propagation in human neuroblastoma cells. Microbiol Immunol 60: 253-260, 2016.
21. Chen J, Wang J, Lin L, He L, Wu Y, Zhang L, Yi Z, Chen Y, Pang X and Liu M: Inhibition of STAT3 signaling pathway by nitidine chloride suppressed the angiogenesis and growth of human gastric cancer. Mol Cancer Ther 11: 277-287, 2012.
22. Mou H, Guo P, Li X, Zhang C, Jiang J, Wang L, Wang Q and Yuan Z: Nitidine chloride inhibited the expression of S phase kinase-associated protein 2 in ovarian cancer cells. Cell Cycle 16: 1366-1375, 2017.
23. Sun M, Zhang N, Wang X, Li Y, Qi W, Zhang H, Li Z and Yang Q: Hedgehog pathway is involved in nitidine chloride induced inhibition of epithelial-mesenchymal transition and cancer stem cells-like properties in breast cancer cells. Cell Biosci 6: 44, 2016.

24. Ou X, Lu Y, Liao L, Li D, Liu L, Liu H and Xu H: Nitidine chloride induces apoptosis in human hepatocellular carcinoma cells through a pathway involving p53, p21, Bax and Bcl-2. Oncol Rep 33: 1264-1274, 2015.
25. Lin J, Shen A, Chen H, Liao J, Xu T, Liu L, Lin J and Peng J: Nitidine chloride inhibits hepatic cancer growth via modulation of multiple signaling pathways. BMC Cancer 14: 729, 2014.
26. Makhey D, Gatto B, Yu C, Liu A, Liu LF and LaVoie EJ: Coralyne and related compounds as mammalian topoisomerase I and topoisomerase II poisons. Bioorg Med Chem 4: 781-791, 1996.
27. Prado S, Michel S, Tillequin F, Koch M, Pfeiffer B, Pierré A, Léonce S, Colson P, Baldeyrou B, Lansiaux A, *et al*: Synthesis and cytotoxic activity of benzo[c][1,7] and [1,8]phenanthrolines analogues of nitidine and fagaronine. Bioorg Med Chem 12: 3943-3953, 2004.
28. Chen G, Umelo IA, Lv S, Teugels E, Fostier K, Kronenberger P, Dewaele A, Sadones J, Geers C and De Grève J: miR-146a inhibits cell growth, cell migration and induces apoptosis in non-small cell lung cancer cells. PLoS One 8: e60317, 2013.
29. Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, *et al*: NCBI GEO: Archive for functional genomics data sets - update. Nucleic Acids Res 41: D991-D995, 2013.
30. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, Barrette T, Pandey A and Chinnaiyan AM: ONCOMINE: A cancer microarray database and integrated data-mining platform. Neoplasia 6: 1-6, 2004.
31. Kolesnikov N, Hastings E, Keays M, Melnichuk O, Tang YA, Williams E, Dylag M, Kurbatova N, Brandizi M, Burdett T, *et al*: ArrayExpress update - simplifying data submissions. Nucleic Acids Res 43: D1113-D1116, 2015.
32. Deng M, Brägelmann J, Schultze JL and Perner S: Web-TCGA: An online platform for integrated analysis of molecular cancer data sets. BMC Bioinformatics 17: 72, 2016.
33. Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, *et al*: Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 6: pl1, 2013.
34. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, *et al*: Proteomics. Tissue-based map of the human proteome. Science 347: 1260419, 2015.
35. Huang W, Sherman BT and Lempicki RA: Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. Nat Protoc 4: 44-57, 2009.
36. Burley SK, Berman HM, Christie C, Duarte JM, Feng Z, Westbrook J, Young J and Zardecki C: RCSB Protein Data Bank: Sustaining a living digital data resource that enables breakthroughs in scientific research and biomedical education. Protein Sci 27: 316-330, 2018.
37. Gao J, Liang L, Zhu Y, Qiu S, Wang T and Zhang L: Ligand and structure-based approaches for the identification of peptide deformylase inhibitors as antibacterial drugs. Int J Mol Sci 17: 17, 2016.
38. Orabi KY, Abaza MS, El Sayed KA, Elnagar AY, Al-Attiyah R and Guleri RP: Selective growth inhibition of human malignant melanoma cells by syringic acid-derived proteasome inhibitors. Cancer Cell Int 13: 82, 2013.
39. He HZ, Wang N, Zhang J, Zheng L and Zhang YM: Tas13D inhibits growth of SMMC-7721 cell via suppression VEGF and EGF expression. Asian Pac J Cancer Prev 13: 2009-2014, 2012.
40. Wurmbach E, Chen YB, Khitrov G, Zhang W, Roayaie S, Schwartz M, Fiel I, Thung S, Mazzaferro V, Bruix J, *et al*: Genome-wide molecular profiles of HCV-induced dysplasia and hepatocellular carcinoma. Hepatology 45: 938-947, 2007.
41. Mas VR, Maluf DG, Archer KJ, Yanek K, Kong X, Kulik L, Freise CE, Olthoff KM, Ghobrial RM, McIver P, *et al*: Genes involved in viral carcinogenesis and tumor initiation in hepatitis C virus-induced hepatocellular carcinoma. Mol Med 15: 85-94, 2009.
42. Satow R, Shitashige M, Kanai Y, Takeshita F, Ojima H, Jigami T, Honda K, Kosuge T, Ochiya T, Hirohashi S, *et al*: Combined functional genome survey of therapeutic targets for hepatocellular carcinoma. Clin Cancer Res 16: 2518-2528, 2010.
43. Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, Thorgeirsson SS, Sun Z, Tang ZY, Qin LX, *et al*: A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. Cancer Res 70: 10202-10212, 2010.
44. Lim HY, Sohn I, Deng S, Lee J, Jung SH, Mao M, Xu J, Wang K, Shi S, Joh JW, *et al*: Prediction of disease-free survival in hepatocellular carcinoma by gene expression profiling. Ann Surg Oncol 20: 3747-3753, 2013.
45. Neumann O, Kesselmeier M, Geffers R, Pellegrino R, Radlwimmer B, Hoffmann K, Ehemann V, Schemmer P, Schirmacher P, Lorenzo Bermejo J, *et al*: Methylome analysis and integrative profiling of human HCCs identify novel protumorigenic factors. Hepatology 56: 1817-1827, 2012.
46. Kim JH, Sohn BH, Lee HS, Kim SB, Yoo JE, Park YY, Jeong W, Lee SS, Park ES, Kaseb A, *et al*: Genomic predictors for recurrence patterns of hepatocellular carcinoma: Model derivation and validation. PLoS Med 11: e1001770, 2014.
47. Villa E, Critelli R, Lei B, Marzocchi G, Cammà C, Giannelli G, Pontisso P, Cabibbo G, Enea M, Colopi S, *et al*: Neoangiogenesis-related genes are hallmarks of fast-growing hepatocellular carcinomas and worst survival. Results from a prospective study. Gut 65: 861-869, 2016.
48. Melis M, Diaz G, Kleiner DE, Zamboni F, Kabat J, Lai J, Mogavero G, Tice A, Engle RE, Becker S, *et al*: Viral expression and molecular profiling in liver tissue versus microdissected hepatocytes in hepatitis B virus-associated hepatocellular carcinoma. J Transl Med 12: 230, 2014.
49. Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J, Soysouvanh F, *et al*: Exome sequencing of hepatocellular carcinomas identifies new mutational signatures and potential therapeutic targets. Nat Genet 47: 505-511, 2015.
50. Mah WC, Thurnherr T, Chow PK, Chung AY, Ooi LL, Toh HC, Teh BT, Sauntharajah Y and Lee CG: Methylation profiles reveal distinct subgroup of hepatocellular carcinoma patients with poor prognosis. PLoS One 9: e104158, 2014.
51. Wang YH, Cheng TY, Chen TY, Chang KM, Chuang VP and Kao KJ: Plasmalemmal Vesicle Associated Protein (PLVAP) as a therapeutic target for treatment of hepatocellular carcinoma. BMC Cancer 14: 815, 2014.
52. Makowska Z, Boldanova T, Adametz D, Quagliata L, Vogt JE, Dill MT, Matter MS, Roth V, Terracciano L and Heim MH: Gene expression analysis of biopsy samples reveals critical limitations of transcriptome-based molecular classifications of hepatocellular carcinoma. J Pathol Clin Res 2: 80-92, 2016.
53. Wijetunga NA, Pascual M, Tozour J, Delahaye F, Alani M, Adeyeye M, Wolkoff AW, Verma A and Greally JM: A pre-neoplastic epigenetic field defect in HCV-infected liver at transcription factor binding sites and polycomb targets. Oncogene 36: 2030-2044, 2017.
54. Grinchuk OV, Yenamandra SP, Iyer R, Singh M, Lee HK, Lim KH, Chow PK and Kuznetsov VA: Tumor-adjacent tissue co-expression profile analysis reveals pro-oncogenic ribosomal gene signature for prognosis of resectable hepatocellular carcinoma. Mol Oncol 12: 89-113, 2018.
55. Cancer Cell Line Encyclopedia C: Genomics of Drug Sensitivity in Cancer C: Pharmacogenomic agreement between two cancer cell line data sets. Nature 528: 84-87, 2015.
56. Tang Z, Li C, Kang B, Gao G, Li C and Zhang Z: GEPIA: A web server for cancer and normal gene expression profiling and interactive analyses. Nucleic Acids Res 45: W98-W102, 2017.
57. Aguirre-Gamboa R, Gomez-Rueda H, Martínez-Ledesma E, Martínez-Torteya A, Chacolla-Huaringa R, Rodriguez-Barrientos A, Tamez-Peña JG and Treviño V: SurvExpress: An online biomarker validation tool and database for cancer gene expression data using survival analysis. PLoS One 8: e74250, 2013.
58. Wang JC: Cellular roles of DNA topoisomerases: A molecular perspective. Nat Rev Mol Cell Biol 3: 430-440, 2002.
59. Chhatriwala H, Jafri N and Salgia R: A review of topoisomerase inhibition in lung cancer. Cancer Biol Ther 5: 1600-1607, 2006.
60. Bush NG, Evans-Roberts K and Maxwell A: DNA topoisomerases. Ecosal Plus 6: 6, 2015.
61. Hou GX, Liu P, Yang J and Wen S: Mining expression and prognosis of topoisomerase isoforms in non-small-cell lung cancer by using Oncomine and Kaplan-Meier plotter. PLoS One 12: e0174515, 2017.
62. Capranico G, Marinello J and Chillemi G: Type I DNA topoisomerases. J Med Chem 60: 2169-2192, 2017.
63. Chen T, Sun Y, Ji P, Kopetz S and Zhang W: Topoisomerase IIα in chromosome instability and personalized cancer therapy. Oncogene 34: 4019-4031, 2015.
64. Ang C, Miura JT, Gamblin TC, He R, Xiu J, Millis SZ, Gatalica Z, Reddy SK, Yee NS and Abou-Alfa GK: Comprehensive multiplatform biomarker analysis of 350 hepatocellular carcinomas identifies potential novel therapeutic options. J Surg Oncol 113: 55-61, 2016.

65. Wong N, Yeo W, Wong WL, Wong NL, Chan KY, Mo FK, Koh J, Chan SL, Chan AT, Lai PB, *et al*: TOP2A overexpression in hepatocellular carcinoma correlates with early age onset, shorter patients survival and chemoresistance. Int J Cancer 124: 644-652, 2009.
66. Panvichian R, Tantiwetrueangdet A, Angkathunyakul N and Leelaudomlipi S: TOP2A amplification and overexpression in hepatocellular carcinoma tissues. BioMed Res Int 2015: 381602, 2015.
67. Isaacs RJ, Davies SL, Sandri MI, Redwood C, Wells NJ and Hickson ID: Physiological regulation of eukaryotic topoisomerase II. Biochim Biophys Acta 1400: 121-137, 1998.
68. Srikantan S, Abdelmohsen K, Lee EK, Tominaga K, Subaran SS, Kuwano Y, Kulshrestha R, Panchakshari R, Kim HH, Yang X, *et al*: Translational control of TOP2A influences doxorubicin efficacy. Mol Cell Biol 31: 3790-3801, 2011.
69. Calderwood SK: A critical role for topoisomerase IIb and DNA double strand breaks in transcription. Transcription 7: 75-83, 2016.
70. Szafran M, Skut P, Ditkowski B, Ginda K, Chandra G, Zakrzewska-Czerwińska J, Jakimowicz D and Topoisomerase I: Topoisomerase I (TopA) is recruited to ParB complexes and is required for proper chromosome organization during Streptomyces coelicolor sporulation. J Bacteriol 195: 4445-4455, 2013.
71. Li TK and Liu LF: Tumor cell death induced by topoisomerase-targeting drugs. Annu Rev Pharmacol Toxicol 41: 53-77, 2001.
72. Hande KR: Clinical applications of anticancer drugs targeted to topoisomerase II. Biochim Biophys Acta 1400: 173-184, 1998.
73. Cort A and Ozben T: Natural product modulators to overcome multidrug resistance in cancer. Nutr Cancer 67: 411-423, 2015.
74. Rodrigues T, Sieglitz F and Bernardes GJ: Natural product modulators of transient receptor potential (TRP) channels as potential anti-cancer agents. Chem Soc Rev 45: 6130-6137, 2016.

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