


The Genomic and Proteomic Profiles of NTRK Genes and Trk Receptors in Liver Hepatocellular Carcinoma

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ABSTRACT

BACKGROUND: The neurotrophic tyrosine kinase (NTRK) gene family includes NTRK1, NTRK2, and NTRK3, which encode tropomyosin receptor kinases TrkA, TrkB, and TrkC, respectively. This study aimed to initially assess the genomic and proteomic profiles of NTRK genes and Trk receptors in liver hepatocellular carcinoma (LIHC).

METHODS: The ONCOMINE, UALCAN, GEPIA, cBioPortal, FusionGDB, SurvivalMeth, and the Human Protein Atlas databases were searched for NTRK gene expression and protein data in LIHC. Immunohistochemistry was used to detect pan-Trk expression across a commercial microarray containing 96 hepatocellular carcinoma (HCC) and 94 para-cancerous tissue spots. A modified histological score (H-score) with a maximum score of 300 was used to quantify immunohistochemical staining for pan-Trk. Student's t- and chi-square tests were the main statistical analyses used.

RESULTS: The transcriptional levels of NTRK genes in LIHC were not significantly different from healthy controls. Using UALCAN and GEPIA, only high expression of NTRK2 was significantly associated with longer disease-free survival ($P=0.004$). The alteration frequencies were low (7% in NTRK1, 1.7% in NTRK2, and 2% in NTRK3). The methylation levels of NTRK genes were all significantly different as analyzed by UALCAN; the high-risk group displayed an unfavorable prognosis compared with the low-risk group for NTRK1 ($P=0.033$) and NTRK3 ($P=0.005$). The median H-score of pan-Trk in HCC and para-cancerous tissues was not statistically different (186.31 ± 23.86 and 192.38 ± 21.06 , $P=0.065$). No differences were observed in clinicopathological features of HCC with the median H-score for pan-Trk expression ($p > 0.05$). The survival rate of patients with pan-Trk expression was also not significantly different.

CONCLUSION: The alteration frequency was low in NTRK genes, including gene fusion and methylation levels. Therefore, pan-Trk expression in HCC tissue has limited value in clinicopathological features and prognosis.

KEYWORDS: Liver hepatocellular carcinoma, NTRK, Trk, bioinformatics, immunohistochemistry

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Introduction

Liver hepatocellular carcinoma (LIHC) was the sixth most diagnosed cancer with a high mortality rate.¹ The prognosis for LIHC is still poor, with a maximum 5-year survival rate of 18% in high-income countries.² Therefore, understanding the pathogenesis of and discovering new therapeutic targets for LIHC may play an important role in developing more precise treatments and improving prognoses.

The neurotrophic tyrosine kinase (NTRK) family comprises three genes, *NTRK1*, *NTRK2*, and *NTRK3*, which encode the tropomyosin receptor kinases TrkA, TrkB, and TrkC, respectively.³ These *NTRK* genes are located on human chromosomes 1q23.1, 9q21.33, and 15q25.3, respectively.⁴ The first-generation Trk inhibitors, larotrectinib, and entrectinib, were approved for

treating *NTRK* fusion-positive solid tumors.⁵ For larotrectinib treatment of *NTRK* fusion-positive cancer, the proportion of patients with an objective response rate was 79% (95% confidence interval (CI): 72%–85%), with 16% of patients having complete responses.⁶ Another Trk inhibitor, entrectinib, was approved by the U.S. Food and Drug Administration (FDA) on August 15, 2019, based on the demonstration of a durable overall response rate of 57% (95% CI: 43%–71%).⁷ Other Trk inhibitors include crizotinib, cabozantinib, lestaurtinib, altiratinib, foretinib, ponatinib, nintedanib, merestinib, MGCD516, PLX7486, DS-6051b, and TSR-011, all of which have been or will be used in clinical trials.^{8,9} Although Trk receptors play an important role in treating *NTRK* fusion-positive solid tumors, little is known about the relationship between Trk receptors and LIHC.



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In this study, we utilized a series of bioinformatic databases to explore the genomic profiles of the *NTRK* gene family, including transcription levels, genetic variation, and protein expression, in LIHC. In addition, immunohistochemistry (IHC) staining was used to explore the relationship between pan-Trk and hepatocellular carcinoma (HCC).

Materials and Methods

ONCOMINE

ONCOMINE (www.oncomine.org) is a translational bioinformatics service that comprises 715 datasets and 86,733 samples for genome-wide expression analyses.¹⁰ Data were extracted to evaluate the expression of *NTRK* genes in LIHC. This study used the default system values during the exploration process. A student's *t*-test was used to analyze differences in the expression of *NTRK* genes in LIHC.

University of Alabama at Birmingham cancer data analysis

The University of Alabama at Birmingham cancer data analysis portal (UALCAN, <http://ualcan.path.uab.edu/analysis.html>), a comprehensive web resource, provides analyses from The Cancer Genome Atlas (TCGA) and MET500 cohort data.¹¹ UALCAN was used in our study to generate graphs depicting gene expression and survival curves. Expression data for the *NTRK* genes were obtained using the expression module. Survival analysis was performed using the survival module. A Student's *t*-test was used to generate *P* values. The *P*-value cut-off was set at 0.05.

Gene expression profiling interactive analysis

Gene Expression Profiling Interactive Analysis (GEPIA, <http://gepia.cancer-pku.cn/index.html>) is an analysis tool developed at Peking University that contains expression data from RNA sequencing data of 9736 tumors and 8587 normal tissue samples.¹² We performed a prognostic analysis of *NTRK* genes with the "Single Gene Analysis" module. The resulting hazard ratios and *P*-values, or Cox *P*-values from the log-rank test, were plotted. Prognostic analysis was performed using Kaplan–Meier curves.

CBioPortal

cBioPortal (www.cbioportal.org), a comprehensive web resource, is used to visualize and analyze cancer genomics data based on the TCGA database.¹³ Genetic alterations were obtained using cBioPortal. Additionally, patterns of gene alterations were visualized, gene alteration frequencies were compared, and all relevant genomic alterations were summarized.

Fusion gene annotation DataBase

Fusion Gene annotation DataBase (FusionGDB, <https://ccsm.uth.edu/FusionGDB>) contains a collection of 48,117 pan-cancer

fusion genes from three representative fusion gene resources: Chimeric Transcripts and RNA-seq data (ChiTaRS 3.1), TumorFusions (an integrative resource for cancer-associated transcript fusions), and TCGA fusions, developed by Gao et al.¹⁴ FusionGDB provides six categories of annotations: FusionGeneSummary, FusionProtFeature, FusionGeneSequence, FusionGenePPI, RelatedDrug, and RelatedDisease.¹⁵

Methylation and clinical analysis

SurvivalMeth (<http://bio-bigdata.hrbmu.edu.cn/survival-meth/>) was used to investigate the effect of DNA methylation-related functional elements on prognosis, and multiple types of commonly used functional elements associated with DNA methylation were considered.¹⁶

The Human Protein Atlas

The Human Protein Atlas (<https://www.proteinatlas.org/>) is a freely accessible website that contains information regarding protein expression patterns in human tissues and cells.¹⁷ We analyzed the protein expression of Trk receptors in liver cancer and normal tissues using immunohistochemistry, as detailed below.

Immunohistochemical staining of pan-Trk in the hepatocellular tissue microarray

A commercial tissue microarray was purchased from Shanghai Outdo Biotech Co., Ltd. (HLiv-HCC197Sur-01; Shanghai, China). Clinical diagnoses and tumor-node-metastasis (TNM) stages were obtained from the patient's clinical data, which were provided by the company. The Union for International Cancer Control's TNM staging system was applied according to the eighth edition of the Cancer Staging Manual. The tissue microarray contained 96 HCC and 94 para-cancerous spots. Microarray samples are described in detail in Table 1. The patients underwent surgical resection between July 2010 and March 2012. All patients with complete clinical and follow-up data until November 2017 provided written informed consent.

Pan-Trk levels in tissue samples were evaluated using IHC, which was performed according to the standard streptavidin-peroxidase method. Briefly, histological slides of tissue samples were deparaffinized using xylene and an alcohol gradient. The antigen was retrieved using citric acid antigen repair buffer (pH 6.0; Wuhan Goodbio Technology Co. Ltd, Hubei, China). Endogenous peroxidase activity was blocked by incubating the slides in a 3% H₂O₂ solution prepared in methanol at room temperature for 25 minute. The slides were washed with phosphate-buffered saline (PBS, pH 7.4) and blocked using a blocking buffer (10% fetal bovine serum in PBS; Boster Bioengineering, Hubei, China). The slides were subsequently incubated with rabbit anti-human polyclonal antibody against pan-Trk (ab76291, dilution 1:500; Abcam, Cambridge, UK), which reacts with a conserved proprietary peptide in the C-terminal portion

Table 1. Clinical characteristics of tissue microarray.

	CANCER	PARA-CANCEROUS
Case number	96	94
Gender		
Male	82	81
Female	14	13
Age, year		
< 50	27	25
≥ 50	69	69
HBV infection		
Yes	79	79
No	11	10
Missing	6	5
Liver cirrhosis		
Yes	70	69
No	20	19
Missing	6	6
Cancer embolus		
Yes	26	26
No	59	57
Missing	11	11
AFP (ng/mL)		
<400	55	54
≥400	28	28
Missing	13	12
CK19		
Yes	30	29
No	66	65
Missing	0	0
TNM stage		
I	19	
II	40	
III	28	
IV	9	

of TrkA, B, and C, at 4°C overnight. Thereafter, the slides were washed and incubated with polyclonal anti-rabbit immunoglobulin G secondary antibody (dilution 1:200; Wuhan Goodbio Technology). Immunocomplexes were detected using 3,3'-diaminobenzidine (Dako, Copenhagen, Denmark). Staining was

monitored under a microscope and terminated when sufficient staining was achieved. The slides were dehydrated and stored. Tissue images were acquired and analyzed using CaseViewer (Danjier, Shandong, China). The histological score (H-score), defined as a continuous variable for each sample, was determined by two pathologists in our hospital blinded to clinical and molecular data and was calculated using the following formula: H-score = (1 × % low intensity cells [1 +]) + (2 × % medium intensity cells [2 +]) + (3 × % high intensity cells [3 +]). H-scores ranging from 0 to 300 indicate stained tumor samples. High and low scores of pan-Trk expression were defined using the median H-score in HCC tissues as the threshold.

Statistical analysis

All statistical analyses were performed using SPSS software (version 22.0; SPSS, Chicago, IL, USA). H-scores were expressed as median ± standard and were analyzed using a Student's *t*-test. Pan-Trk expression levels and clinicopathological features of patients with HCC were analyzed using a chi-square test. The roles of pan-Trk in survival were assessed using the Kaplan–Meier method.

Results

Expression of *NTRK* genes in patients with LIHC

NTRK1, *NTRK2*, and *NTRK3* expression patterns were studied using the ONCOMINE database. We first explored the transcriptional levels of *NTRK* genes in LIHC and normal tissue using ONCOMINE (Figure 1). Based on the data from ONCOMINE, the transcriptional levels of *NTRK* genes in LIHC tissues were not significantly different from the normal controls ($P=0.148$ for *NTRK1*, $P=0.616$ for *NTRK2*, and $P=0.972$ for *NTRK3*). We assessed the expression levels of *NTRK* genes in 371 cases of LIHC and 50 normal controls using UALCAN, which were not significantly different between groups ($P=0.244$ for *NTRK1*, $P=0.078$ for *NTRK2*, and $P=0.681$ for *NTRK3*) (Figure 1D to F).

Prognostic value of *NTRK* mRNA in patients with LIHC

UALCAN and GEPIA were used to evaluate the prognostic value of differentially expressed *NTRK* genes in the progression of LIHC. The survival curves generated from UALCAN showed no differences in survival between different expression levels of *NTRK* genes ($P=0.15$ for *NTRK1*, $P=0.37$ for *NTRK2*, and $P=0.17$ for *NTRK3*) (Figure 2A to C). Overall survival (OS) and disease-free survival (DFS) curves were further explored by GEPIA and are shown in Figure 2D to I. Neither the differential expression of *NTRK1* nor that of *NTRK3* were significantly associated with OS ($P=0.94$, 0.87 , respectively) or DFS ($P=0.68$, 0.42 , respectively). High expression of *NTRK2* was significantly associated with longer DFS ($P=0.004$), but not OS ($P=0.87$).

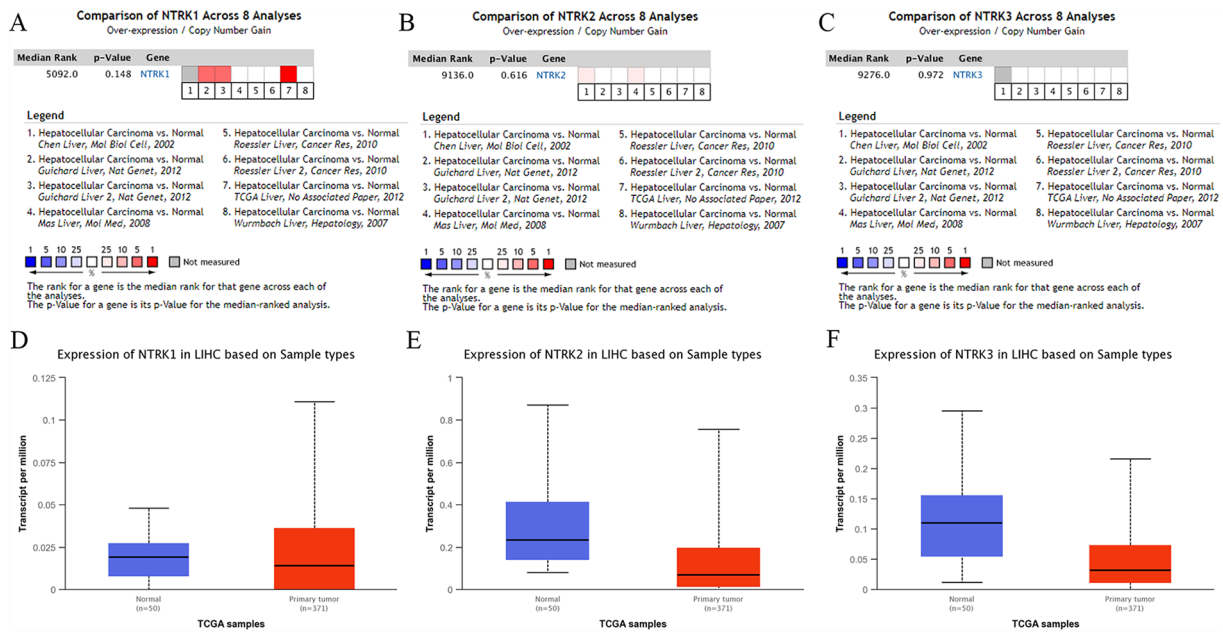


Figure 1. The expression levels of *NTRK* genes in the ONCOMINE (A-C) and UALCAN (D-E) databases.

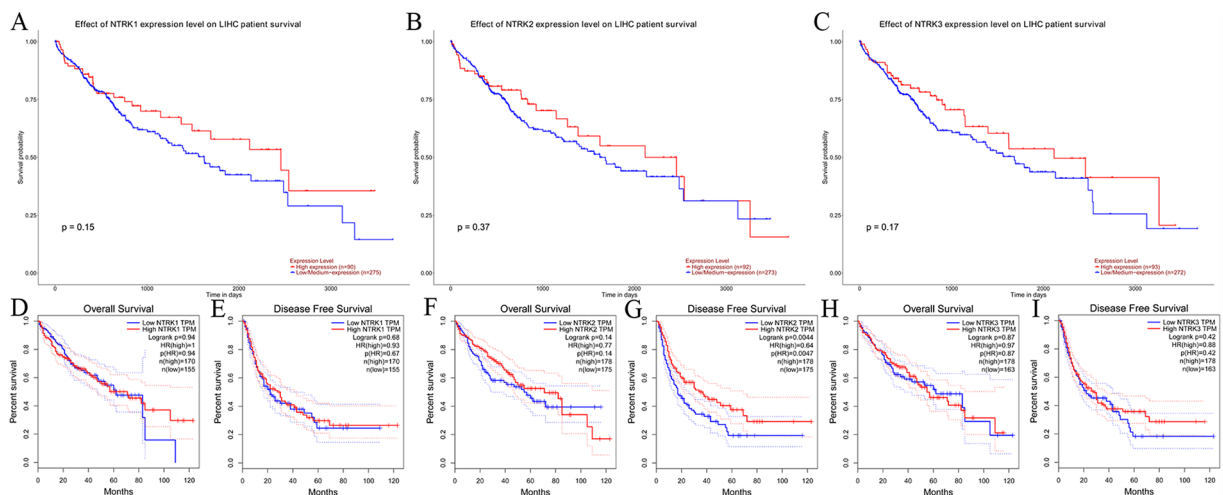


Figure 2. The prognostic value of *NTRK* mRNA in patients with LIHC as determined by using the UALCAN and GEPIA databases. A-C: Survival curves from UALCAN. D-I: Overall survival (OS) and disease-free survival (DFS) analysis by GEPIA.

Genetic alteration analysis

OncoPrint in cBioPortal was used to explore the genetic alterations of *NTRK* genes in LIHC using data from 1438 patients across six studies. As a result, the alteration frequency of *NTRK1* was determined to be 7% (altered/profiled = 102/1375) and was due to amplifications (n = 91) and mutations (n = 11). The alteration frequency of *NTRK2* was 1.7% (altered/profiled = 24/1375) and was due to mutations (n = 22), multiple alterations (n = 1), and a deep deletion (n = 1). The alteration frequency of *NTRK3* was 2% (altered/profiled = 28/1375) and was due to mutations (n = 25) and amplifications (n = 3). The results are shown in Figure 3A to D.

As FusionGDB aims to provide a resource or reference for the functional annotation of fusion genes in cancer for better

therapeutic targets, a fusion gene analysis through this database was used to identify 16 *NTRK1*, 8 *NTRK2*, and 15 *NTRK3* fusion genes (Table 2).

The methylation levels of *NTRK* genes were analyzed using UALCAN. Hyper- and hypomethylation are generally indicated by beta values of 0.7–0.5 and 0.3–0.25, respectively. Compared to normal tissues, *NTRK1* had a median beta value of 0.292 (0.331 for normal tissues, $P = 1.840 \times 10^{-6}$, Figure 4A), *NTRK2* had a median beta value of 0.144 (0.106 for normal tissues, $P = 1.624 \times 10^{-12}$, Figure 4B), and *NTRK3* had a median beta value of 0.089 (0.054 for normal tissues, $P = 1.624 \times 10^{-12}$, Figure 4C). Survival related to methylation in LIHC was explored using SurvivalMeth. Detailed information about probe IDs and DNA methylation levels of *NTRK* genes in the normal and LIHC groups are presented in Figure 4D to F. The

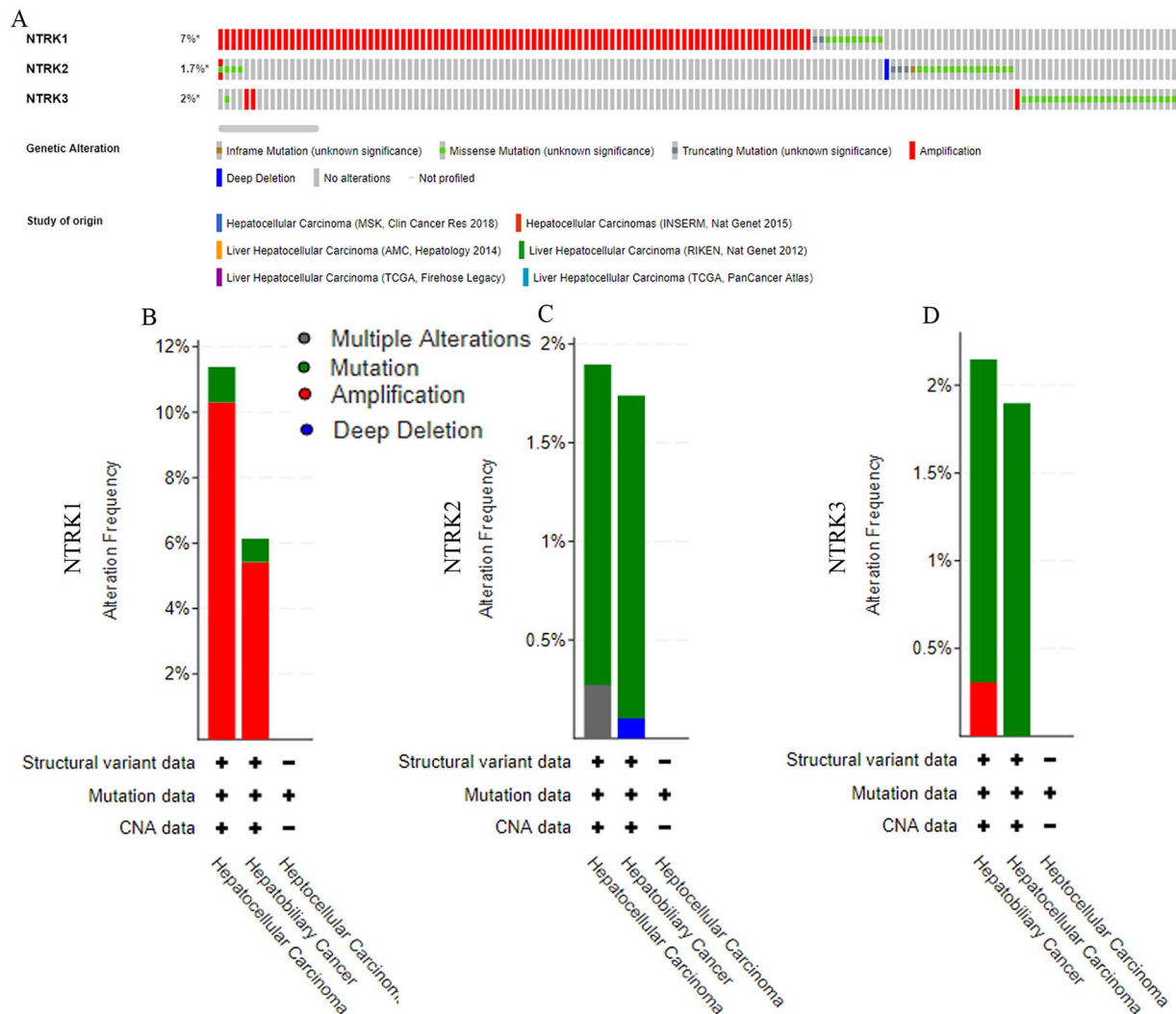


Figure 3. Genetic alteration analysis of LIHC in cBioPortal. A: general overview of genetic alterations. B-D: Proportions of alteration frequencies and genetic types.

high-risk group displayed an unfavorable prognosis compared with the low-risk group for *NTRK1* ($P=0.033$) and *NTRK3* ($P=0.005$) but not for *NTRK2* ($P=0.061$) (Figure 4E to I).

Immunohistochemistry staining data from the Human Protein Atlas

Most cancer tissues exhibited weak to moderate cytoplasmic immunoreactivity to Trk receptors, often with a granular pattern for TrkA (Figure 5A). Of the eight patients examined, one had high TrkA expression and six had medium expression levels (Figure 5B). A few cases of urothelial, lung, pancreatic, and head and neck cancers showed moderate to strong cytoplasmic positivity for TrkB, while the other cancers were mainly negative (Figure 5C). No patients with liver cancer had positive staining for TrkB (Figure 5D). Most cancer cells were negative for TrkC. Lymphomas and colorectal, cervical, and liver cancer tissues showed weak to moderate cytoplasmic and nuclear staining for TrkC in a fraction of cells (Figure 5E). One of the

four patients with liver cancer showed a medium TrkC expression level (Figure 5F).

Expression levels of pan-Trk in HCC and para-cancerous tissues

IHC staining images showing pan-Trk expression levels in HCC and para-cancerous tissues are presented in Figure 6. Positive staining was observed in the cytoplasmic and membranous areas. In HCC tissue, the median H-score of pan-Trk was 186.31 ± 23.86 . The median H-score was 192.38 ± 21.06 in para-cancerous tissues. No statistical significance was observed using a Student's *t*-test ($P=0.065$). After dividing pan-Trk expression into high and low expression groups according to the median value of pan-Trk, the value indexes of sex, age, hepatitis B virus (HBV) infection, liver cirrhosis, cancer embolus status, alpha-fetoprotein (AFP) level, CK19 expression, and TNM stage of patients with HCC were tested. No differences were observed in these value indexes for high or

Table 2. The fusion gene analysis of *NTRK* genes in FusionGDB.

FUSIONGID	FUSIONGENE	HGENE	HGID	TGENE	TGID
2554	ARHGEF25_NTRK1	ARHGEF25	115557	NTRK1	4914
6254	CD74_NTRK1	CD74	972	NTRK1	4914
6756	CEL_NTRK1	CEL	1056	NTRK1	4914
11820	EPHB2_NTRK1	EPHB2	2048	NTRK1	4914
17721	IRF2BP2_NTRK1	IRF2BP2	359948	NTRK1	4914
19900	LMNA_NTRK1	LMNA	4000	NTRK1	4914
22024	MIR548F1_NTRK1	MIR548F1	100302192	NTRK1	4914
22397	MPRIP_NTRK1	MPRIP	23164	NTRK1	4914
24134	NFASC_NTRK1	NFASC	23114	NTRK1	4914
25015	NTRK1_DYNC2H1	NTRK1	4914	DYNC2H1	79659
31952	RPL7A_NTRK1	RPL7A	6130	NTRK1	4914
35894	SQSTM1_NTRK1	SQSTM1	8878	NTRK1	4914
36123	SSBP2_NTRK1	SSBP2	23635	NTRK1	4914
37852	TFG_NTRK1	TFG	10342	NTRK1	4914
39236	TPM3_NTRK1	TPM3	7170	NTRK1	4914
42540	ZBTB7B_NTRK1	ZBTB7B	51043	NTRK1	4914
991	AFAP1_NTRK2	AFAP1	60312	NTRK2	4915
25016	NTRK2_AFAP1	NTRK2	4915	AFAP1	60 312
25017	NTRK2_LAP3	NTRK2	4915	LAP3	51 056
25018	NTRK2_NTRK2	NTRK2	4915	NTRK2	4915
25019	NTRK2_RASEF	NTRK2	4915	RASEF	158 158
25881	PAN3_NTRK2	PAN3	255967	NTRK2	4915
35895	SQSTM1_NTRK2	SQSTM1	8878	NTRK2	4915
39475	TRIM24_NTRK2	TRIM24	8805	NTRK2	4915
1322	AKAP13_NTRK3	AKAP13	11214	NTRK3	4916
11565	EML4_NTRK3	EML4	27436	NTRK3	4916
12186	ETV6_NTRK3	ETV6	2120	NTRK3	4916
12895	FAT1_NTRK3	FAT1	2195	NTRK3	4916
20444	LYN_NTRK3	LYN	4067	NTRK3	4916
25020	NTRK3_ACTR8	NTRK3	4916	ACTR8	93 973
25021	NTRK3_ETV6	NTRK3	4916	ETV6	2120
25022	NTRK3_LOXL2	NTRK3	4916	LOXL2	4017
25023	NTRK3_NTRK3	NTRK3	4916	NTRK3	4916
25024	NTRK3_PEA1	NTRK3	4916	PEAK1	79 834
25025	NTRK3_RBPMS	NTRK3	4916	RBPMS	11 030
25026	NTRK3_SLC8B1	NTRK3	4916	SLC8B1	80 024
30836	RBPMS_NTRK3	RBPMS	11 030	NTRK3	4916
35839	SPTAN1_NTRK3	SPTAN1	6709	NTRK3	4916
41558	VPS18_NTRK3	VPS18	57 617	NTRK3	4916

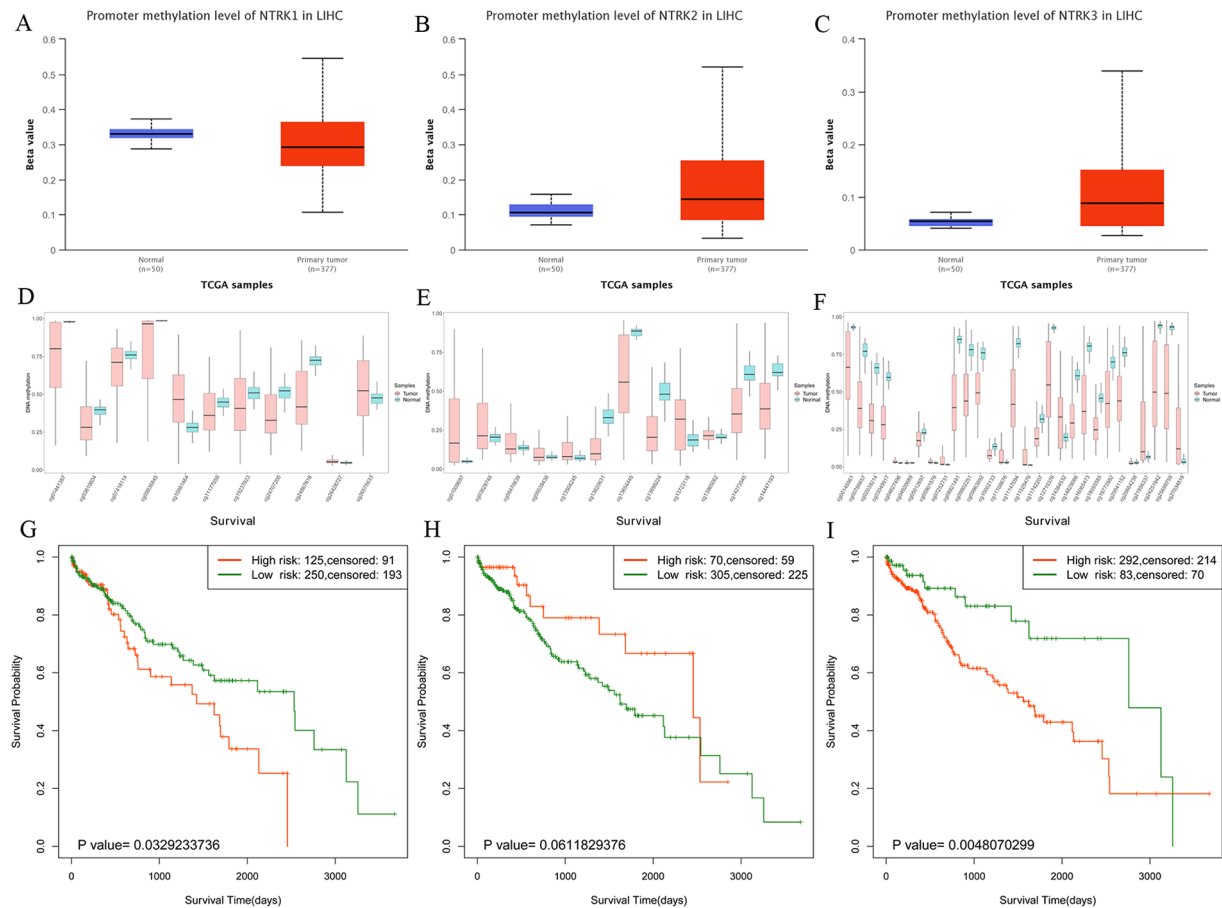


Figure 4. Methylation levels of *NTRK* genes and survival analysis in the UALCAN database. A-C: Methylation levels of *NTRK* genes in LIHC and normal groups. D-F: Methylation levels of *NTRK* genes presented by this study. G-I: Survival of LIHC in relation to methylation of *NTRK* genes.

low expression of pan-Trk ($P > 0.05$, Table 3). After follow-up, 49 of 96 patients survived, and 47 died. According to the Kaplan–Meier survival analysis, the survival rates of patients with high vs low pan-Trk expression were not significantly different ($P = 0.392$, Figure 6).

Discussion

There were 390,000 liver cancer-related deaths in China in 2020, second only to lung cancer.¹⁸ Typically, only 5–15% of patients are eligible for surgical removal. Neither current ablation therapies nor chemotherapy effectively improves the outcomes of this devastating disease.¹⁹

In the ONCOMINE database, the transcriptional levels of *NTRK* genes in LIHC tissues were not significantly different from those in normal individuals. The survival curves generated from UALCAN showed no relationship between the expression of *NTRK* genes and survival. Further exploration of the OS and DFS curves using GEPIA showed that neither high nor low transcriptional levels of *NTRK1* or *NTRK3* were significantly associated with OS ($P = 0.94$ and 0.87 , respectively) or DFS ($P = 0.68$ and 0.42 , respectively). Only high expression of *NTRK2* was significantly associated with longer DFS ($P = 0.004$). The alteration frequencies were very low in all *NTRK* genes (*NTRK1*, 7%, *NTRK2*, 1.7%, and *NTRK3*, 2%).

Fusions involving *NTRK1*, *NTRK2*, or *NTRK3* are the most common mechanisms of oncogenic Trk activation.²⁰ *NTRK* fusions can be diagnosed using fluorescence in situ hybridization, reverse transcription polymerase chain reaction, DNA-based next-generation sequencing (NGS), and RNA-based NGS.^{21,22} Hsiao et al previously summarized the occurrence of *NTRK* fusion genes in cancer through different studies which found *NTRK1-LMNA*, *NTRK1-RABGAP1L*, and *NTRK1-TPM3* in cholangiocarcinoma; however, no *NTRK2* or *NTRK3* fusion genes were detected. Furthermore, there were no *NTRK* fusion genes associated with hepatocellular carcinoma.²³ However, analyses with FusionGDB revealed that *NTRK* gene fusions were diverse, including 16 *NTRK1* fusions, 8 *NTRK2* fusions, and 15 *NTRK3* fusions.

Compared to normal tissues, *NTRK1* was hypomethylated, and *NTRK2* and *NTRK3* were hypermethylated; these differences were statistically significant. The high-risk group displayed an unfavorable prognosis compared with the low-risk group for *NTRK1* ($P = 0.033$) and *NTRK3* ($P = 0.005$) but not for *NTRK2* ($P = 0.061$). Choi et al²⁴ found that higher Trk expression in tissues occurred in hepatocellular carcinoma cases (21 of 288 (7.3%)) with recurrence-free survival ($P = 0.092$) and OS ($P = 0.079$); no *NTRK* fusion genes were detected in 16 samples overexpressing Trk receptors. Another study showed

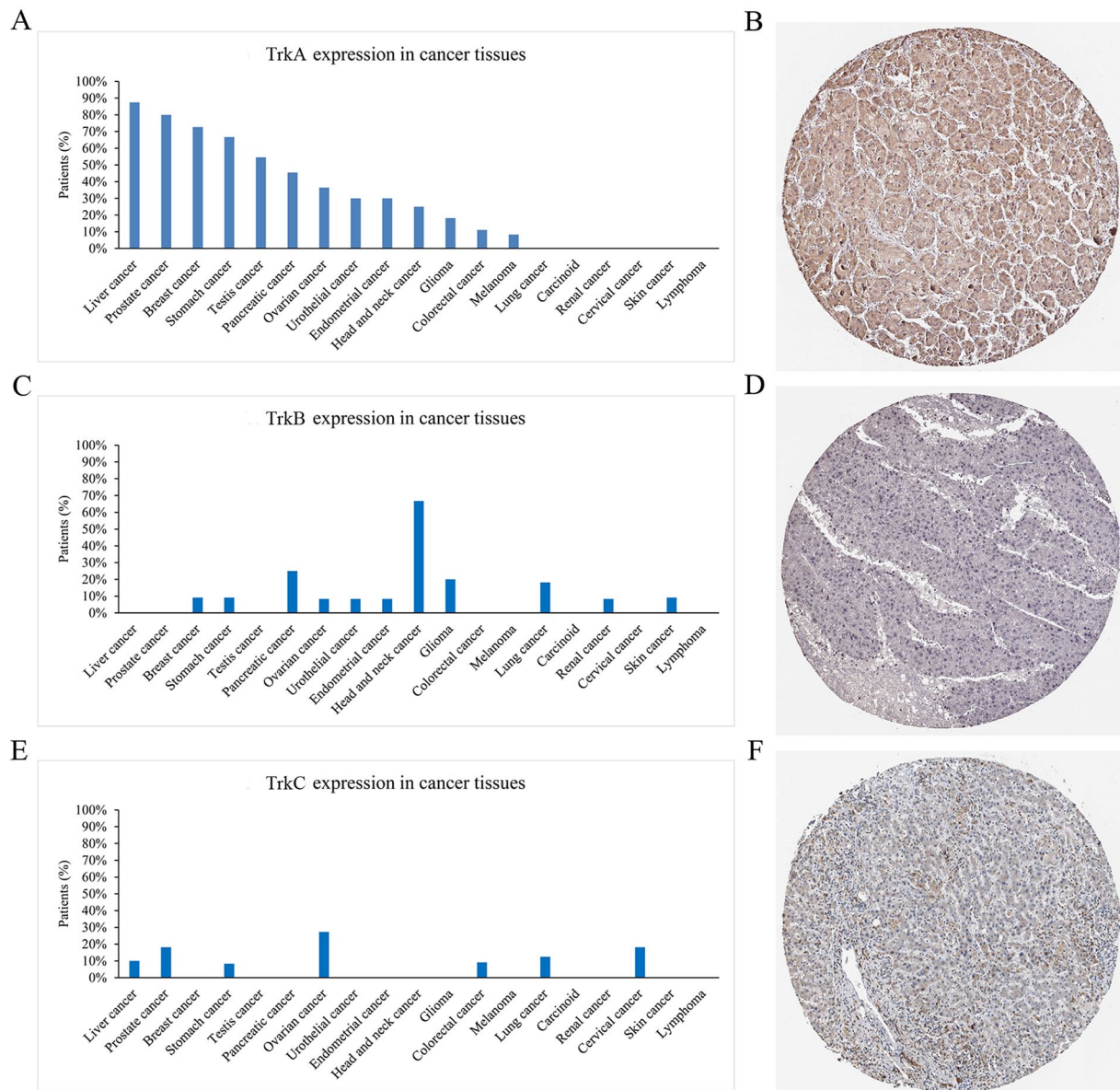


Figure 5. Trk receptor staining in cancer. A, C, and E: Proportions of Trk expression in cancer. B, D, and F: Representative images of Trk expression in liver cancer.

that the positive expression rate of TrkB in hepatocellular carcinoma tissues was 55.4% (36/65), significantly correlated with advanced stages of hepatocellular carcinoma.²⁵ Our study found that the differences in pan-Trk expression in LIHC and para-cancerous tissues were not statistically significant. Zhang and Liao²⁶ found that 12/110 (10.9%) cases of hepatocellular carcinoma showed weak cytoplasmic Trk expression by IHC, while all other hepatocellular carcinoma, including 69 intrahepatic cholangiocarcinomas, were negative for Trk. Our study may have observed no statistical difference in pan-Trk expression in LIHC and para-cancerous tissues due to weak or rare expression levels. Additionally, no differences were observed in the sex, age, HBV infection, liver cirrhosis, cancer embolus statuses or AFP level, CK19 expression, and TNM stage of patients with HCC with the median H-score for pan-Trk

expression. In addition, the survival rate of patients with pan-Trk expression was not significantly different.

To further our understanding of the role *NTRK* genes play in LIHC, we used the extensive experimental data made available through a series of bioinformatic databases. As different databases validated our results, our findings were compelling. However, there were some limitations to our work. For example, gene fusion and methylation statuses of *NTRK* genes are important indicators for LIHC, but we did not expand our research further. Additionally, though an IHC analysis was used to explore pan-Trk expression in HCC tissues, some patient information is lacking in China, such as HBV infection status and alcohol consumption levels. This is due to a patient's right to refuse some tests, especially those regarding HBV infection, liver cirrhosis, and cancer embolus statuses or AFP

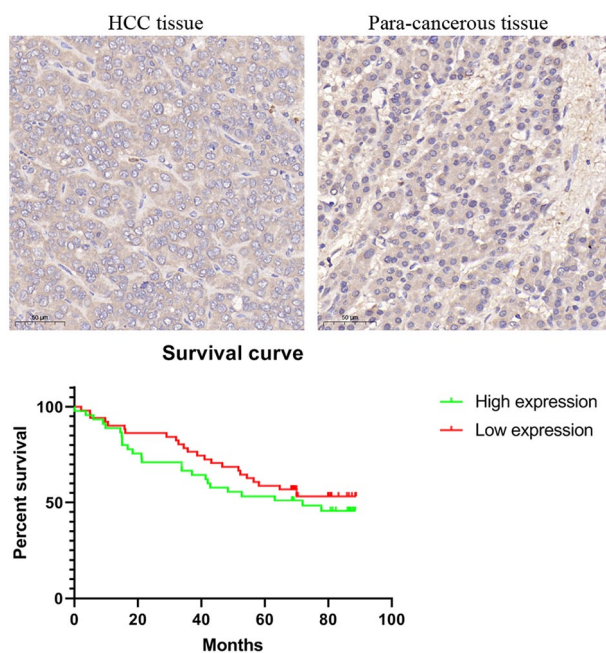


Figure 6. Immunohistochemical staining and survival analysis of HCC and para-cancerous tissues.

Table 3. Associating the expression of pan-Trk with clinicopathological features in patients with hepatocellular carcinoma.

VARIABLES	NUMBER	PAN-TRK EXPRESSION (MEDIAN 186.31)		χ^2	P
		HIGH (45)	LOW (51)		
Case number	96				
Gender				0.106	0.744
Male	82	39	43		
Female	14	6	8		
Age, year				0.568	0.451
<50	27	11	16		
≥50	69	34	35		
HBV infection				0.052	0.820
Yes	79	26	53		
No	11	4	7		
Liver cirrhosis				0.003	0.955
Yes	70	32	38		
No	20	9	11		
Cancer embolus				1.622	0.203
Yes	26	14	12		
No	59	23	36		
AFP (ng/mL)				0.722	0.396
<400	55	25	30		

(Continued)

Table 3. (Continued)

VARIABLES	NUMBER	PAN-TRK EXPRESSION (MEDIAN 186.31)		χ^2	P
		HIGH (45)	LOW (51)		
≥ 400	28	10	18		
CK19				1.800	0.180
Yes	30	12	18		
No	60	33	27		
TNM stage				1.246	0.264
I, II	59	25	34		
III, IV	37	20	17		

AFP, alpha-fetoprotein; HBV, hepatitis B virus.

levels, for financial or other reasons. HBV infection is the leading cause of LIHC worldwide. China has the world's largest burden of HBV infection, as nearly 23,355,000 patients infected with HBV (approximately 29.0% of global HBV infections) occurred in China in 2019.^{27,28}

Conclusion

We systemically analyzed the relationships between *NTRK* gene expression and LIHC using bioinformatics and IHC. The expression levels of *NTRK* genes in LIHC tissues were not significantly different. Only high expression of *NTRK2* was significantly associated with longer DFS. The alteration frequencies, including gene fusion events, are very low in *NTRK* genes. The methylation levels of all *NTRK* genes were significantly different between LIHC and normal tissues, and both *NTRK1* and *NTRK3* modifications may be related to the survival rate for patients with LIHC. However, we found that the expression of pan-Trk in HCC tissue has limited value in clinical characteristics and prognosis.

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Author Contributions

Xuefeng Fang and Ying Yuan designed and supervised this project. Hejing Wang and Lina Qi performed bioinformatic analyses and wrote the article. Hejing Wang performed the immunohistochemical staining and analysis. Chenhan Zhong provided advice and supervision of the analytic process. Xuefeng Fang and Ying Yuan amended the writing of the article. All authors participated in the writing, review, and final approval of the manuscript.

Ethical Statement

This study was approved by the Institutional Review Board of The Second Affiliated Hospital, Zhejiang University School of Medicine (202112202).

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