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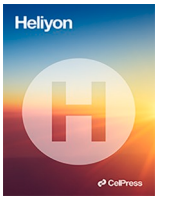

Research article

Comprehensive analysis of INTS family related to expression, prognosis, diagnosis and immune features in hepatocellular carcinoma

Bingyu Wang [a,1], Zifei Du [a,1], ChongSen Lin [c], Dandan Liu [d], Jiewen Guo [a,**], Jiawei Shi [b,***], Xiaobo Wang [b,*]

[a] Department of Science and Education, The Affiliated TCM Hospital of Guangzhou Medical University, Guangzhou, Guangdong, China
[b] Department of Spinal Surgery, Nanfang Hospital, Southern Medical University, Guangzhou, Guangdong, China
[c] Department of Orthopedics, Huizhou Hospital of Guangzhou University of Chinese Medicine, Huicheng, Guangdong, China
[d] Department of Respiratory Medicine, Taixing Hospital of TCM, Taixing, Jiangsu, China

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ABSTRACT

Purpose: The integrator subunit (INTS) family, a group exclusive to metazoans, participates in various biologic processes. However, their roles in hepatocellular carcinoma (HCC) remain largely unexplored.

Methods: Public databases were utilized to investigate the transcriptional and protein expression, and clinical relevance of the INTS family in HCC. Meanwhile, the effects of INTS13 knockdown and overexpression on cell proliferation and apoptosis were studied using HCC cell lines.

Results: The mRNA expression of most INTSs were higher in tumor than normal tissues. Higher expression of INTS1/2/3/4/7/8/9/11/12/13 were correlated with poorer overall survival (OS) in Kaplan-Meier Survival Analysis. Multivariate analysis revealed higher level of INTS13 was an independent prognostic factor for shorter OS. Furthermore, genetic alteration of INTS3/6/7/8/9/10 were found in HCC patients and was associated with shorter disease-free survival and progression-free survival. INTS1/2/3/5/7/11/13/14 were associated with activation of tumor-induced immune response and immune infiltration in HCC. Knockdown of INTS13 inhibited cell proliferation and induced apoptosis in HCC cell lines, while overexpression of INTS13 had the opposite effect.

Conclusion: Our results indicate that INTS13 is an independent prognostic biomarker in HCC. Furthermore, INTS13 enhances cell proliferation and decreases cell apoptosis in HCC cell lines leading to a poorer OS in HCC patients.

1. Introduction

Primary liver cancer ranks as the sixth most commonly diagnosed cancer and stands as the third leading cause of cancer-related

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: 2019620682@gzhmu.edu.cn (J. Guo), 276562820@qq.com (J. Shi), wxb581990@126.com (X. Wang).
[1] These authors contributed equally to this work.

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mortality globally [1]. Hepatocellular carcinoma (HCC) constitutes approximately 90 % of primary liver cancer cases [2]. Despite significant advancements in HCC treatment, including local ablation, surgical resection, liver transplantation, and immunotherapy [3], the 5-year overall survival (OS) for most HCC patients remains low [4]. In the United States, only 18 % of HCC patients survive 5 years post-diagnosis [5]. Therefore, the identification of reliable biomarkers for early diagnosis and for predicting therapeutic response is essential to enhance the prognosis of HCC patients.

The integrator subunits (INTSs), a metazoan-specific protein family comprising 14 members, play important roles in various biologic processes. INTSs regulate the cleavage of the extended 3′-end of Uridine-rich small nuclear RNAs, essential for the biogenesis of spliceosomal snRNPs and the 3′-end formation of enhancer RNA, to regulate the transcriptional activation of neighboring protein-encoding genes. Among the INTSs, INTS6, initially identified as the deleted in cancer 1 protein, has been identified as a tumor suppressor gene in serval tumors, including non-small cell lung carcinomas, esophageal squamous cell carcinoma, nasopharyngeal carcinoma, prostate cancer and HCC [6–10]. However, in colorectal cancer, INTS6 was found higher expression in cancer tissues and it promoted the growth of tumor cells by facilitating G1/S cell cycle transition [11]. Other INTS family members also contribute to cancer progression; for example, INTS3 has been shown to promote HCC development [12], and genetic polymorphisms in INTS10 are implicated in the progression from persistent hepatitis B virus (HBV) infection to HCC [13]. Moreover, INTS8 has been found to accelerate HCC cell growth by upregulating the TGF-β signaling pathway [14]. These findings indicate the significant role of INTS family members in HCC development.

In this study, we fully examined the role of INTSs in HCC using transcriptomic data from the Cancer Genome Atlas (TCGA) database. We investigated the expression, prognosis, immune infiltration, and genetic mutations of INTS family members in HCC patients. Additionally, functional studies of INTS13 were conducted on Hep3B and Huh7 cell lines, and gene set enrichment analysis (GSEA) was utilized to elucidate the potential mechanisms by which INTS13 influences HCC.

2. Material and methods

2.1. Transcriptomic and protein expression of INTSs in HCC

Transcriptomic data of HCC were downloaded from TCGA database. The R language (version 3.6.3) and R package "ggplot2" were performed to analyze and visualize the data from TCGA, respectively. The Clinical Proteomic Tumor Analysis Consortium (CPTAC) analysis available through the UALCAN portal (http://ualcan.path.uab.edu/analysis-prot.html) to explore the protein expression levels of INTSs in HCC [15].

2.2. Kaplan-Meier (K-M) survival analysis and Cox regression analysis

The package "survival" (version 3.6) in R was used to conducted K-M survival analysis for INTSs, assessing OS, disease-specific survival (DSS) and progression-free interval (PFI). The OS was also analyzed in subset groups, stratified by age ($\leq$60 and $>$ 60), stage (stage I + II and stage III + IV), grade (grade I + II and grade III + IV). The cohort was divided into high-expression and low-expression groups based on the cutoff value with the lowest p-value. Univariate Cox regression and multivariate Cox regression were further applied to investigate the prognostic value of INTSs in HCC.

2.3. Receiver operating characteristic (ROC) curve analysis

The package "survival" (version 3.6) in R was also used to explore the ROC curve analysis. An area under the ROC curve (AUC) exceeding 0.8 was considered indicative of high diagnostic accuracy.

2.4. CBioPortal

cBioPortal (www.cbioportal.org) serves as an open-source platform that facilitates the exploration, visualization, and analysis of multidimensional cancer genomics and clinical data [16]. Mutations from genomic identification of significant targets in cancer (GISTIC) and mRNA expression z-scores (RNASeq V2 RSEM) with a z-score threshold ±1.8 were used to analyzed the genetic mutations of INTSs. The impact of genetic mutations in INTSs on OS, disease-free survival (DFS) and progression-free survival (PFS) in HCC patients were evaluated and were presented in K-M plots. Statistical significance between survival curves was determined using a log-rank test, with a p-value of less than 0.05 denoting significance.

2.5. Immune cell infiltration analysis

The immune scores of patient samples from the TCGA database were calculated by the ESTIMATE algorithm. The examination of the effects of INTSs on immune cell infiltration and the correlation between INTSs expression and the abundance of tumor-infiltrating immune cells using algorithm of "ssGSEA". Statistical significance was assessed using the Wilcoxon rank-sum test and Spearman's rank correlation coefficients.

2.6. Cell culture and transfection procedures

Human HCC cell lines, Hep3B and Huh7, were acquired from ScienCell Research Laboratories and cultured in DMEM supplemented with 10 % fetal bovine serum (Gibco, Carlsbad, CA, USA). Both cell lines were maintained in a humidified incubator at 37 °C with 5 % CO_2. INTS13 overexpression plasmids and INTS13 short hairpin RNA (shRNA) were purchased from Ribo. Transfections were performed using Lipofectamine RNAiMAX (ThermoFisher, USA) according to the manufacturer's instructions. The target sequences for shRNA were as follows: CCGGCCACGAAAGTCAGGTTCTAAACTC.

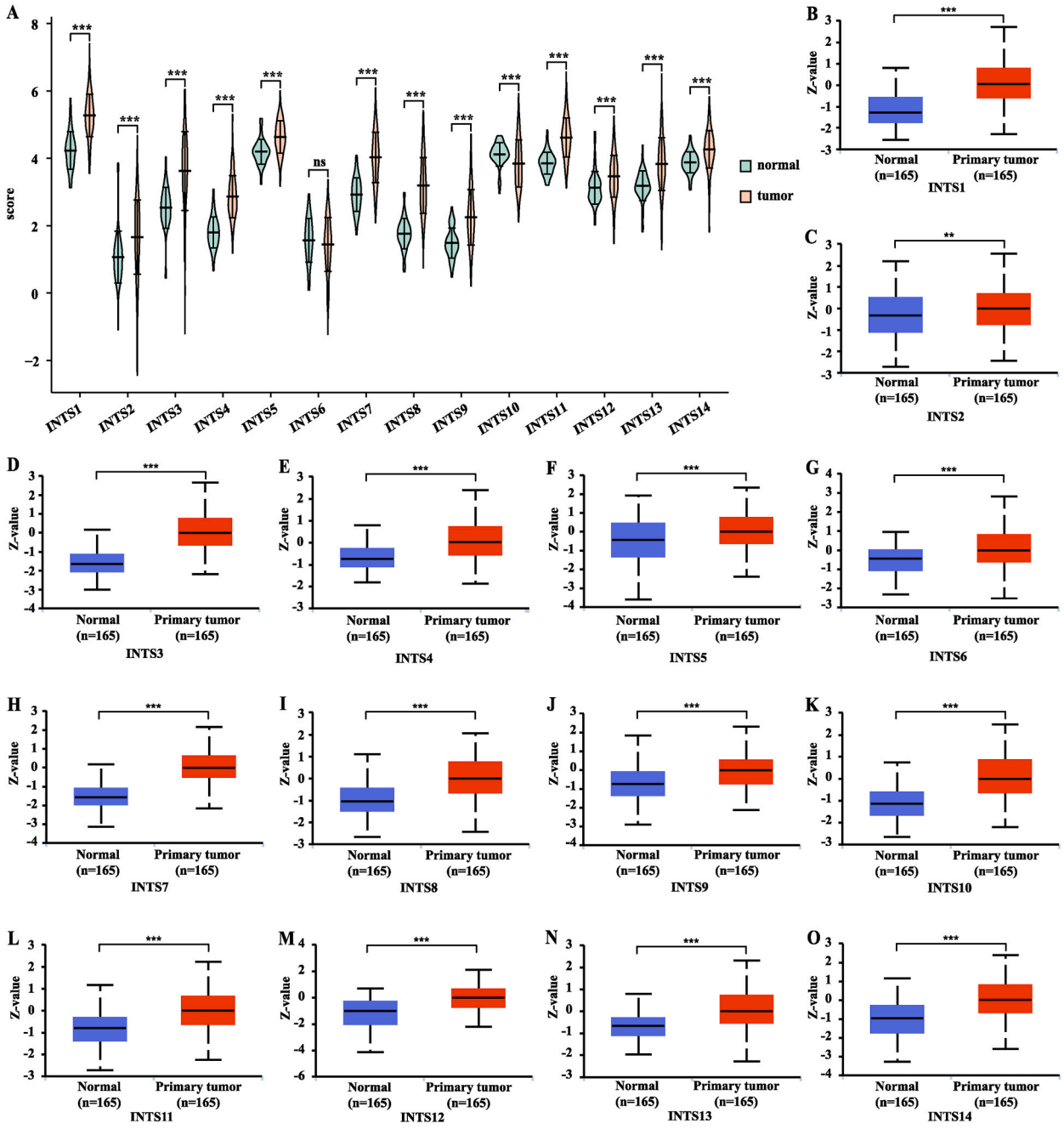

Fig. 1. Transcriptional and protein expression of INTSs in HCC across different datasets. **A** The mRNA expression of INTSs in HCC from the TCGA dataset. **B–O** Protein expression of the 14 INTS members in primary HCC tissues versus normal samples using the UALCAN segment of the CPTAC dataset. ns, no significance, *p < 0.05, **p < 0.01, ***p < 0.001.

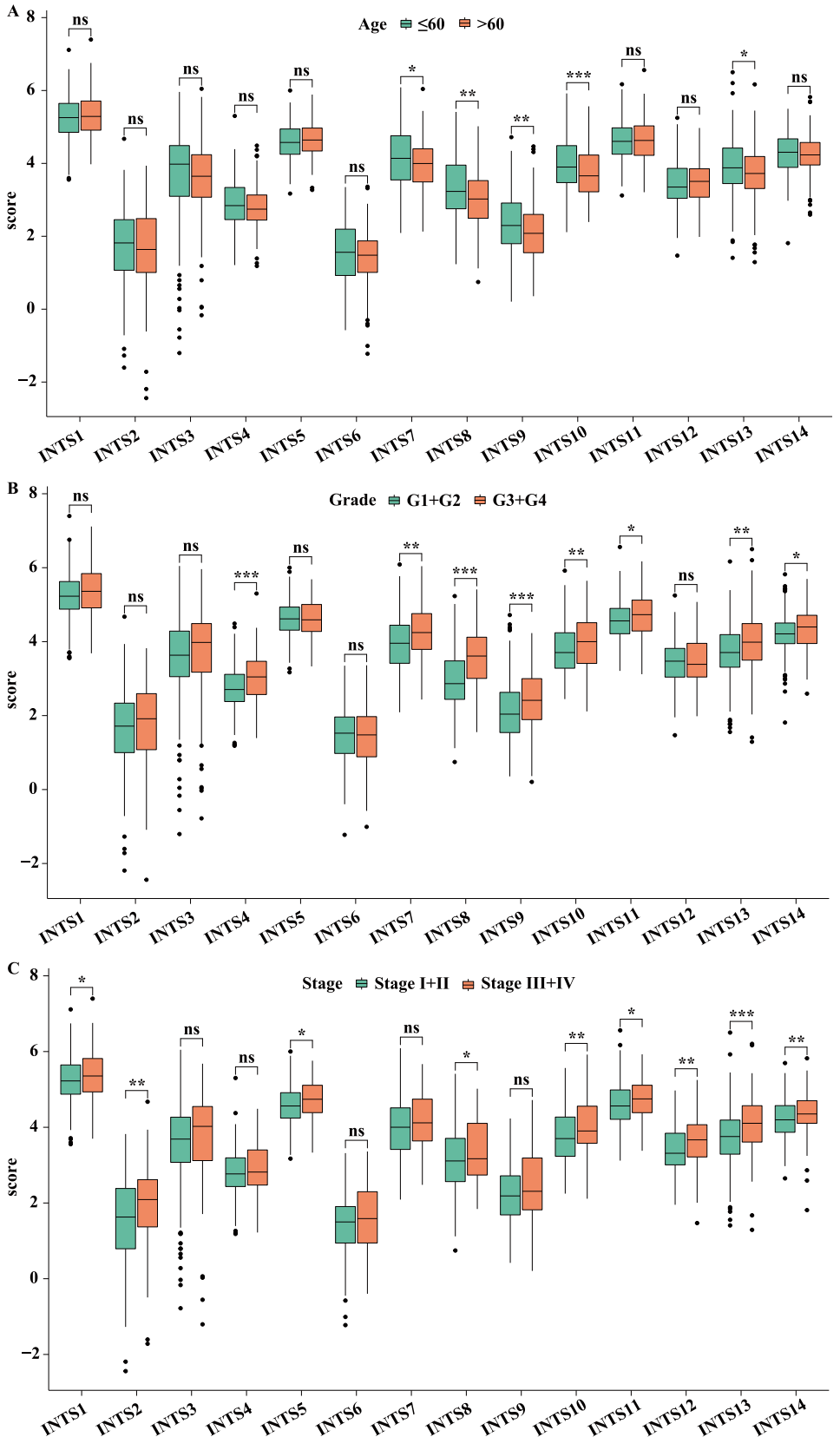

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Fig. 2. The mRNA expression of INTSs are analyzed with respect to age **(A)**, patient tumor grade **(B)** and the patients' individual cancer stage **(C)**. ns, no significance, *p < 0.05, **p < 0.01, ***p < 0.001.

2.7. Cell proliferation assay

The Cell Counting Kit-8 (CCK-8) assay was utilized to evaluate cell viability. Two human HCC cell lines, including Hep3B and Huh7, were seeded in 48-well plates at a density of 1 × 10^4 cells per well. After transfecting with sh-INTS13 or OE-INTS13 plasmids, cells were incubated with CCK-8 reagent (Dojindo, Kumamoto, Japan) at 37 °C for 1.5 h. The optical density of samples at 24, 48, 72 and 96 h was measured using a microplate reader at a wavelength of 450 nm.

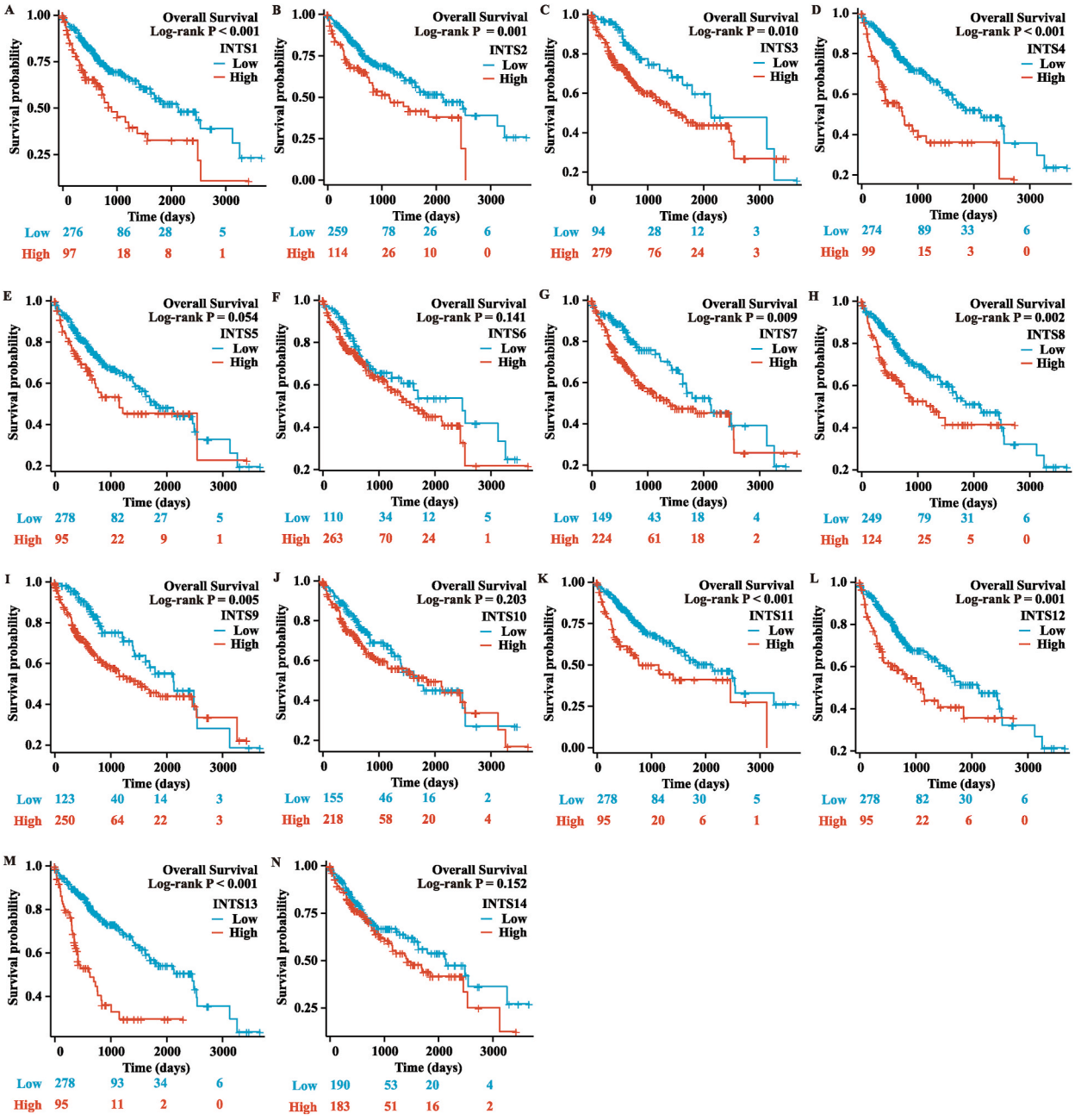

Fig. 3. Kaplan-Meier curve analysis for detecting prognostic value of the mRNA expression of INTSs in HCC patients. **A-N** The Kaplan-Meier curves in all HCC patients stratified by high and low expression of INTS1-14. p < 0.05.

2.8. Western blot analysis

Cells were lysed using RIPA buffer, and total protein concentrations were determined using BCA assays (Thermo Fisher Scientific, Main St, MA, USA) as per the manufacturer's guidelines. Proteins were denatured and subjected to 10 % SDS-PAGE, then transferred to PVDF membranes (Millipore, Darmstadt, Germany). The membranes were blocked with 5 % nonfat milk for 1 h at room temperature, and incubated with primary antibodies overnight at 4 °C, followed by suitable horseradish peroxidase-conjugated secondary antibodies. Proteins were detected using the Chemi Doc XRS System with Immobilon Western Chemiluminescent HRP substrate (Millipore, Darmstadt, Germany). The primary antibodies are indicated as follows: BCL2, Abcam, Cat#ab182858; BAX, Abcam, Cat#ab182733;

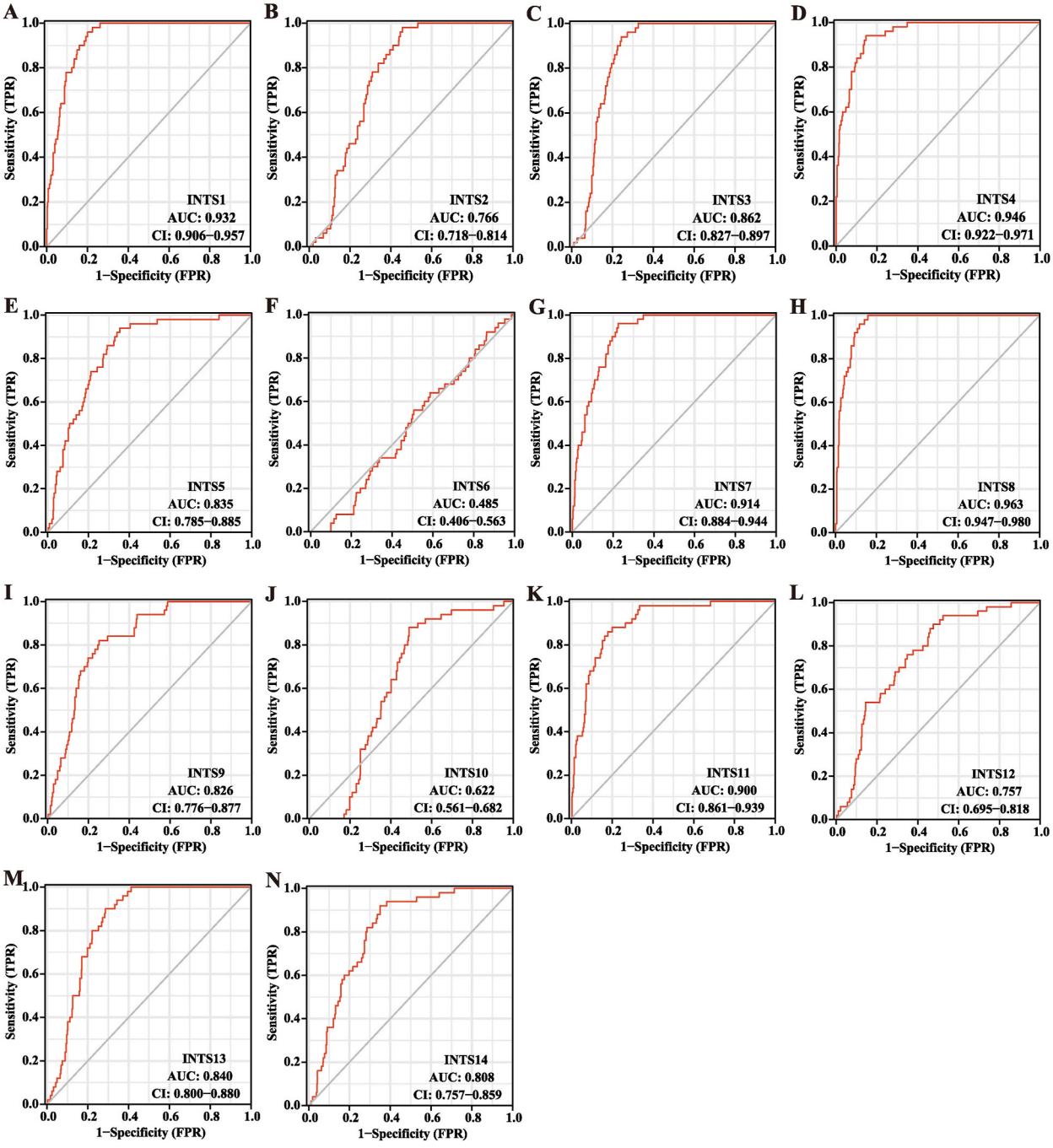

Fig. 4. The receiver operating characteristic (ROC) curve of the risk score in HCC. **A-N** The ROC curve of the risk score evaluating its diagnostic potential for HCC based on expression of INTSs.

Caspase-3, Abcam, Cat#ab184787; INTS13, AntibodySystem, Cat# PHK91701; GAPDH, Abcam, Cat# ab8245.

2.9. Cell-light 5-ethynyl-2-deoxyuridine (EdU) assay

To assess cell proliferation, we utilized the EdU Apollo567 In Vitro kit (Ribobio, China). After transfection for 24 h, cells were seeded into 6-well plates at a density of 2 × 10^5 cells per well. Following a 12-h cultivation period, the cells were transfected for an additional 24 h and then cultured for 40 more hours. Subsequently, the cells were incubated with the EdU working solution for 2 h, fixed with 4 % paraformaldehyde, permeabilized, washed, and stained with 1 × Apollo solution and 1 × Hoechst33342 solution as per the manufacturer's instructions. The results were analyzed using microphotographs taken with a fluorescence microscope.

2.10. Statistical methods

The expression of INTSs between the high and low immune score groups was compared using an unpaired *t*-test. Cox regression analysis was performed using R version 3.6.1, assessed the association between mRNA expression of the INTSs and patient survival. Univariate Cox regression evaluated the influence of clinical parameters and mRNA expression on HCC patient survival, with genes showing a p-value ≤0.05 included in subsequent multivariate analysis. Statistical analyses and figure generation were conducted using GraphPad Prism (version 7.0), with a p-value <0.05 considered statistically significant.

3. Results

3.1. The mRNA and protein level of INTSs in HCC

As shown in Fig. 1A, the mRNA levels of INTS1/2/3/4/5/7/8/9/10/11/12/13/14 were significantly higher in HCC tissues compared to normal tissues, while INTS6 mRNA level exhibited no significant difference. To validate our findings, the protein level of INTS were investigated using CPTAC dataset. The results showed that the protein levels of INTSs were significantly higher in HCC tissues than normal tissues (Fig. 1B-O).

3.2. mRNA expression of INTSs in different subgroups of HCC

To further elucidate the role of INTS family members in HCC, we analyzed their expression across various age groups, grades, and stages (Fig. 2A–C). The results revealed that the significantly higher mRNA expressions of INTS7/8/9/10/13 in young patients (≤60 years old) compared to old patients (>60 years old) (Fig. 2A). The grade of the tumor refers to the abnormal degree of the tumor cells under the microscope. Patients with grade III and IV expressed significantly higher INTS4/7/8/9/10/11/13/14 than those in patients with grade I and II (Fig. 2B). The tumor stage refers to the size of the tumor and/or whether the tumor has spread. HCC patients with stage III and IV tumors expressed higher levels of INTS1/2/5/8/10/11/12/13/14 compared to those with stage I and II tumors

Table 1
Univariate and multivariate analysis of overall survival in 370 HCC patients.

Variables	Univariate analysis			Multivariate analysis		
	Hazard Ratio	CI 95 %	P	Hazard Ratio	CI 95 %	P
Age (≤60/>60)	1.03	1.01–1.04	0.004	1.03	1.01–1.05	0.005
BMI (≤26/>26)	1.03	1–1.06	0.093	1.02	0.98–1.05	0.317
gender (M/F)	1.32	0.86–2.02	0.199			
grade (I + II/III + IV)	1.13	0.91–1.41	0.259			
HBV (Y/N)	1.4	0.91–2.15	0.122			
HCV (Y/N)	1.74	1.07–2.83	0.026	1.86	1.09–3.17	0.024
AJCC stage (I + II/III + IV)	1.22	0.96–1.57	0.11			
INTS1 (high/low)	1.01	1–1.02	0.037*	1	0.99–1.01	0.55
INTS2 (high/low)	1.05	0.98–1.12	0.17			
INTS3 (high/low)	1	0.99–1.02	0.625			
INTS4 (high/low)	1.03	0.98–1.08	0.255			
INTS5 (high/low)	1.01	0.98–1.03	0.485			
INTS6 (high/low)	0.95	0.83–1.08	0.44			
INTS7 (high/low)	1	0.98–1.03	0.834			
INTS8 (high/low)	1.04	1–1.08	0.029*	1.01	0.96–1.06	0.688
INTS9 (high/low)	1.03	0.97–1.1	0.348			
INTS10 (high/low)	0.99	0.96–1.02	0.568			
INTS11 (high/low)	1	0.98–1.02	0.73			
INTS12 (high/low)	1.03	0.99–1.07	0.106			
INTS13 (high/low)	1.03	1.01–1.05	<0.001*	1.03	1.01–1.06	0.007*
INTS14 (high/low)	0.99	0.97–1.02	0.65			

HCC Hepatocellular carcinoma, CI Confidence interval. *p < 0.05.

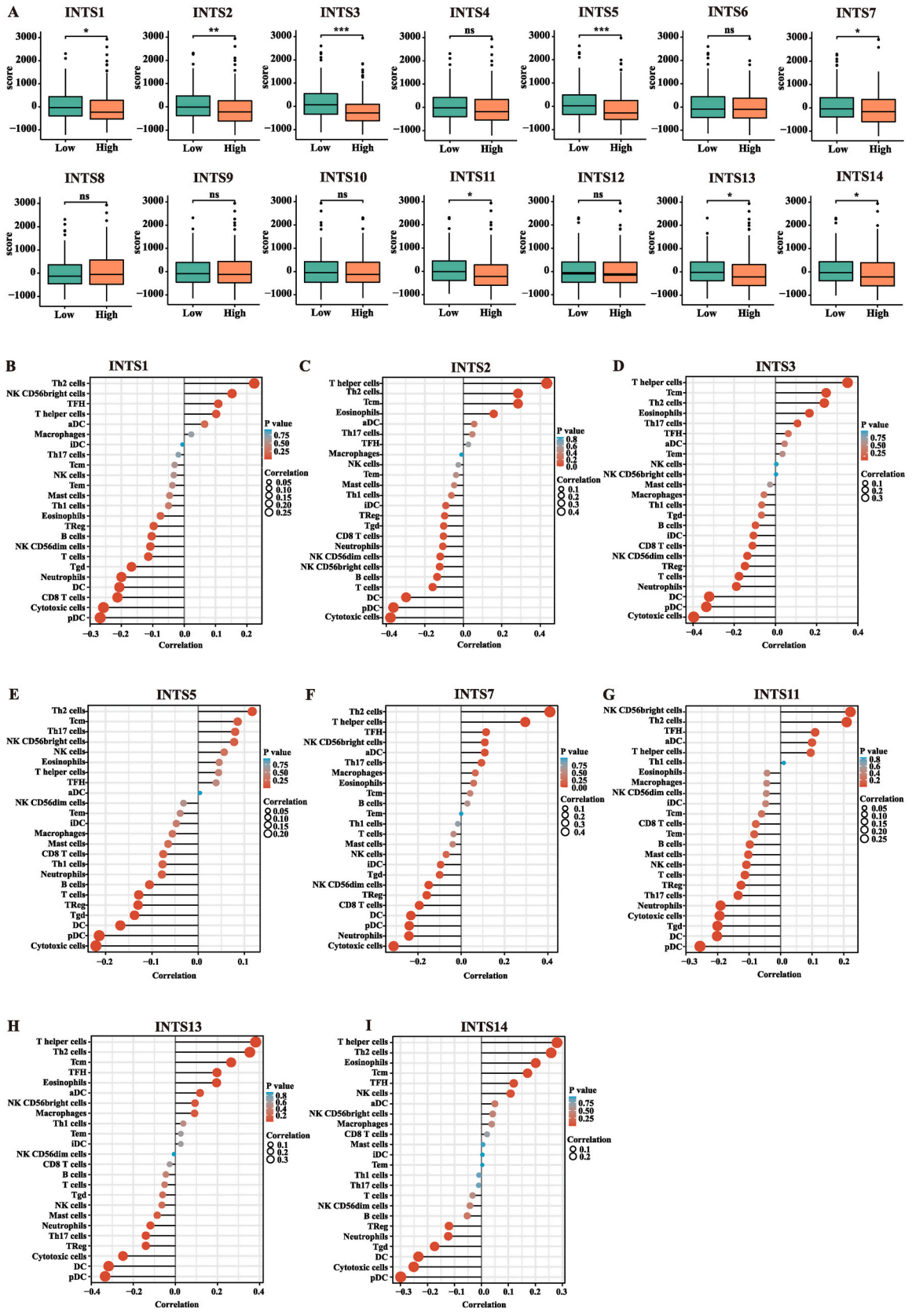

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Fig. 5. Correlation between INTSs expression and the level of immune infiltration in HCC. **A** INTSs expression between the high and low immune score groups of HCC patients. **B–I** The relation between INTS1/2/3/5/7/11/13/14 and immune cells in HCC represent as lollipop chart. ns, no significance, *p < 0.05, **p < 0.01, ***p < 0.001.

(Fig. 2C).

3.3. Survival prognostic value of INTSs in HCC

The correlation between the mRNA levels of INTS1–14 and the survival of HCC patients was investigated. The K-M curves and log-rank test analysis showed that higher levels of INTS1/2/3/4/7/8/9/11/12/13 were correlating with shorter OS, while no significant differences in OS were found for INTS5/6/10/14 (Fig. 3). To refine our understanding of the prognostic value of INTSs in HCC, DSS and PFI between high and low expression groups of INTSs were compared. The results showed that higher mRNA levels of INTS1/2/3/4/7/8/13 were significantly associated with worse DSS, with no significant differences for INTS5/6/9/10/11/12/14 in terms of DSS (Fig. S1). Moreover, higher expression of INTS1/2/4/5/8/11/12/13 was related to worse PFI, with no significant associations between INTS3/6/7/9/10/14 expression and PFI (Fig. S2). The survival curves were further compared across different age, grade, and stage subgroups for members with varying gene expressions (Figs. S3–S5). The results indicated that higher mRNA expression of INTS13 was associated with poor OS in the subgroup of patients over 60 years old (Fig. S3E), while the mRNA expression of other INTSs did not show significant relationships with OS across different ages (Figs. S3A–D). The mRNA levels of INTSs were also not significantly related to OS by grade (Figs. S4A–H), and the only mRNA expression of INTS1 showed a significant association with prognosis in HCC patients with stage I and II HCC (Fig. S5A), while the levels of other INTS members were not significantly related to OS in different stage (Figs. S5B–I) (see Fig. 4).

3.4. Independent prognostic value of mRNA expression of INTSs in terms of OS in HCC patients

Transcriptomic data and clinical characteristics of HCC samples from the TCGA database were shown in Supplementary Table 1. Univariate analysis of all patients with HCC revealed that advanced age (HR = 1.03, 95 % CI: 1.01–1.04, p = 0.004), and high mRNA expression of INTS1 (HR = 1.01, 95 % CI: 1–1.02, p < 0.05), INTS8 (HR = 1.04, 95 % CI: 1–1.08, p < 0.05), and INTS13 (HR = 1.03, 95 % CI: 1.01–1.05, p < 0.001) were significantly associated with shorter OS (Table 1).

Multivariate analysis showed that only high mRNA expression of INTS13 (HR = 1.03, 95 % CI: 1.01–1.06, p = 0.007) was independently associated with shorter OS among all HCC patients (Table 1). These findings demonstrated that INTS13 is an independent prognostic factor for OS in all HCC patients.

3.5. Diagnostic value of mRNA expression of INTS genes in HCC

The ROC analysis showed that INTS1/3/4/5/7/8/9/11/13 exhibited high diagnostic potential in discriminating HCC from normal tissues, with an AUC of 0.932 (INTS1, 95 % CI: 0.906–0.957), 0.862 (INTS3, 95 % CI: 0.827–0.897), 0.946 (INTS4, 95 % CI: 0.922–0.971), 0.835 (INTS5, 95 % CI: 0.785–0.885), 0.914 (INTS7, 95 % CI: 0.884–0.944), 0.963 (INTS8, 95 % CI: 0.947–0.980), 0.826 (INTS9, 95 % CI: 0.776–0.877), 0.900 (INTS11, 95 % CI: 0.861–0.939), and 0.840 (INTS13, 95 % CI: 0.800–0.880), while INTS2/6/10/12/14 have no diagnostic effective in HCC, with low AUC values of 0.766 (INTS2, 95 % CI: 0.718–0.814), 0.485 (INTS6, 95 % CI: 0.406–0.563), 0.622 (INTS10, 95 % CI: 0.561–0.682), 0.757 (INTS12, 95 % CI: 0.695–0.818), and 0.808 (INTS14, 95 % CI: 0.757–0.859) in diagnosis HCC.

3.6. Relationship between expression of INTSs and immune infiltration in HCC

Immune scores for each HCC sample were calculated from the TCGA database. Based on these scores, samples were classified into high and low immune score groups using the median value as a threshold (Fig. 5A). The expression levels of INTS1/2/3/5/7/11/13/14 were significantly lower in the high immune score group, suggesting an involvement of these genes in immune infiltration within HCC. The mRNA level of INTS1 showed no substantial correlation with immune cells in HCC (Fig. 5B). However, INTS2 expression correlated positively with T helper cell infiltration (r = 0.434, p < 0.001) and negatively with the infiltration of pDC (r = −0.363, p < 0.001) and cytotoxic cells (r = −0.379, p < 0.001) (Fig. 5C). INTS3 demonstrated a positive correlation with T helper cells (r = 0.350, p < 0.001) and a negative correlation with the infiltration of DC (r = −0.322, p < 0.001), pDC (r = −0.336, p < 0.001) and cytotoxic cells (r = −0.397, p < 0.001) (Fig. 5D). The mRNA level of INTS5 has no substantial correlated with immune cells in HCC (Fig. 5E). INTS7 showed a strong positive association with Th2 cells (r = 0.409, p < 0.001), and a negative correlation with cytotoxic cells (r = −0.312, p < 0.001) (Fig. 5F). The mRNA level of INTS11 showed no substantial correlation with immune cells in HCC (Fig. 5G). INTS13 was strongly positively correlated with T helper cells (r = 0.382, p < 0.001) and Th2 cells (r = 0.354, p < 0.001), and negatively with DC (r = −0.318, p < 0.001) and pDC (r = −0.334, p < 0.001) (Fig. 5H). INTS14 expression negatively correlated with pDC (r = −0.300, p < 0.001) (Fig. 5I).

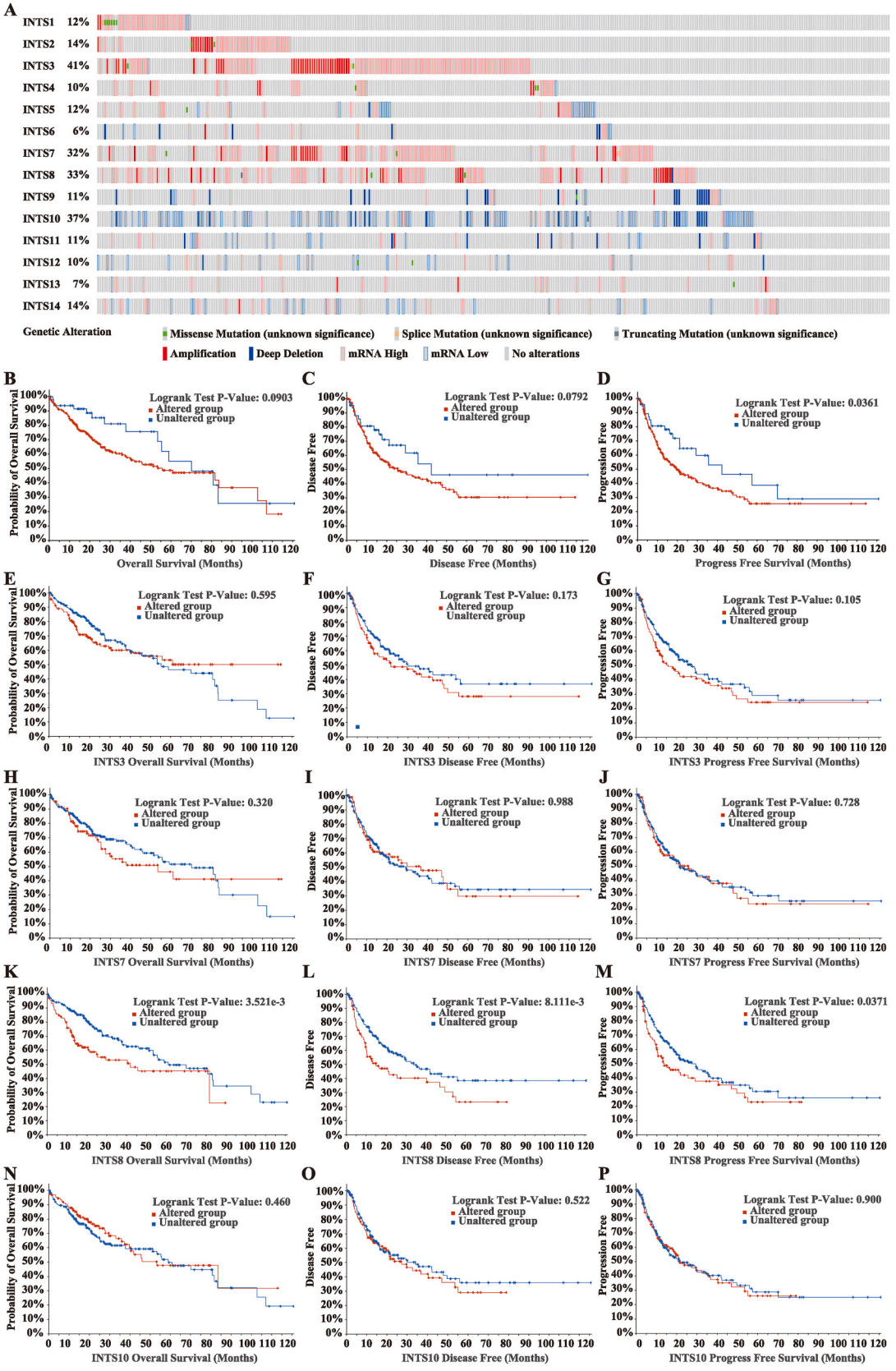

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Fig. 6. Genetic mutations of INTSs. **A** Genetic mutation analysis of INTSs via cBioPortal. **B-D** Relationship between genetic mutations in INTSs and Overall Survival (**B**), Disease-Free Survival (**C**), and Progression-Free Survival (**D**).

3.7. Genetic mutations of INTSs and their associations with OS, DFS, and PFS in HCC patients

Genetic mutations of INTSs were investigated using the cBioportal database. As shown in Fig. 6A, a high mutation rate of INTSs was observed in HCC patients, with alterations in INTS members identified in 299 out of 348 samples (85.9 %), with INTS3 exhibiting the highest frequency of mutations at approximately 41 %. INTS3/7/8/10 also displayed mutation rates exceeding 30 %. These genetic alterations were not significantly associated with OS (Fig. 6B, $p = 0.093$), yet they correlated with shorter DFS (Fig. 6C, $p = 0.099$) and PFS (Fig. 6D, $p = 0.036$). These results indicated that mutations of INTSs significantly impacted DFS and PFS in patients with HCC.

Additionally, the correlations between genetic alterations of INTSs, where mutation rates exceeded 30 %, and OS, DFS, and PFS were analyzed (Fig. 6E-P). Notably, only the genetic alterations of INTS7 were significantly associated with shorter OS (Fig. 6K, $p = 0.0035$), DFS (Fig. 6L, $p = 0.0081$), and PFS (Fig. 6M, $p = 0.0371$). However, the genetic alterations of INTS3, INTS7, and INTS10 did not show significant associations with OS, DFS, and PFS (Fig. 6E–J, N-P).

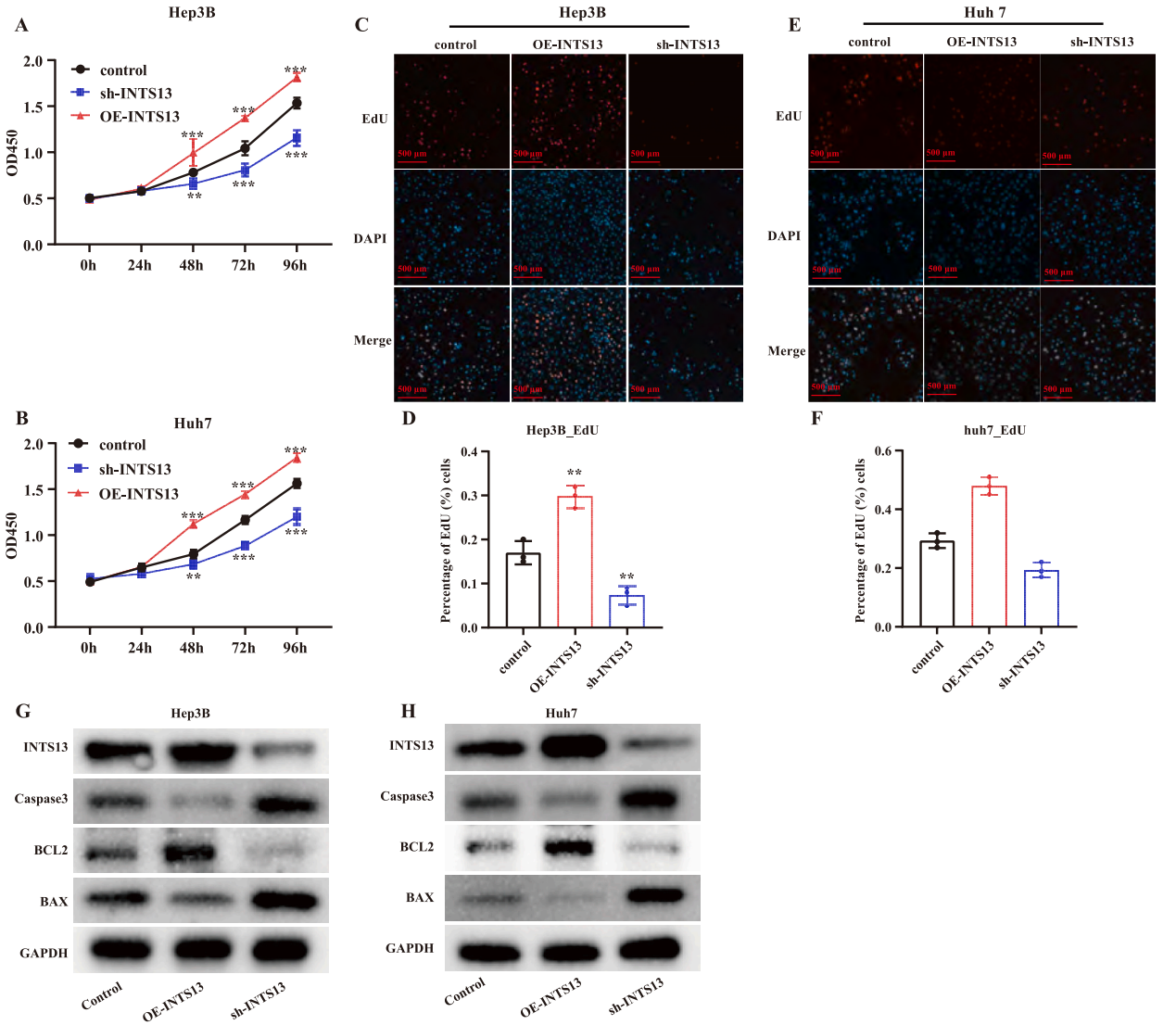

Fig. 7. Functional experiments were performed in HCC cell lines with knockout and overexpression of INTS13. **A, B** CCK-8 cell proliferation assay results in Hep3B and Huh7 cells as the indicated treatment. **C–F** Cell proliferation of Hep3B and Huh7 cells measured by EdU assays and quantification of EdU results after indicated treatment. **G, H** The expression of apoptosis-related proteins was analyzed in Hep3B and Huh7 cells analyzed by western blotting.

3.8. INTS13 promotes cell proliferation and inhibits cellular apoptosis in vitro

Since INTS13 mRNA expression was significantly associated with prognosis in HCC patients, as revealed through univariate and multivariate analyses, we further investigated the oncogenic function of INTS13. Cellular functional experiments were performed in two HCC cell lines. CCK8 assay showed that sh-INTS13 significantly reduced cell viability in both Hep3B and Huh7 cells, whereas OE-INTS13 enhanced cell viability (Fig. 7A–B). The EdU assay results suggested that sh-INTS13 inhibited cell proliferation, while OE-INTS13 increased cell proliferation (Fig. 7C–F). A significant increase in apoptotic markers including Caspase3 and BAX and a significant decrease in anti-apoptotic marker BCL2 were found in cells with INTS13 knockdown, whereas INTS13 overexpression had the opposite effects (Fig. 7G–H).

4. Discussion

Given the high invasiveness and substantial mortality associated with HCC, the early diagnosis and accurate prediction of prognosis for HCC patients present significant challenges. These factors play crucial roles in clinical decision-making. This study comprehensively explored the mRNA expression, prognostic role, and immune infiltration activities of all INTSs in HCC. Generally, mRNA expressions of INTSs were elevated in HCC tissues. A high mutation rate of INTSs in HCC patients was found and was associated with shorter DFS and PFS. Notably, INTS13 emerged as an independent prognostic factor for OS, PFI and DSS across all HCC patients. Our experiments demonstrated that sh-INTS13 reduced HCC cell proliferation and increased apoptosis, whereas OE-INTS13 promoted cell proliferation and inhibited apoptosis.

Early diagnosis and identification of prognostic factors are crucial in improving outcomes for cancer patients [17]. Privious studies indicate that higher expression of INTSs indicated worse OS in various cancers, including INTS1 in malignant mesothelioma [18] INTS2 in breast cancer [19] and INTS7 in lung adenocarcinoma (LUAD) [20]. Consistent with these findings, our results suggest that higher expression of most INTSs indicates poor prognosis, with INTS1/3/4/5/7/8/9/11/13 demonstrating strong diagnostic potential. Moreover, high expression of INTS1 was associated with shorter OS in early-stage patients, whereas high levels of INTS13 correlated with favorable OS. Given the strong association between defective DNA damage repair and tumorigenesis, and the potential role of INTSs in impairing DNA damage repair [21,22], it is unsurprising that INTSs are closely related to malignant biological behavior. Further, our univariate and multivariate analyses investigated the function of INTS13, revealing its role in inhibiting apoptosis and promoting cell proliferation, thus enhancing tumor growth and leading to poor prognosis in HCC patients.

The expression levels of INTS members vary across cancers. Most INTS members were upregulated in tumors, such as INTS5 in osteosarcoma [23], INTS7 in LUAD [24], INT11 in esophageal adenocarcinoma [25], INTS14 in prostate cancer [26]. Besides, INTS8 has been reported to be upregulated in various human cancers, including gastric cancer (GC), tongue squamous cell carcinoma, and renal papillary cell carcinoma [27–29], while INTS13 shows high expression in rectum adenocarcinoma, lung cancer small cells, and cholangiocarcinoma [30]. Conversely, INTS1 and INTS10 are found to be downregulated in GC samples negative for *Helicobacter pylori* [31] and in oral squamous cell carcinoma [32], respectively. In this study, analysis of the TCGA database revealed that mRNA levels of INTS1/2/3/4/5/7/8/9/10/11/12/13/14 and protein levels of all INTSs were higher in HCC tissues than in normal tissues, indicating their regulatory function in cancer. Further research revealed that most INTSs were significantly upregulated in patients with higher cancer stage, higher tumor grade and older age. The differential expression of genes in tumor versus normal tissues underscores their potential involvement in tumor progression [33], positioning INTSs as potential biomarkers and therapeutic targets in HCC.

The tumor microenvironment (TME) plays a crucial role in the development and progression of HCC, with distinct immune features correlating significantly with response to immune therapy and tumor behavior [34]. INTS12, for instance, has been identified as a participant in the immunotherapy process for pancreatic cancer [35]. In this study, we analyzed the correlation between INTSs expression and TME scores, tumor immune cell infiltration, and immune subtypes. We found that INTS2/3/7/13/14 exhibited a significant positive correlation with the infiltration of specific immune cells in HCC. These findings revealed that high expression of INTS2/3/7/13/14 in HCC tissues is associated not only with tumor progression and poor prognosis but also with enhanced immune cell infiltration, potentially leading to a highly immunosuppressive tumor microenvironment while possibly improving anti-tumor immune responses.

Genomic instability is one of the hallmark of tumor cells, characterized by a mutation rate higher than normal. Although genomic instability is a double-edged sword, it also offers therapeutic opportunities [36]. Lim B et al. reported that INTS2 mutations in GC patients were associated with poorer survival probabilities compared to those without such mutations [37]. In our study, INTS3/6/7/8/9/10 exhibited high mutation rates. Previous study has shown that INTS3 is particularly responsive to DNA damage [38], which is found to be closely related to genomic instability [39]. Furthermore, higher mutation rates in INTSs correlated with poor DFS and PFS, indicating that genetic alterations in INTSs may promote cancer initiation and accelerate tumor progression.

Several limitations of this study should be noted. Firstly, most of our results were based on publicly available expression datasets, and more prospective data are needed to validate the clinical applicability. Secondly, although we validated the effect of INTS13 on HCC cell proliferation and apoptosis in vitro, additional experiments are necessary to confirm the roles of other INTS members in HCC.

5. Conclusion

In summary, findings of our study illustrated the mRNA and protein expression, diagnostic and prognostic values and immune filters of INTS members in HCC from a bioinformatics perspective. The results indicate that several INTSs were related to clinical outcomes of patients with HCC. Especially, INTS13 emerges as an independent prognostic biomarker for HCC. It has been shown to

promote cell proliferation and inhibit apoptosis in two HCC cell lines. Further well-designed investigations are required to elucidate the significance of our findings and thus develop the clinical utility of INTSs.

Data availability

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

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CRediT authorship contribution statement

Bingyu Wang: Writing – original draft, Formal analysis, Data curation. **Zifei Du:** Writing – original draft, Formal analysis, Data curation. **ChongSen Lin:** Methodology, Data curation. **Dandan Liu:** Validation, Formal analysis. **Jiewen Guo:** Writing – review & editing, Supervision. **Jiawei Shi:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Funding acquisition, Formal analysis. **Xiaobo Wang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Funding acquisition, Formal analysis, Data curation.

Declaration of competing interest

We declared that there were no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled.

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Graphical abstracts draw by figdraw.

Abbreviations

HCC	Hepatocellular carcinoma
INTS	Integrator subunit
K-M	Kaplan-Meier
ROC	Receiver operating characteristic
TIMER	Tumor immune estimation resource
OS	Overall survival
TCGA	The Cancer Genome Atlas
GSEA	Gene set enrichment analysis
CPTAC	Clinical Proteomic Tumor Analysis Consortium
HBV	Hepatitis B virus
GISTIC	Genomic Identification of Significant Targets in Cancer
DSS	Disease-specific survival
PFI	Progression-free interval
PFS	Progression-free survival
DFS	Disease-free survival
ESTIMATE	Estimation of STromal and Immune cells in Malignant Tumor Tissues using Expression data
GSVA	Gene set variation analysis
KEGG	Kyoto Encyclopedia of Genes and Genomes
DC	Dendritic cells
pDC	plasmacytoid Dendritic cells
Th2	T helper 2
shRNA	short hairpin RNA
OE-INTS13	overexpressed-INTS13
NC	Negative Control
CCK-8	Cell Counting Kit-8
HRP	Horseradish peroxidase
ssGSEA	single-sample GSEA
GC	Gastric cancer
LUAD	Lung adenocarcinoma

TME tumor microenvironment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e30244.

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