

High hnRNP AB expression is associated with poor prognosis in patients with colorectal cancer

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Abstract. Heterogeneous ribonucleoprotein AB (hnRNP AB) is a member of the heterogeneous nuclear ribonucleoprotein family, which serves important functions in gene expression and signal transduction. However, the expression and clinicopathological significance of hnRNP AB in colorectal cancer (CRC) remain to be elucidated. To investigate the expression and clinical significance of hnRNP AB in CRC, hnRNP AB expression levels were analysed in two independent cohorts of patients with CRC. The results of reverse transcription-quantitative PCR, immunohistochemistry and western blot analysis demonstrated that hnRNP AB was upregulated in CRC tissues compared with the corresponding adjacent normal tissues. Immunohistochemical analyses indicated that a high expression of hnRNP AB was significantly associated with preoperative carcinoembryonic antigen (CEA; $P < 0.001$) and carbohydrate antigen 19-9 ($P = 0.014$) levels, tumour size ($P = 0.022$) and infiltration ($P = 0.026$), lymph node metastasis ($P < 0.001$) and Tumour-Node-Metastasis stage ($P < 0.001$). Univariate and multivariate Cox survival analyses revealed that hnRNP AB expression and preoperative CEA levels were significant independent factors affecting overall survival in patients with CRC ($P < 0.05$). According to the Kaplan-Meier model, patients with CRC with high hnRNP AB expression exhibited significantly poorer prognosis compared with those with low hnRNP AB expression ($P < 0.001$). In conclusion, the results of the present study demonstrated that hnRNP AB expression may serve an important role in the progression of CRC and that hnRNP AB may be considered a predictor of prognosis for patients with CRC.

Introduction

Colorectal cancer (CRC) was reported as the third most common cancer and the second leading cause of cancer-associated mortality worldwide in 2018, with >1.8 million new cases of CRC expected to be diagnosed, and 881,000 deaths estimated to occur, and accounting for 1 in 10 cancer cases and deaths (1). In the USA, localized stage CRC, with a 5 year survival rate of 90%, was diagnosed in 39% of all patients with CRC between 2006 and 2012 (2); however, the 5 year survival rate declined to 14% in patients diagnosed with distant-stage CRC (2). Therefore, raising awareness, early detection and improvements in treatment are necessary for the prevention and treatment of CRC. The identification of useful biomarkers and the determination of the mechanisms involved in the development of CRC may have potential value for improving the outcomes of CRC.

Nuclear heterogeneous ribonucleoprotein AB (hnRNP AB) is a member of the heterogeneous nuclear ribonucleoprotein family (hnRNPs). hnRNPs are a large class of RNA-binding proteins that accumulate in the nuclei of eukaryotic cells and participate in the regulation of mRNA transcription, splicing, editing, translation, stability and localization (3-5). A number of hnRNPs are associated with normal biological processes and various neurodegenerative diseases, such as spinal muscular atrophy, amyotrophic lateral sclerosis, Alzheimer's disease and frontotemporal lobe dementia (3). Sinnamon *et al* (6) have reported that hnRNP AB may serve a key role in the maintenance, differentiation and survival of neuronal stem cells by activating glutamate signalling pathways. Lampason *et al* (7) demonstrated that hnRNP AB promoted the migration of normal neural cells by regulating exocrine gland-secreted peptide 8 transcription during the development of the central nervous system in mouse models. Other studies have revealed that abnormal hnRNP expression serves an important role in the occurrence and development of malignant tumours, including lung cancer (8), hepatocellular carcinoma (9) and CRC (10).

hnRNP AB, which was first purified from the human HeLa cell 40S core particle (11), comprises four paralogues, A1, A2/B1, A3 and A0 (12), which serve an important role in tumour development and progression (13-15). Liu *et al* (8) demonstrated that the expression of hnRNP A1 in lung cancer tissues was significantly higher compared with that in adjacent

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normal tissues and that the depletion of hnRNP A1 from lung cancer cells could induce cell cycle arrest in G0/G1 phase and inhibit lung cancer cell proliferation. Meredith *et al* (16) revealed that hnRNP A2/B1 knockdown inhibited long non-coding (lnc)RNA HOTAIR-dependent breast cancer cell migration and invasion by reducing the expression of junctional adhesion molecule 2 and protocadherin 10. Kuranaga *et al* (17) reported that hnRNP A2 regulated the alternative mRNA splicing of tumour protein P53 inducible nuclear protein 2 to promote invasive CRC cell migration. Taken together, these results suggested that hnRNP AB may serve a crucial role in tumourigenesis and progression of various types of cancer.

The expression and clinicopathological significance of hnRNP AB in CRC remain to be elucidated. The aim of the present study was to investigate the association between hnRNP AB expression in cancer tissues of patients with CRC and their clinicopathological features and prognosis. The effect of hnRNP AB on the progression of CRC and its potential as an independent prognostic indicator of overall survival was explored.

Materials and methods

Patients and follow-up. Two independent cohorts of patients with CRC were enrolled in the present study. To investigate the expression of hnRNP AB in CRC tissues and its association with the occurrence of CRC, 30 tumour and paired adjacent normal tissues (cohort 1, snap-frozen tissues) were collected from patients with CRC (mean age, 58.4; age range, 38-76 years; sex distribution, 17 males and 13 females) undergoing curative resection between January 2018 and April 2018 at the Affiliated Hospital of Zunyi Medical University (Zunyi, China) for RT-qPCR, immunohistochemistry and western blot analysis. The adjacent normal tissues were collected 5 cm from the lesions. The tissue samples were snap-frozen in liquid nitrogen immediately following resection and stored at -80°C.

To evaluate the prognostic role of hnRNP AB in CRC, 184 paraffin-embedded tumour specimens were obtained from patients with CRC (mean age, 58.0; age range, 21-81 years; sex distribution, 107 males and 77 females), who underwent curative resection at the Affiliated Hospital of Zunyi Medical University between January 2012 and January 2014 (cohort 2, paraffin tissues). These patients were monitored following surgery until January 30th, 2019, with a median follow-up period of 60 months. The clinicopathological data of cohort 2 that were collected include: Age, sex, preoperative carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA19-9) levels, tumour site, tumour size, differentiation status, tumour infiltration, lymph node metastasis and Tumour-Node-Metastasis (TNM) stage. Preoperative CEA and CA19-9 levels were measured using a Beckman DXI800I Analyzer (Beckman Coulter, Inc.). According to the manufacturer's instructions, the cut-off value for normal CEA is <3.5 µg/l, and that for normal CA19-9 is <35 kU/l. Pathological TNM staging was performed according to the 8th edition of the Union for International Cancer Control (UICC) (18). Detailed clinicopathological features of cohort 2 are presented in Table I. None of the patients received treatment, including radiation or chemotherapy, prior to surgery. Ethical approval for the use of human subjects was obtained from the Research

Ethics Committee of the Affiliated Hospital of Zunyi Medical University, and written informed consent was obtained from the patients or their guardians.

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted from tissues using TRIzol® reagent (Beijing Solarbio Science & Technology Co., Ltd.). RNA integrity was analysed using a NanoDrop instrument (NanoDrop Technologies; Thermo Fisher Scientific, Inc.). RNA was reverse transcribed to cDNA with a PrimeScript® RT reagent kit (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. qPCR was performed on a CFX-96 Real-Time PCR system (Bio-Rad Laboratories, Inc.) with SYBR® Premix Ex Taq™ II (Takara Biotechnology Co., Ltd.) according to the manufacturer's protocol. The thermocycling conditions were: Denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 15 sec and annealing/elongation at 60°C for 30 sec. GAPDH was used as an internal control. The primer sequences were as follows: hnRNP AB forward, 5'-AAGAAGTCTATCAGCAGCAGCAGTATG-3' and reverse, 5'-CTCCACCTCCACCACCACCTC-3'; GAPDH forward, 5'-ATGACATCAAGAAGGTGGTGAAGCAGG-3' and reverse, 5'-GCGTCAAAGGTGGAGGAGTGGGT-3'. The mRNA expression changes were calculated using the $2^{-\Delta\Delta Cq}$ method (19).

Immunohistochemistry (IHC). All CRC tissues were reviewed by two histopathologists. Consecutive 4 µm sections were mounted on 3-aminopropyltriethoxysilane-coated slides (Shanghai BioChip Co., Ltd.). Immunohistochemistry was performed using a Novolink Polymer Detection System two-step protocol (Novocastra Laboratories, Ltd.) according to the manufacturer's instructions. Tissues underwent microwave antigen retrieval in 10 mM citrate-phosphate buffer (pH 6.0; cat. no. ZLI-9064; OriGene Technologies, Inc.) at medium heat in a microwave for 14 min. Subsequently, the tissues were incubated with 3% hydrogen peroxide for 10 min at room temperature to inhibit endogenous peroxidase activity, followed by incubation with bovine serum albumin (BSA) for 10 min at room temperature to block non-specific binding. Next, tissues were incubated with hnRNP AB primary antibody (1:100; cat. no. ab199724; Abcam) at 4°C overnight, followed by a 25 min incubation with MaxVision™ horseradish peroxidase-Polymer anti-Mouse/Rabbit IHC secondary antibody (not diluted; cat. no. KIT-5020; Fuzhou Maixin Biotech Co., Ltd.) at 37°C. The tissue sections were treated with 3,3-diaminobenzidine chromogenic liquid (cat. no. ZLI-9018; OriGene Technologies, Inc) was added to the sections, incubated for 3 min at room temperature and stopped with water. The sections were counterstained with haematoxylin (cat. no. KGA223; KeyGEN bioTECH) for 25 sec at room temperature and washed with phosphate buffered solution (PBS) three times. This was followed by incubation with 1% hydrochloric acid alcohol for 30 sec and dehydration with a gradient of ethanol (70% ethanol for 5 min; 95% ethanol for 5 min; and 100% ethanol for 5 min) and treated with xylene for 3 min at room temperature. Finally, the sections were sealed with neutral gum and examined under a light microscope (magnifications x100,

Table I. Clinicopathological characteristics of patients with CRC.

Characteristics	Patients (N=184)	
	N	%
Age, years		
<60	88	47.8
≥60	96	52.2
Sex		
Male	107	58.2
Female	77	41.8
Preoperative CEA, µg/l		
<3.5	94	51.1
≥3.5	90	48.9
Preoperative CA19-9, kU/l		
<35	140	23.9
≥35	44	76.1
Tumour site		
Rectum	129	70.1
Colon	55	29.9
Tumour size, cm		
<5.0	70	62.0
≥5.0	114	38.0
Differentiation status		
Well-differentiated	55	29.9
Moderately/poorly differentiated	129	70.1
Tumour infiltration		
T1+T2	39	21.2
T3+T4	145	78.8
Lymph node metastasis		
No	78	42.4
Yes	106	57.6
TNM stage		
I-II	81	44.0
III+IV	103	56.0

T1, tumour invades the submucosa; T2, tumour invades the muscularis propria; T3, tumour invades the subserosa, non-peritonealised pericolic or perirectal tissues; T4, tumour directly invades other organs or structures and/or perforates the visceral peritoneum; TNM, Tumour-Node-Metastasis; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

x200 and x400). The expression levels were quantified using Image-Pro Plus v6.0 software (Media Cybernetics, Inc.). The mean densities were calculated as the ratio of integrated absorbance to total area. The median density of hnRNP AB staining was determined and used as a cut-off value for subsequent analyses.

Western blot analysis. Freshly frozen tissues were homogenized in RIPA buffer (high) (cat. no. R0010; Beijing Solarbio Science & Technology Co., Ltd.), and the protein concentration

was quantified with a BCA Protein Assay kit (cat. no. PC0020; Beijing Solarbio Science & Technology Co., Ltd.). SDS-PAGE loading buffer and dithiothreitol (cat. no. P1040; Beijing Solarbio Science & Technology Co., Ltd.) were added to equal amounts of protein (40 µg per lane), and were separated under reducing conditions by sodium dodecyl sulfate polyacrylamide gel electrophoresis on a 10% gel. The protein was transferred onto a nitrocellulose membrane overnight at 30 V using a wet transfer system. The membrane was blocked in 5% skimmed milk in TBS with 0.03% Tween 20 (TBST) for 1 h at room temperature, and incubated overnight at 4°C with anti-hnRNP AB (1:1,000; cat. no. ab199724; Abcam) and anti-GAPDH (1:2,000; cat. no. ab181602; Abcam) primary antibodies. Subsequently, the membrane was washed with TBST and incubated in a HRP-conjugated antibody solution (1:5,000; cat. no. 7074; Cell Signaling Technology, Inc.) for 1 h at room temperature. The membrane was washed with TBST three times and visualized using an enhanced chemiluminescence reagent (cat. no. abs920; Absin Bioscience, Inc.). Semi-quantitative analysis of absorbance values of bands was conducted using Image-Pro Plus 6.0 software (Media Cybernetics, Inc.).

Statistical analysis. Statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Inc., Chicago, IL, USA). Quantitative data between groups were analysed by Student's t-test or one-way ANOVA followed by Tukey's post hoc test. Categorical data were analysed by Pearson's χ^2 test or Fisher's exact test. Univariate and multivariate analyses were performed using Cox proportional hazard regression models. Overall survival (OS) time was calculated by the Kaplan-Meier method, and differences were analysed by the log-rank test. A value of $P < 0.05$ was considered statistically significant. All P-values were two-sided.

Results

hnRNP AB is upregulated in human CRC tissues. To evaluate the expression of hnRNP AB in CRC tissues, RT-qPCR and IHC were performed on 30 pairs of fresh-frozen human CRC tissues and adjacent normal tissues from cohort 1. RT-qPCR revealed that hnRNP AB mRNA expression levels were significantly higher in CRC tissues compared with those in adjacent normal tissues ($P < 0.01$; Fig. 1A). IHC analysis demonstrated the predominant nuclear expression of hnRNP AB in CRC tissue samples, which exhibited high hnRNP AB protein levels compared with the corresponding adjacent normal tissues (hnRNP AB protein mean density 0.0448 ± 0.005 in tumour tissues vs. 0.007 ± 0.002 in adjacent normal tissues; $P < 0.01$; Fig. 1B and C). In addition, western blot analysis was performed in 30 paired CRC and adjacent normal tissues, and hnRNP AB protein expression was significantly upregulated in tumours compared with adjacent normal tissues ($P < 0.01$; Fig. 1D and E). These results suggested that hnRNP AB may be upregulated in human CRC tissues.

Association between hnRNP AB expression and clinicopathological characteristics of patients with CRC. To investigate the association between hnRNP AB expression and patient clinicopathological characteristics, hnRNP AB

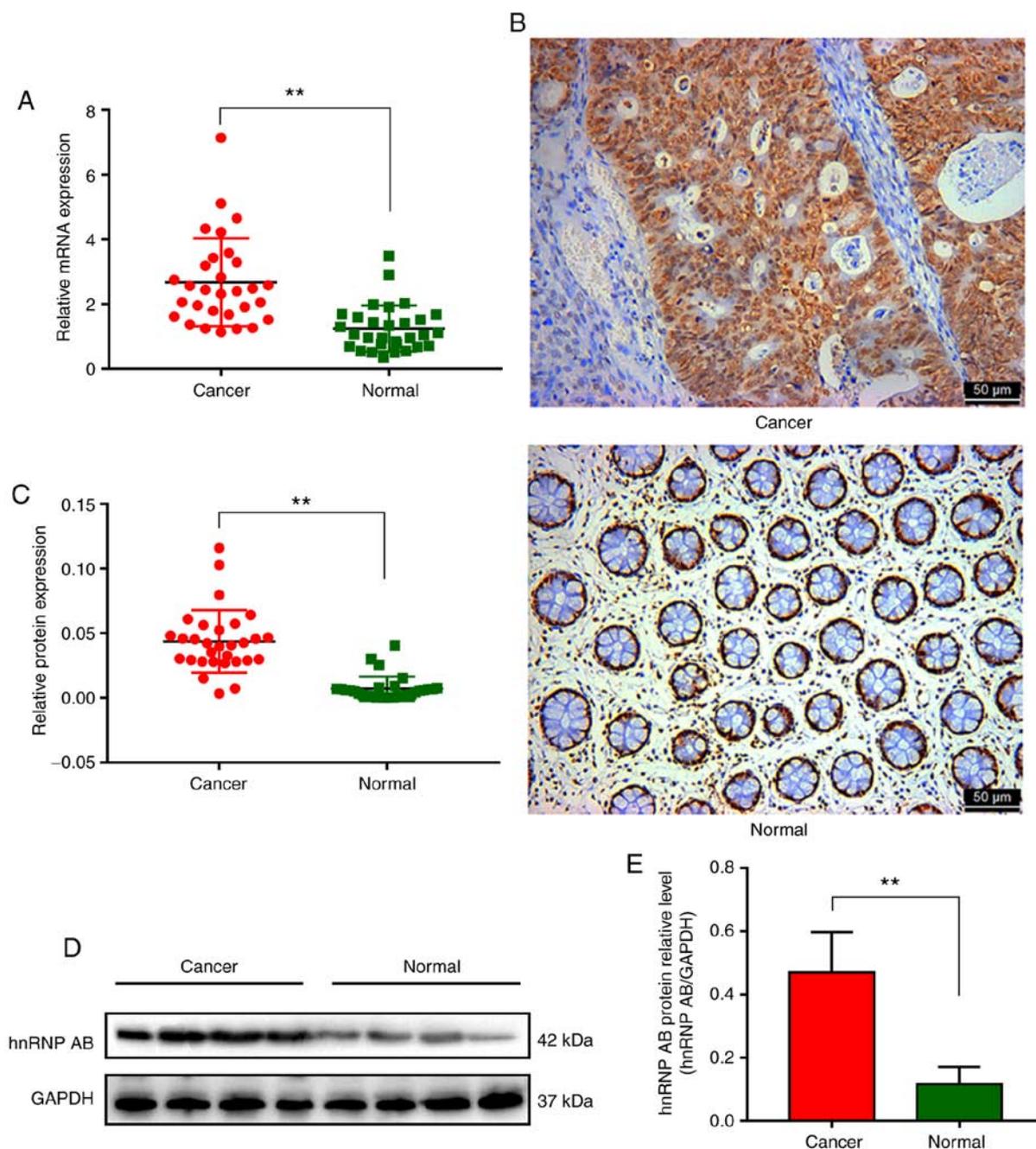


Figure 1. hnRNP AB mRNA and protein expression is upregulated in CRC tissues. (A) The mRNA expression levels of hnRNP AB in CRC tissues were detected by reverse transcription-quantitative PCR. (B) Expression patterns of hnRNP AB expression in CRC tissues and adjacent normal tissues were detected by immunohistochemistry. Scale bar, 50 μ m; magnification x200. (C) Semiquantitative analysis of hnRNP AB expression in colorectal cancer tissues and adjacent normal tissues. (D) Representative results of hnRNP AB protein expression in 4 paired CRC tissues and adjacent normal tissues. (E) The protein level of hnRNP AB was detected using western blot analysis and quantified. Statistical analyses were performed using paired Student's t-test (n=30). **P<0.01. CRC, colorectal cancer; hnRNP AB, heterogeneous ribonucleoprotein AB.

protein expression levels were detected in 184 CRC tissue specimens in cohort 2, which included all TNM stages, using IHC (Fig. 2A). The protein expression levels of hnRNP AB in stage I, II, III and IV CRC tissues were 0.0276 ± 0.0042 , 0.0328 ± 0.0043 , 0.0497 ± 0.0137 and 0.0592 ± 0.0148 , respectively, as detected by IHC (Fig. 2B). The results revealed that the difference of hnRNP AB protein expression between stage I and II CRC tissues was not statistically significant ($P=0.233$; Fig. 2B); however, the expression of hnRNP AB gradually increased between stages II, III and IV ($P<0.01$;

Fig. 2B). hnRNP AB expression levels in stage I+II, which are considered the early stage of CRC, and stage III+IV, which refer to lymph node or organ metastasis and indicate the late stage of CRC, were then compared; the protein levels of hnRNP AB in late-stage tissues were significantly higher compared with those in early-stage CRC tissues ($P<0.01$; Fig. 2C). The median density of hnRNP AB expression in patients was calculated and used as a cut-off to divide patients into high and low hnRNP AB expression groups. Pearson's χ^2 test demonstrated that hnRNP AB was

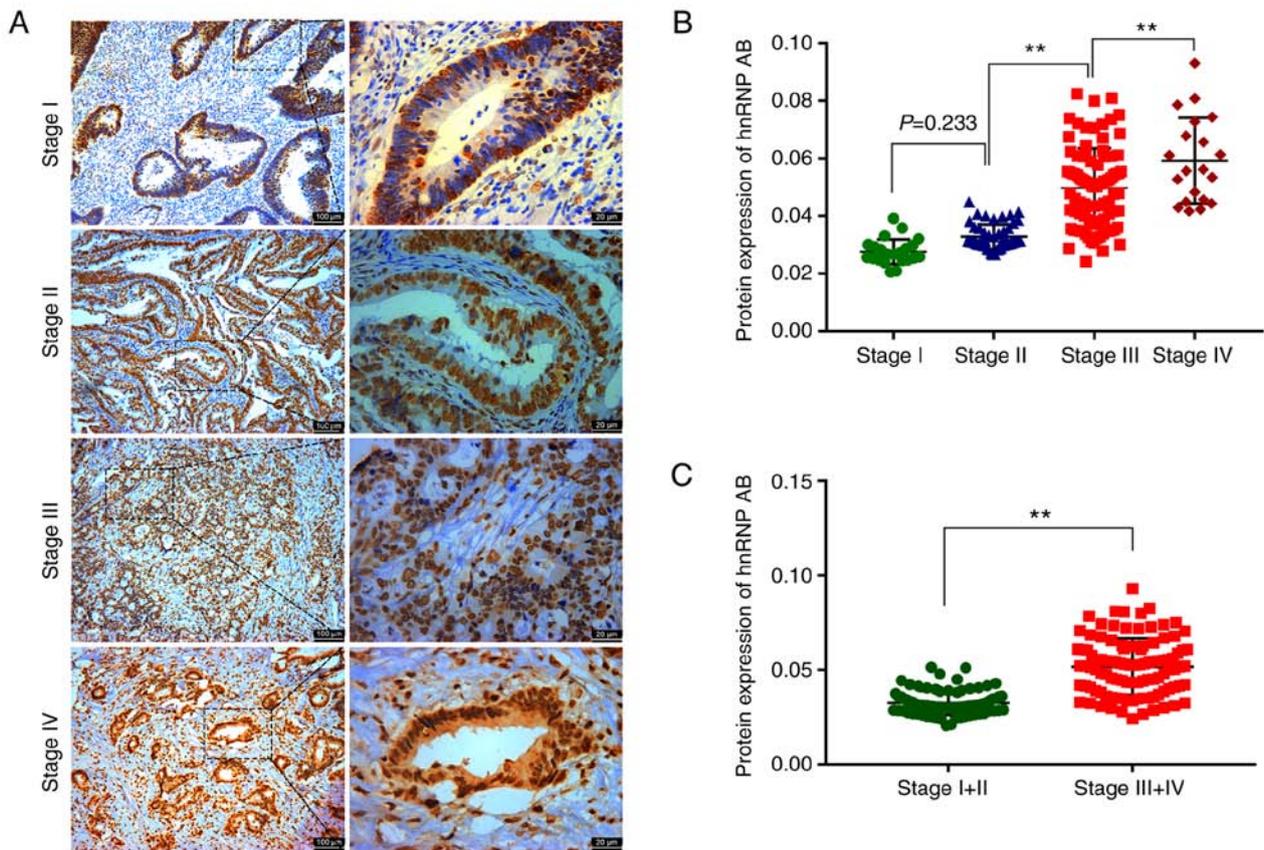


Figure 2. hnRNP AB protein levels in each stage of CRC detected by IHC. (A) Expression patterns of hnRNP AB protein expression in cancer tissues from patients with various stages of CRC. Scale bars, 100 μ m (left) and 20 μ m (right), magnification x100 (left) and x400 (right). (B) The difference of hnRNP AB protein expression between CRC stage I and II was not statistically significant, but the expression of hnRNP AB was gradually increased between stages II, III and IV. (C) The protein levels of hnRNP AB in late-stage (stage III+IV) CRC tissues were significantly higher compared with those in early-stage (stage I+II) CRC tissues. ** P <0.01. CRC, colorectal cancer; hnRNP AB, heterogeneous ribonucleoprotein AB.

significantly associated with the preoperative CEA level (P <0.001), the preoperative CA19-9 level (P =0.014), tumour size (P =0.022), tumour infiltration (P =0.026), lymph node metastasis (P <0.001) and TNM stage (P <0.001) (Table II). Other clinical characteristics, such as age (P =0.184), sex (P =0.370), tumour site (P =0.334) and tumour differentiation (P =0.197), were not associated with the expression of hnRNP AB protein (Table II).

Prognostic significance of hnRNP AB expression in CRC.

To determine whether hnRNP AB may be used as an independent risk factor for the poor prognosis of patients with CRC, conventional clinicopathological factors and hnRNP AB protein expression were analysed using Cox univariate and multivariate hazard regression models. The results of the univariate analysis indicated an association between the OS rates of patients with CRC and the preoperative CEA (P <0.001) and CA19-9 (P <0.001) levels, tumour size (P =0.028), differentiation (P =0.022) and infiltration (P =0.044), lymph node metastasis (P <0.001), TNM stage (P <0.001) and high hnRNP AB expression (P <0.001) (Table III). The multivariate Cox regression analysis revealed that the preoperative CEA level [hazard ratio (HR)=1.941; P =0.002] and hnRNP AB expression (HR=1.659; P =0.013) were independent prognostic factors for OS (Table IV). Kaplan-Meier survival analysis demonstrated that patients with low hnRNP AB expression

exhibited significantly longer OS compared with those with high hnRNP AB expression (P <0.001; Fig. 3A). In addition, patients with a low preoperative CEA level (<3.5 μ g/l) experienced significantly improved OS compared with those with a high preoperative CEA level (\geq 3.5 μ g/l; P <0.001; Fig. 3B).

Discussion

The results of the present study demonstrated that hnRNP AB was expressed at higher levels in CRC tissues compared with corresponding adjacent normal tissues in cohort 1 and that high expression of hnRNP AB in CRC was significantly associated with the serum CA19-9 level, tumour size, depth of tumour invasion, lymph node metastasis and TNM stage. High expression of hnRNP AB was associated with poor prognosis in cohort 2.

Previous studies have demonstrated that the upregulation of hnRNP AB subfamily members is associated with oncogene expression, cancer cell proliferation and invasion, apoptosis and epithelial-mesenchymal transition (EMT), which serve important roles in the occurrence and progression of various types of cancer, including lung (8,20), breast (21,22), pancreatic (23,24) and CRC (25,26). Xuan *et al* (20) reported that hnRNP A2/B1 promoted cell proliferation by activating cyclooxygenase-2 signalling in non-small cell lung cancer. Zhou *et al* (22) demonstrated that the expression of hnRNP

Table II. Association between hnRNP AB expression and clinicopathological characteristics in patients with CRC (n=184).

Characteristics	Tumour hnRNP AB expression			P-value
	Low	High	χ^2	
Age, years				
<60	49	39	2.178	0.184
≥60	43	53		
Sex				
Male	50	57	1.094	0.370
Female	42	35		
Preoperative CEA, $\mu\text{g/l}$				
<3.5	64	30	25.142	<0.001 ^a
≥3.5	28	62		
Preoperative CA19-9, kU/l				
<35	78	63	6.828	0.014 ^a
≥35	14	29		
Tumour site				
Rectum	61	68	1.271	0.334
Colon	31	24		
Tumour size, cm				
<5.0	43	27	5.903	0.022 ^a
≥5.0	49	65		
Differentiation status				
Well differentiated	32	23	1.967	0.197
Moderately/poorly differentiated	60	69		
Tumour infiltration				
T1+T2	25	12	5.717	0.026 ^a
T3+T4	67	80		
Lymph node metastasis				
No	52	26	15.044	<0.001 ^a
Yes	40	66		
TNM stage				
I+II	54	29	15.718	<0.001 ^a
III+IV	38	63		

^aP<0.05, Pearson's χ^2 test. hnRNP AB, heterogeneous ribonucleoprotein AB; T1, tumour invades the submucosa; T2, tumour invades the muscularis propria; T3, tumour invades the subserosa, non-peritonealized pericolic or perirectal tissues; T4, tumour directly invades other organs or structures and/or perforates the visceral peritoneum; TNM, Tumour-Node-Metastasis; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

A2/B1 in primary invasive breast cancer was identified in 48/85 (56.5%) samples, whereas in normal breast tissue it was identified in 7/72 (9.7%) samples; additionally, exposure to drug retinoids, at doses known to inhibit cell proliferation, led to decreased expression of hnRNP A2/B1. It has been speculated that hnRNP A2/B1 may be associated with breast cancer carcinogenesis; Xuan *et al* (21) demonstrated that the knockdown of hnRNP A1 significantly induced cell death and decreased cell invasion by regulating the splicing of CD44 in breast cancer. Brandi *et al* (24) revealed that mitochondrial uncoupling protein 2, which is a mitochondrial

anion transporter protein, promoted the proliferation of cancer cells by inducing the expression of hnRNP A2/B1 in pancreatic cancer. Dai *et al* (23) demonstrated that hnRNP A2/B1 promoted EMT and increased the proliferation and invasion of pancreatic cancer cells by activating the ERK/Snail signalling pathway.

Accumulating studies have demonstrated that hnRNP AB subfamily members are associated with the occurrence of CRC (26,27). Huang *et al* (27) reported that hnRNP A1 is involved in the progression of CRC by regulating cellular metabolism via the HOXB-AS3A peptide. Roda *et al* (26)

Table III. Univariate analyses of prognostic factors and OS (n=184).

Variables	OS		
	HR	95% CI	P-value
Age, years (<60 vs. ≥60)	1.188	0.825-1.712	0.354
Sex (Male vs. Female)	0.849	0.587-1.229	0.386
Preoperative CEA, μg/l (<3.5 vs. ≥3.5)	2.893	1.980-4.227	<0.001 ^a
Preoperative CA19-9, kU/l (<35 vs. ≥35)	2.441	1.641-3.630	<0.001 ^a
Tumour site (Rectum vs. Colon)	0.966	0.651-1.436	0.966
Tumour size, cm (<5 vs. ≥5)	1.542	1.049-2.266	0.028 ^a
Tumour differentiation (Well differentiated vs. Moderately/poorly differentiated)	1.638	1.076-2.496	0.022 ^a
Tumour infiltration (T1+T2 vs. T3+T4)	1.677	1.014-2.772	0.044 ^a
Lymph node metastasis (No vs. Yes)	1.922	1.307-2.826	0.001 ^a
TNM stage (I+II vs. III+IV)	2.285	1.557-3.353	<0.001 ^a
hnRNP AB expression (Low vs. High)	2.437	1.679-3.537	<0.001 ^a

^aP<0.05, Cox proportional hazards regression. OS, overall survival; HR, hazard ratio; CI, confidence interval; hnRNP AB, heterogeneous ribonucleoprotein AB; T1, tumour invades the submucosa; T2, tumour invades the muscularis propria; T3, tumour invades the subserosa, non-peritonealised pericolic or perirectal tissues; T4, tumour directly invades other organs or structures and/or perforates the visceral peritoneum; TNM, Tumour-Node-Metastasis; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

Table IV. Multivariate analyses of prognostic factors and OS (n=184).

Variables	OS		
	HR	95% CI	P-value
Preoperative CEA, μg/l (<3.5 vs. ≥3.5)	1.941	1.267-2.973	0.002 ^a
Preoperative CA19-9, kU/l (<35 vs. ≥35)	1.235	0.763-1.997	0.390
Tumour size, cm (<5 vs. ≥5)	1.080	0.709-1.645	0.720
Tumour differentiation (Well differentiated vs. Moderately/poorly differentiated)	1.345	0.870-2.079	0.183
Tumour infiltration (T1+T2 vs. T3+T4)	0.956	0.548-1.669	0.875
Lymph node metastasis (No vs. Yes)	1.031	0.533-1.996	0.927
TNM stage (I+II vs. III+IV)	1.348	0.680-2.669	0.392
hnRNP AB (Low vs. High)	1.659	1.111-2.476	0.013 ^a

^aP<0.05, Cox proportional hazards regression. OS, overall survival; HR, hazard ratio; CI, confidence interval; hnRNP AB, heterogeneous ribonucleoprotein AB; T1, tumour invades the submucosa; T2, tumour invades the muscularis propria; T3, tumour invades the subserosa, non-peritonealised pericolic or perirectal tissues; T4, tumour directly invades other organs or structures and/or perforates the visceral peritoneum; TNM, Tumour-Node-Metastasis; CEA, carcinoembryonic antigen; CA 19-9, carbohydrate antigen 19-9.

demonstrated that hnRNP A1 acetylation was induced in KRAS A146T allele CRC HCT116 cells following epidermal growth factor (EGF) treatment, whereas the levels of acetyl-hnRNP A1 did not change following EGF treatment in KRAS G13D-unresponsive HCT116 cells, suggesting that hnRNP A1 acetylation, which changes according to KRAS mutational status, is involved in the carcinogenic pathway in CRC.

A recent study (28) has reported that hnRNP AB interacts with the lncRNA PCAT19 to activate a series of cell cycle

genes that promote prostate cancer growth and metastasis. Zhou *et al* (9) demonstrated that the overexpression of hnRNP AB in CRC cells triggered the EMT transcription factor Snail, which resulted in EMT, and increased the proliferation and invasion of liver cancer cells. In the present study, the expression of hnRNP AB protein in 30 CRC and adjacent normal tissues was analysed by RT-qPCR, immunohistochemistry and western blotting, which revealed that the expression of hnRNP AB protein in CRC tissues was significantly higher compared with that in adjacent normal tissues. In addition,

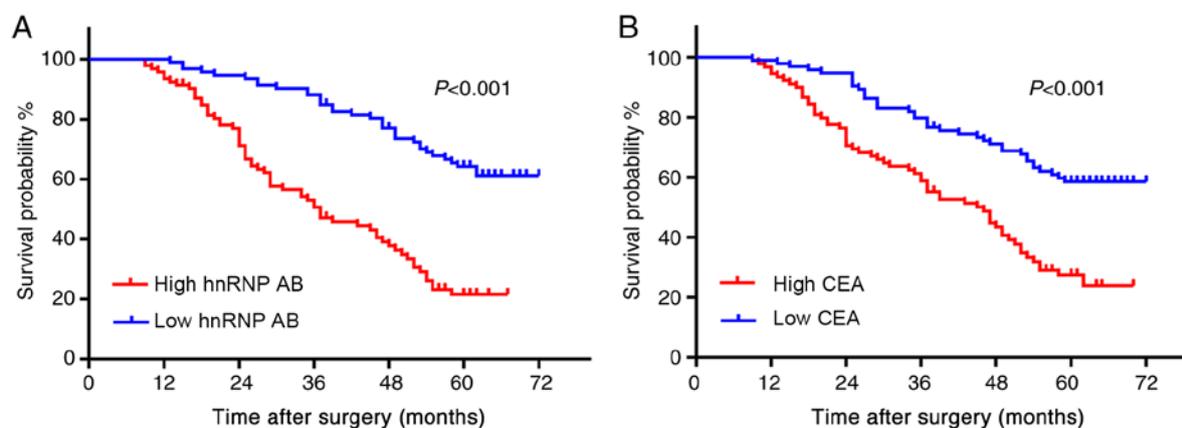


Figure 3. Kaplan-Meier analysis of overall survival of patients with CRC. (A) Patients with high hnRNP AB expression exhibited poorer prognosis compared with those with low hnRNP AB expression. (B) Patients with a high preoperative CEA level exhibited poorer prognosis compared with those with a low preoperative CEA level. CRC, colorectal cancer; hnRNP AB, heterogeneous ribonucleoprotein AB; CEA, carcinoembryonic antigen.

increased expression of hnRNP AB was observed at later stages compared with early-stage CRC tissues in cohort 2. These results indicated that hnRNP AB may be involved in the carcinogenesis and progression of CRC.

As hnRNP AB was detected in CRC tissues in the present study, further experiments were performed to determine whether there was any association between hnRNP AB expression, preoperative CEA and CA19-9 levels, tumour location and differentiation, depth of tumour invasion, lymph node and distant metastasis and TNM staging and OS rate, which may indicate hnRNP AB as an independent prognostic factor of CRC. The results demonstrated that the expression of hnRNP AB in CRC was associated with the preoperative CEA and CA19-9 levels, tumour size and infiltration, lymph node metastasis and TNM stage. The protein levels of hnRNP AB in late-stage (stage III+IV) CRC tissues were significantly higher compared with those in early-stage (stage I+II) tissues. Consistent with these results, Ma *et al* (29) reported that the expression of hnRNP A1 in normal colorectal, adenoma, CRC and CRC with liver metastasis tissues exhibited an increasing trend paralleled with the progression of CRC. Univariate Cox survival analysis in the present study indicated an association between the OS rates of patients with CRC and the preoperative CEA and CA19-9 levels, tumour size, differentiation and infiltration, lymph node metastasis, TNM stage and hnRNP AB expression. Previous studies reported that CEA was an independent prognostic factor and that elevated preoperative serum CEA levels were associated with poor outcomes in CRC (30,31). In the present study, multivariate Cox survival analysis revealed that hnRNP AB and the preoperative CEA level were significant independent factors affecting OS in patients with CRC. Although several studies have reported that elevated serum levels of CA19-9 are associated with CRC recurrence and a poor prognosis (32,33), the preoperative CA 19-9 level was not a significant independent factor affecting OS in patients with CRC in the present study. The Kaplan-Meier survival curve demonstrated that the OS time of the hnRNP AB low expression group was significantly longer compared with that of the high expression group. These findings suggested that hnRNP AB expression may be an independent prognostic marker for CRC. Park *et al* (34) reported that the

hnRNP AB subfamily member hnRNP A1 did not associate with poor prognosis in CRC; however, consistent with our results, Ma *et al* (29) reported a lower survival rate in patients with CRC with high hnRNP A1 expression levels in cancer tissues. Hope *et al* (25) demonstrated that high hnRNP A1 expression in patients with CRC was associated with advanced TNM stage, a low OS rate and poor prognosis. Zhou *et al* (9) reported that patients with hepatocellular carcinoma who expressed low hnRNP AB levels exhibited a better prognosis compared with those with high hnRNP AB levels.

Recently, Yang *et al* (35) used lncRNA microarrays to screen for hnRNP AB-regulated lnc-ELF209, the expression of which was negatively correlated with that of hnRNP AB and inhibited the progression of hepatocellular carcinoma. Warns *et al* (36) reported that an exon containing a binding site of hnRNP AB served an important role in ribosome localization, which is an important mechanism for chromatin and alternative splicing during EMT. One of the EMT regulatory factors, such as Snail, Slug or zinc finger E-box-binding homeobox 1, may be the downstream gene, which would be affected in response to the alteration in hnRNP AB. The results of the present study demonstrated that hnRNP AB was associated with the occurrence and development of malignant tumours in CRC, and the underlying molecular mechanism of hnRNP AB in CRC will be the focus of our next study.

In conclusion, the results of the present study demonstrated that hnRNP AB expression may serve an important role in the occurrence and progression of CRC and that hnRNP AB may be considered a prognostic predictor for patients with CRC, which may help improve the prognosis and quality of life of patients with CRC.

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Availability of data and materials

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

Authors' contributions

JMZ and KMW conceived and designed the experiments. JMZ, HJ, TY, XBL and GXZ performed the experiments and data statistics. JMZ, HJ, TY and KMW discussed and interpreted the data. ZJM wrote the manuscript. KMW supervised the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The present study was approved by the Ethics Committee of the Affiliated Hospital of Zunyi Medical University (Zunyi, China). Written informed consent was obtained from the patients or their guardians.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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