Original Article
Enhanced RBM8A expression in human hepatocellular carcinoma

Rong Liang[1], Yan Lin[1], Xue-Xing Yan[1], Jia-Zhou Ye[1], Yong-Qiang Li[1], Hai-Hong Ye[2]

[1]First Department of Chemotherapy, Affiliated Tumour Hospital of Guangxi Medical University, Nanning, China; [2]Department of Hepatobilliary Surgery, Affiliated Minzu Hospital of Guangxi Medical University, Nanning 530001, China

Abstract: Objective: To investigate the expression level of RBM8A and its significance in HCC. Methods: RT-PCR method, immunohistochemistry and Western blot assay were performed to detect RBM8A expression in 105 cases of HCC tissues, adjacent tissues and 67 cases of normal liver tissues; The relationship between RBM8A expression and clinicopathological features of HCC was analyzed. Results: RT-PCR showed that: RBM8A mRNA expression in HCC tissues was significantly higher than that in adjacent tissues and normal liver tissues (P<0.05). Immunohistochemistry showed that: the positive rates of RBM8A in hepatocellular carcinoma tissues, adjacent tissues, normal liver tissues were 85%, 61.67%, 5.71% (P<0.05); western blotting showed that: Gray values of RBM8A in HCC tissues, adjacent tissues and normal tissues were 1.86 ± 0.36; 1.35 ± 0.32; 0.95 ± 0.35 (P<0.05). The correlation analysis of RT-PCR, immunohistochemistry results and clinical features of liver cancer patients showed that: RBM8A protein expression was positively correlated with HBsAg, tumor size, TNM stage, Edmondson pathological grade (P<0.05). The overall OS and PFS of patients with low expression of RBM8A were prolonged compared with those with high RBM8A expression (P<0.05). Conclusion: RBM8A expression in HCC tissues was significantly higher than that in adjacent and normal tissues; RBM8A may be related to the occurrence and development of HCC.

Keywords: RBM8A, hepatocellular carcinoma

Introduction

Primary Liver Cancer (PLC) is one of the world's common gastrointestinal tumors [1]. China is a big country of liver cancer; PLC incidence rate ranks No. 2 in all cancers; the mortality is only second to lung cancer [2]. Among them, hepatocellular Carcinoma (HCC, hereinafter referred to as liver cancer) accounts for about 70-85% percent [3]. The incidence and development of liver cancer is a multi-gene, multi-step, multi-stage process; its invasion and metastasis are related with the dysfunction of a series of related genes; the key genes or protein products in this process have become the focus of biomedical research. Bursch et al in a series of experimental studies of liver cancer [4-7] found that in the initiation, triggering and progression of liver cancer, there was abnormal apoptosis, revealing the close relationship between apoptosis and liver cancer development. Early in 1999, domestic scholars Xu Hong Yu et al [8]

proposed that overexpression of Bcl-2 and low expression of Bax in HCC could inhibit cell apoptosis to cause imbalance between cell proliferation and apoptosis, leading to tumorigenesis. Then some researchers detected human liver cancer tissue and found that [9], apoptotic index of invasive liver cancer and liver metastases was significantly lower than that of non-invasive and non-metastatic liver cancer. These studies showed that apoptosis regulatory mechanism disorders-induced apoptosis blocking was undoubtedly very important for liver cancer cell transfer. So looking for liver cancer-related apoptosis gene and exploring its molecular mechanism of regulating tumor apoptosis are the forefront topics of liver cancer research, with great scientific significance.

Newly discovered RNA binding motif protein 8A (RBM8A) (also known as Y14) is one member of RNA binding motif protein (RBM) family [10]. Such protein is an RNA-binding protein, associ-

Table 1. Primers and reaction conditions of RT-PCR

Genes	Primers	Annealing temperature	cycles	Amplified fragment length (bp)
RBM8A	5'-AGATGGCGGACGTGCTAGA-3'	60	40	176
	5'-CTCCACGCTGTCATAATCCTCA-3'			
β-actin	5'-TGGCACCCAGCACAATGAA-3'	60	40	186
	5'-CTAAGTCATAGTCCGCCTAGAAGCA-3'			

ated with gene expression, cell cycle regulation and stability, regulation of apoptosis, RNA splicing and translation; its role has attracted widespread attention [11-15]. Recent studies on the mechanism of apoptosis have found that, deletion of RBM8A gene can induce apoptosis; RBM8A is an important modulator of apoptosis. Ishigaki et al [16] in the research of human cervical carcinoma cell line (HeLa) and human lung adenocarcinoma cell line (A549) found that, after RBM8A genes were knocked out, tumor cell activity was inhibited; cell cycle was arrested in M phase; abnormal central body, a characteristic of tumor cell apoptosis, appeared; these further confirmed that deletion of RBM8A gene can directly regulate the cell cycle, leading to tumor cell apoptosis; on the other hand, the deletion of RBM8A gene can down-regulate the expression levels of multiple pro-apoptotic genes such as the Bcl-Xs, Bim, and Mcl1S [17, 18]. Obviously, RBM8A gene is involved in multiple aspects of apoptosis. Other studies have confirmed that RBM8A gene could regulate the splicing of different mRNAs, closely related with tumor development.

At present, the relationship between liver cancer and RBM8A is rarely reported. ZINDY et al [19] had found that as one of the genes involved in liver cancer cell extracellular matrix remodeling, metabolism and post-transcriptional gene regulation, expression of RBM8A gene in HCC increased, but without in-depth study. Therefore, this study took RBM8A as the research object, detected and analyzed the relationship of RBM8A expression intensity with clinicopathological features and prognosis to explore its expression and significance in hepatocellular carcinoma.

Materials and methods

Materials

Source of specimen: 105 cases of pathologically confirmed liver cancer patients receiving surgical resection and in Guangxi Medical University Cancer Hospital from March 2012 to December 2014 were selected. Where in adjacent tissues were taken from the tissues 1.5 cm from tumor margin; 67 cases of normal liver tissues were drawn from the tissues more than 5 cm from hemangioma tissue.

Reagents and instruments: RBM8A antibody (Santa Cruz, USA); Trizol (Invitrogent company); reverse transcription kit and quantitative PCR kit (Takara Biotechnology Inc.); fluorescence quantitative PCR instrument Agilent Mx3000P; Nanodrop; conventional reagents, cold centrifuges and refrigerators were provided by the laboratory of Cancer Hospital of Guangxi Medical University.

Methods

RT-PCR to detect mRNA level: RNA was extracted by Trizol; After the quantitation of total RNA by a UV spectrophotometer, reverse transcription was performed according to reverse transcription kit instructions to obtain cDNA; Mx3000P real-time PCR instrument was used to detect RBM8A mRNA Levels. Primers were designed and synthesized by TAKARA Company; internal reference was β-actin; the reaction system was 20 ul; primer sequences and reaction conditions were listed in **Table 1**.

Western blotting to detect protein leve: lRIPA lysis buffer was used to extract tissue protein; Protein concentrations were determined by BCA method. 10 ul sample was subjected to SDS-PAGE gel electrophoresis to isolate protein, and then the protein was transferred to PVDF membranes by semi-dry electric transfer apparatus, blocked with skimmed milk powder, incubated with RBM8A primary antibody at 4°C overnight and β-actin secondary antibody at room temperature for 2 h before ECL developing and fixing. RBM8A molecular size was 24 KDa; β-actin protein size was 44 KDa. Bio-Rad imaging gel imager was used to capture images and analyze the results.

Immunohistochemistry assay to detect protein levels: Immunohistochemical two-step staining
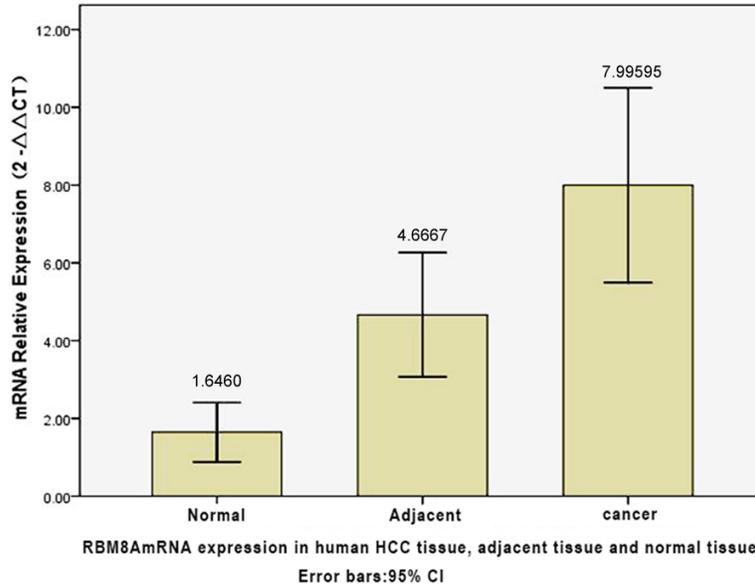
Figure 1. RBM8A mRNA expression in human HCC tissue, adjacent tissue and normal tissue.

According to the number of chromogenic cells × color intensity: 0-2 negative (-); >2 - positive, 3-4 weak positive (+), 5-7 moderate positive (++), ≥8 strong positive (+++). Western Blotting results: The film was scanned; Bio-Rad's Imaging Analysis software was used to analyze gray value (integrated absorbance, IA); IA target gene/IA β-actin represented the relative expression of RBM8A.

Statistical analysis

SPSS16.0 statistical software was used for analysis. Measurement data were expressed as x ± s; count data were indicated by the number of cases. The group in line with homogeneity of variance was analyzed by independent t-test or ANOVA; if there was variance arrhythmia, natural logarithm conversion was performed before independent t-test or ANOVA. Count data were analyzed using Pearson X^2 test, continuity correction X^2 test or Fisher's exact test. The results of immunohistochemistry were analyzed using Ordinal-logistic regression analysis. Correlation analysis was performed using Spareman rank correlation. $P < 0.05$ was considered statistically significant.

Results

RBM8A mRNA expression in human HCC tissue, adjacent tissue and normal tissue

RT-PCR analysis showed that, the average relative expression levels of RBM8A mRNA in cancer tissues, adjacent tissues and normal tissues were 7.99595 ± 5.49764, 4.6667 ± 3.78338, and 1.6460 ± 1.53852, respectively; the results showed that in terms of the expression of RBM8A mRNA, HCC > adjacent tissues > normal tissues (**Figure 1**), and the difference was statistically significant (P<0.05).

RBM8A protein expression in human HCC tissue, adjacent tissue and normal tissue

Western Blotting assay was used to detect the RBM8A mRNA expression in human HCC tis-

method was used; conventional dewaxing and hydration of slices were performed; endogenous peroxidase was inactivated at room temperature; high-temperature antigen retrieval was performed; then the sample was blocked with normal goat serum, incubated with 1:50 RBM8A primary antibody at 4°C overnight and Secondary Antibodies; After DAB staining, hematoxylin re-staining was performed. Positive tissue sections were used as positive controls; PBS buffer instead of primary antibody was used as the negative control.

Result determination: After completing RT-PCR, the relative expression level was calculated by 2-ΔΔCT (ΔΔCT = [CT (target gene) - CT (β-actin-) experimental group] - [CT (target gene) - CT (β-actin) control group]); normal liver cells 7702 were used as controls. Immunohistochemical film reading was performed by two pathologists; semi-quantitative analysis was performed using thirteen-point scoring method: according to the proportion of coloring cells in slices to score: 0-no cell was stained; 1-the ratio of coloring cells was less than 10%; 2-the ratio of coloring cells was more than 10% but less than 50%; 3-the ratio of coloring cells was more than 50% but less than 80%; 4-the ratio of coloring cells was more than 80 percent. According to cell color intensity to score: 0-no coloration; 1-pale yellow (weak staining); 2-brownish yellow (moderate staining); 3-tan (strong staining).

Table 2. Gray value of RBM8A expression in each group

| | Cases | RBM8A/β-actin gray value | |
		Mean ± standard deviation	P-value
HCC tissues	105	1.86 ± 0.36	
Adjacent tissues	105	1.35 ± 0.32	0.000*
Non-cancerous tissues	67	0.95 ± 0.35	0.000#

Note: Compared with HCC tissues, adjacent tissues *P = 0.00, Non-cancerous tissues #P = 0.00.
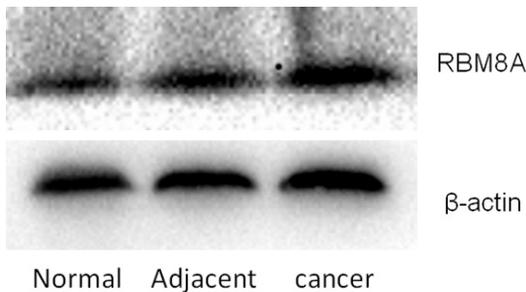
Figure 2. RBM8A protein expression in human HCC tissue, adjacent tissue and normal tissue.

sue, adjacent tissue and normal tissue; the results showed that RBM8A had been expressed in three kinds of tissues.

Results showed that, the average relative gray values in cancer tissues, adjacent tissues and normal tissues were 1.86 ± 0.36, 1.35 ± 0.32 and 0.95 ± 0.35, respectively; The statistical analysis showed that: RBM8A protein expression in HCC tissues was higher than that in adjacent tissues and non-cancerous tissues, and RBM8A protein expression in adjacent tissues was higher than that in non-cancerous tissues; the difference was statistically significant (P = 0.00), shown in **Table 2**; **Figure 2**.

Immunohistochemistry

Compared with the negative control (**Figure 3A**), RBM8A was lightly expressed in non-cancerous tissue (**Figure 3B**); its expression can also be found in adjacent tissues, and the degree of intensity was stronger compared with non-cancerous tissues (**Figure 3C**); high expression (**Figure 3D**) was observed in hepatocellular carcinoma tissues; protein was mainly located in cytoplasm of cancer cells, showing dark brown yellow or brown granular staining.

RBM8A positive expression rates in HCC tissues, adjacent tissues and non-cancerous tissues were 87.62% (92/105), 60.95% (64/105), 4.47% (3/67). RBM8A staining intensity in each group was shown in **Table 3**.

Immunohistochemistry showed that, there were significant differences in RBM8A expression levels in HCC tissues, paraneoplastic and non-cancerous tissues (P = 0.00). RBM8A expression level in HCC tissues was higher than that in adjacent and non-cancerous tissues, and the level in adjacent tissues was higher than that in non-cancerous tissues.

The relationship between RBM8A expression and clinical index

With 7.99595 as the boundary of RBM8A mRNA expression in HCC, 105 cases were divided into RBM8A low expression group (85 cases) and high expression group (20 cases); high expression positive rate was 80.95%. And the relationship of RBM8A mRNA and protein levels with clinical pathological features was analyzed, shown in **Tables 4**, **5**.

There were statistically significant differences in RBM8A protein expression between different groups of HBsAg, tumor diameter, TNM stage and Edmondson pathologic grade (*P*<0.05.) RBM8A mRNA high expression was significantly correlated with HbsAg and Edmondson pathologic grade; while in terms of tumor diameter and TNM stage, there was no significant difference (P>0.05). RBM8A expression increased in groups of HbsAg and Edmondson pathologic grade.

Further nonparametric rank correlation analysis of RBM8A protein expression in Edmondson pathologic grade groups showed that: P = 0.00, shown in **Table 5**. Spearman correlation coefficient was 0.992, suggesting that liver cancer histological grade was positively correlated with RBM8A expression intensity; the lower degree of differentiation, the stronger RBM8A expression.

Survival analysis

For the 105 cases, follow-up time was 3-620 days; 0 lost cases; a total of 105 cases were
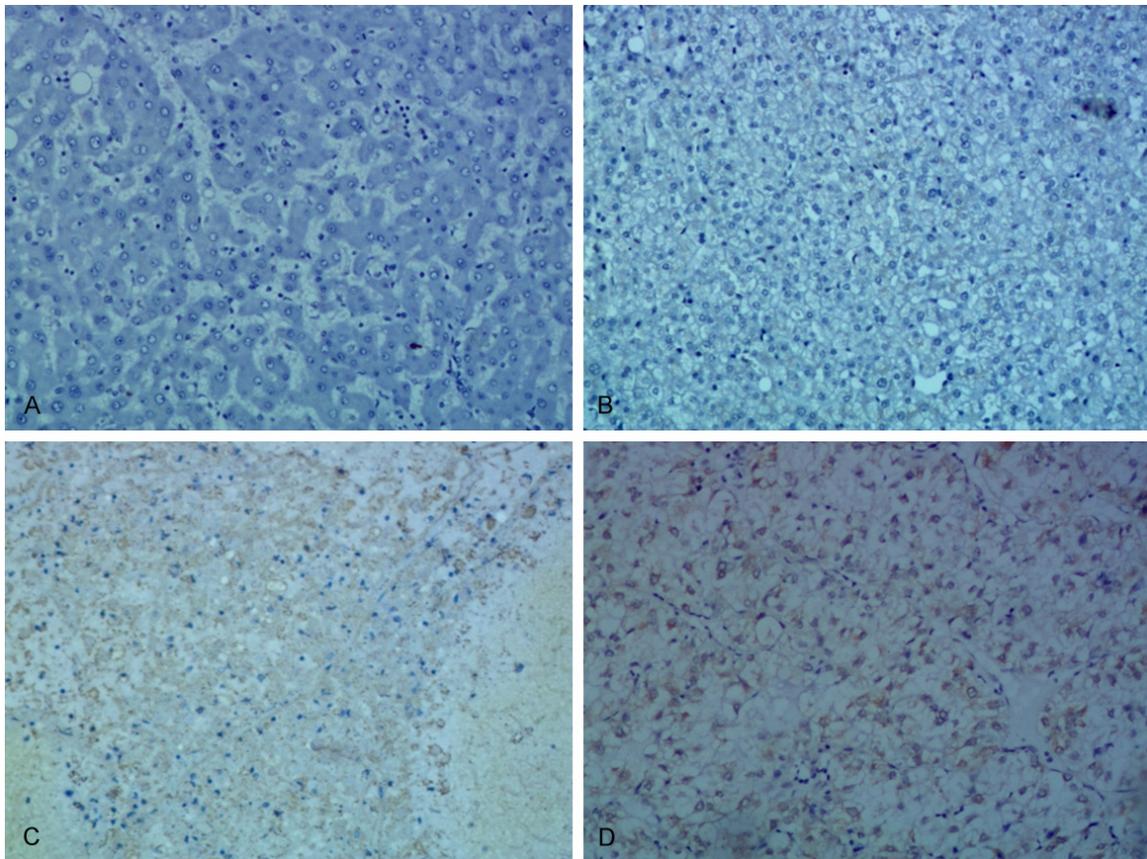
Figure 3. Immunohistochemistry was used to detect RBM8A expression in the negative control group (A), non-cancerous tissue (B), adjacent tissues (C) and HCC tissues (D), ×200.

Table 3. RBM8A staining intensity in HCC tissues, adjacent tissues and non-cancerous tissues

	Cases	Strong positive	Positive	Weak positive	Negative	Positive rate	P value
HCC tissues	105	18	46	28	13	87.62%	
Adjacent tissues	105	3	28	33	41	60.95%	0.00*
Non-cancerous tissues	67	0	1	2	64	4.47%%	0.00#

Note: Compared with HCC tissues, adjacent tissues *P = 0.00, Non-cancerous tissues #P = 0.00.

included in the statistics; follow-up rate was 100%. The RBM8A expression of all patients were divided into high expression group (positive and strongly positive, n = 64) and low expression group (negative and weak positive, n = 41). Kaplan-Meier survival curve method was used to analyze and calculate the OS and PFS in high expression group and low expression group, with the Log-rank test to detect whether there were significant differences between the two groups.

The results showed that: in RBM8A low expression and high expression groups, the median

OS were 229 days and 160 days (P = 0.000) respectively (**Figure 4A**); the median PFS were 114 days and 90 days, respectively (P = 0.001) (**Figure 4B**). Therefore, the overall OS and PFS of patients with low expression of RBM8A was significantly longer (P<0.005).

Discussion

RBM8A is a RNA binding protein of 26 kDa; the corresponding gene in the chromosome is 14q21-q23, which encodes four transcriptions; it is widely expressed in cells, shuttling in cytoplasm and nucleus [20]. Compared with other

Table 4. The relationship between the strength of RBM8A expression in HCC tissue and clinical index

| Items | Cases | RBM8A mRNA | | | RBM8A protein | | |
		High expression	Low expression	P-value	Positive	Positive	P-value
Sex				0.104			0.144
Male	78	66	12		71	7	
Female	27	19	8		21	6	
Age				0.079			0.463
≥60 years	35	25	10		29	6	
<60 years	70	60	10		63	7	
AFP ($\mu \cdot gL^{-1}$)				0.516			0.433
≥400	78	62	16		70	8	
<400	27	23	4		22	5	
HBsAg				0.000			0.000
Positive	82	73	9		81	1	
Negative	23	12	11		11	12	
Tumor diameter (CM)				0.248			0.013
≥10	28	20	8		27	1	
5-10	50	41	9		39	11	
<5	27	24	3		26	1	
Cirrhosis				0.329			0.58
Yes	58	45	13		54	4	
No	47	40	7		38	9	
Portal vein tumor thrombosis				0.128			0.276
Yes	42	31	11		35	7	
No	63	54	9		57	6	
Child-pugh score				0.283			0.670
A	43	34	9		37	6	
B	58	49	9		52	6	
C	4	2	2		3	1	
Edmondsom Pathology							
Grades				0.004			0.003
I	20	10	10		12	8	
II	45	40	5		42	3	
III	38	33	5		36	2	
IV	2	2	0		2	0	
TNM stages				0.153			0.000
I	24	16	8		15	9	
II	60	51	9		58	2	
III	21	18	3		19	2	
IV	0						

genes in the family, currently RBM8A's complete structure and specific mechanism of action are rarely reported in the literature; it has been known that its main biological role is to participate in the formation of exon-junction biding complex (EJC) [21]; meanwhile, it is also the core protein of nonsense codon-mediated mRNA decay (NMD), involved in NMD-mediated mRNA monitoring. NMD is a monitoring mechanism to ensure the stability of RNA in eukaryotic cells; it can quickly clear the abnormal transcripts containing premature termination codons (PTC) and reduce the generation of hazardous truncated proteins [22-24]. In 2001,

Table 5. Relationship between the strength of RBM8A protein expression and Edmondson pathological grade

Edmondson Pathological grade		RBM8A/β-actin gray value	
I	20	1.41 ± 0.51	
II	45	1.63 ± 0.89	0.004[#]
III	38	2.21 ± 0.63	0.001[##]
IV	2	2.50 ± 0.71	0.031[###]

Edmondson pathological grade, [#]: Between grade II and grade III, P = 0.004. [##]: Between grade I and grade III, P = 0.001. [###]: Between grade I and grade IV, P = 0.031.

Noensie and Dietz [25] proposed to use NMD mechanism in cancer research, using drugs or siRNA to inhibit NMD, thereby identifying the mutant gene. Chinese scholars also raised that the incidence of malignant tumors was related with the stability of mRNA [26]; therefore, looking for the NMD interfering with cancer cells is likely to open new avenues for cancer treatment. RBM8A, as a core component of NMD, may play an important role in cell malignant transformation process.

So far high RBM8A gene expression has been found in a variety of malignancies, including: primary liver cancer, pleural mesothelioma and multiple myeloma [18, 19, 27-31]. It has been found in further studies that RBM8A may be involved in tumor regulation mechanism through following ways:

In human lung adenocarcinoma, RBM8A gene-knockout cells were arrested in G2/M phase; RBM8A-Mago complex affected the positioning of the central body thereby preventing tumor cell mitosis; it mediated apoptosis by regulating cell cycle [16, 32]. Meanwhile, as the core component of EJC, RBM8A deletion can down-regulate the expression levels of pro-apoptotic genes such as Bcl-xS and Mcl1 in Bcl-2 family; it was closely related with disorders in apoptosis regulation mechanism; so it can mediate metastasis of HCC [17].

Three cell pathways, Ras/MAPK, JAK/STAT3 and NF-κB, had been confirmed to be closely associated with liver cancer, synergistically involved in the development, invasion and metastasis of liver cancer [33-35]. RBM8A deletion would result in the following changes in above three pathways [36-39]: 1, MAPK protein synthesis was reduced, inhibiting Ras/ MAPK pathway and leading to cell apoptosis in advance; 2, JAK/STAT3 pathway was inhibited; DNA-binding activity of STAT3 decreased in tandem; 3, phosphorylation in TNF-α/STAT3 pathway was reduced, resulting in weakened effect of NF-κB. Therefore, as the common molecular of three signaling pathways, RBM8A genes play a very important role in controlling cell growth and apoptosis molecular network.

Our research from cell and histology levels confirmed that the expression level of RBM8A in HCC tissues was significantly higher than that in adjacent tissues and normal liver tissues, indicating that RBM8A can be used as a meaningful histopathological marker, providing an iconic protein marker for diagnosis of liver cancer. One of the mechanisms of malignancy is high-frequency nonsense mutation of oncogenes; these nonsense mutants after initiating NMD pathway can lead to deletion of tumor suppressor gene expression because the translation is terminated in advance. High expression of RBM8A in HCC confirmed that mechanism of action of NMD was more active, indirectly identifying that PTC gene mutation level in hepatocellular carcinoma was significantly higher than that in normal liver cancer. The expression of RBM8A in adjacent tissues was significantly higher than that in normal tissues, whether the difference was caused by the presence of micro-metastases in adjacent tissues remains to be further studied.

RBM8A expression level is associated with HbsAg expression. Epidemiological data show that hepatitis B virus infection is the primary risk factor of HCC in China; almost all HBV-related liver cancers have hepatitis B virus integration; hepatitis B virus integrated in the liver cell genome accelerates the development of liver fibrosis and liver cell inflammation, thus promoting liver cancer [40, 41]. This study found that, in HbsAg positive group, gene and protein levels of RBM8A were significantly increased, most likely participating HbsAg gene integration and at the protein level interacting with the hepatitis B surface antigen to jointly promote the occurrence of liver cancer.

Histological grade and TNM stage of HCC are clinically important indicators to assess the degree of malignancy and prognosis of liver cancer; they are important for prognosis judgement [42]. The results of this study showed

A

B
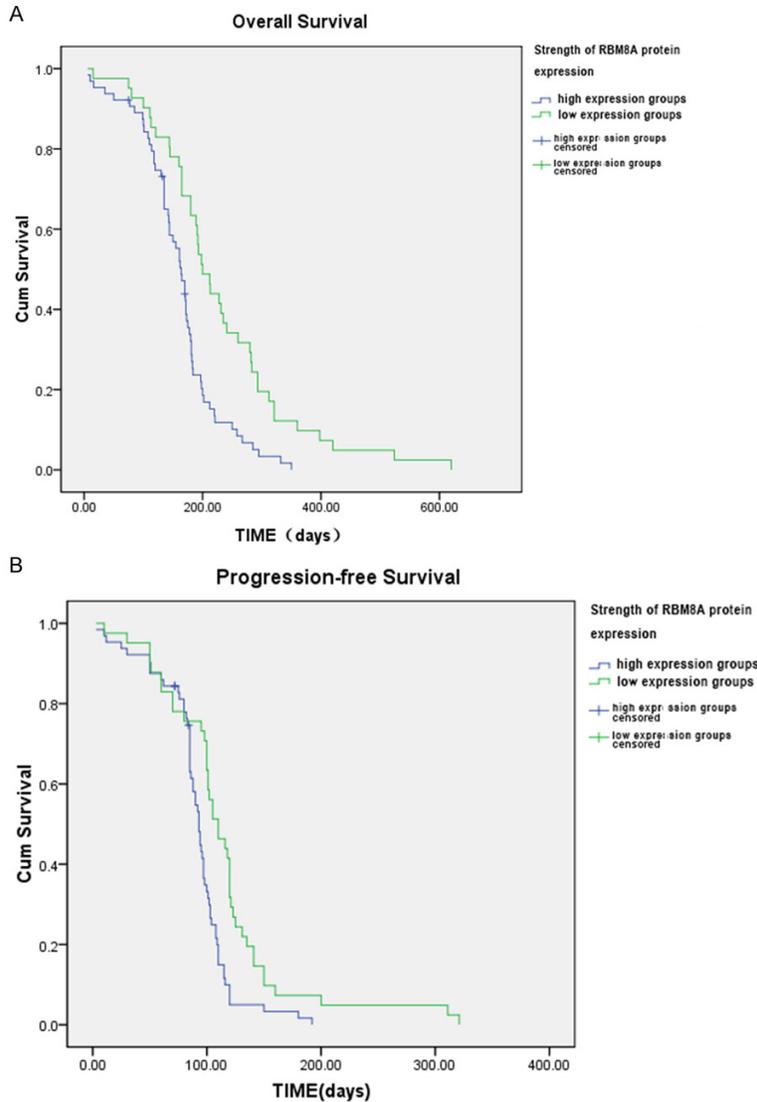
Figure 4. Strength of RBM8A protein expression and survival analysis.

and the clinical follow-up study was conducted. Survival analysis showed that, in low RBM8A expression group, median OS and the median PFS were was 229 days and 114 days respectively, higher than those (160 and 90 days) high RBM8A expression group; the difference was statistically significant. So RBM8A may be used as a prognostic factor of HCC, which was closely related with the recurrence and metastasis, and survival of patients.

In summary, in this study, we confirmed that the expression of RBM8A was elevated in HCC. There were statistically significant differences in RBM8A expression between different groups of HBsAg, tumor diameter, TNM stage and Edmondson pathologic grade, likely to help determine the degree of pathological differentiation and clinical stage. Meanwhile, in the survival analysis, we found that OS and PFS of patients with low expression of RBM8A were longer than those with high expression of RBM8A. Our study revealed the possible correlation between RBM8A and liver cancer development; strengthening the in-depth study of the molecular mechanisms will contribute to provide new ways for tumor targeted therapy.

that: the expression level of RBM8A in advanced hepatocellular carcinoma was higher than that in early stage; while in histology, the lower the histological grade, the higher the RBM8A expression. Kim et al [28] have demonstrated that in cervical cancer patients with lymph node metastasis RBM8A level was higher than that in those without lymph node metastasis (P<0.009), indicating that RBM8A may become a clinical molecular marker of early diagnosis and assessment of risk transfer of liver cancer.

Based on immunohistochemistry results, patients were divided into high RBM8A expression group and low RBM8A expression group,

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Hai-Hong Ye, Department of Hepatobilliary Surgery, Affiliated Minzu Hospital of Guangxi Medical University, No. 232 Mingxiu Road East, Nanning 530001, Guangxi, China. Tel: 0771-5335155; E-mail: yehhvip@163.com

References

[1] Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. Global cancer statistics 2012. CA Cancer J Clin 2015; 65: 87-108.

[2] Chen JG, Chen WQ, Zhang SW, Zheng RT, Zhu J and Zhang YH. Incidence and mortality of liver cancer in China: an analysis on data from the Nationd Registration System between 2003 and 2007. Zhong Hua Liu Xing Bing Xue Za Zhi 2012; 33: 547-53.

[3] Perz JF, Armstrong GL, Farrington LA, Hutin YJ and Bell BP. The contributions of hepatitis B virus and hepatitis C virus infections to cirrhosis and primary liver cancer worldwide. J Hepatol 2006; 45: 529-538.

[4] Bursch W, Grasl-Kraupp B, Ellinger A, Török L, Kienzl H, Müllauer L and Schulte-Hermann R. Active cell death: role in hepatocarcinogenesis and subtypes. Biochem Cell Biol 1994; 72: 669-675.

[5] Bursch W, Lauer B, Timmermann-Trosiener I, Barthel G, Schuppler J and Schulte-Hermann R. Controlled death (apoptosis) of normal and putative preneoplastic cells in rat liver following withdrawal of tumor promoters. Carcinogenesis 1984; 5: 453-458.

[6] Bursch W, Paffe S, Putz B, Barthel G and Schulte-Hermann R. Determination of the length of the histological stages of apoptosis in normal liver and in altered hepatic foci of rats. Carcinogenesis 1990; 11: 847-853.

[7] Schulte-Hermann R, Bursch W, Kraupp-Grasl B, Oberhammer F, Wagner A and Jirtle R. Cell proliferation and apoptosis in normal liver and preneoplastic foci. Environ Health Perspect 1993; 101 Suppl 5: 87-90.

[8] Xu HY and Guan XL. The Bcl-2 and Bax protein expression in primary liver cancer and the relationship between cell apoptosis index. Ha Er Bin Yi Ke Da Xue Xue Bao 1999; 33: 436-438.

[9] Kong XB, Liang LJ and Huang JF. Hepatocellular carcinoma (HCC) apoptosis, p21 and PCNA expression and the clinical outcomes of research. Ai Zheng 1999; 426-429.

[10] Salicioni AM, Xi M, Vanderveer LA, Balsara B, Testa JR, Dunbrack RL Jr and Godwin AK. Identification and Structural Analysis of Human RBM8A and RBM8B: Two Highly Conserved RNA-Binding Motif Proteins That Interact with OVCA1, a Candidate Tumor Suppressor. Genomics 2000; 69: 54-62.

[11] Bechara EG, Sebestyén E, Bernardis I, Eyras E and Valcárcel J. RBM5, 6, and 10 differentially regulate NUMB alternative splicing to control cancer cell proliferation. Mol Cell 2013; 52: 720-733.

[12] Boman K, Segersten U, Ahlgren G, Eberhard J, Uhlén M, Jirström K and Malmström PU. Decreased expression of RNA-binding motif protein 3 correlates with tumour progression and poor prognosis in urothelial bladder cancer. BMC Urol 2013; 13: 17.

[13] Ehlén A, Brennan DJ, Nodin B, O'Connor DP, Eberhard J, Alvarado-Kristensson M, Jeffrey IB, Manjer J, Brändstedt J, Uhlén M, Pontén F and Jirström K. Expression of the RNA-binding protein RBM3 is associated with a favourable prognosis and cisplatin sensitivity in epithelial ovarian cancer. J Transl Med 2010; 8: 78.

[14] Fushimi K, Ray P, Kar A, Wang L, Sutherland LC and Wu JY. Up-regulation of the proapoptotic caspase 2 splicing isoform by a candidate tumor suppressor, RBM5. Proc Natl Acad Sci U S A 2008; 105: 15708-15713.

[15] Jögi A, Brennan DJ, Rydén L, Magnusson K, Fernö M, Stål O, Borgquist S, Uhlen M, Landberg G, Påhlman S, Pontén F and Jirström K. Nuclear expression of the RNA-binding protein RBM3 is associated with an improved clinical outcome in breast cancer. Mod Pathol 2009; 22: 1564-1574.

[16] Ishigaki Y, Nakamura Y, Tatsuno T, Hashimoto M, Shimasaki T, Iwabuchi K and Tomosugi N. Depletion of RNA-binding protein RBM8A (Y14) causes cell cycle deficiency and apoptosis in human cells. Exp Biol Med (Maywood) 2013; 238: 889-897.

[17] Michelle L, Cloutier A, Toutant J, Shkreta L, Thibault P, Durand M, Garneau D, Gendron D, Lapointe E, Couture S, Le Hir H, Klinck R, Elela SA, Prinos P and Chabot B. Proteins associated with the exon junction complex also control the alternative splicing of apoptotic regulators. Mol Cell Biol 2012; 32: 954-967.

[18] Sudo H, Tsuji AB, Sugyo A, Kohda M, Sogawa C, Yoshida C, Harada YN, Hino O and Saga T. Knockdown of COPA, identified by loss-of-function screen, induces apoptosis and suppresses tumor growth in mesothelioma mouse model. Genomics 2010; 95: 210-216.

[19] Zindy PJ, L'Helgoualc'h A, Bonnier D, Le Béchec A, Bourd-Boitin K, Zhang CX, Musso O, Glaise D, Troadec MB, Loréal O, Turlin B, Léger J, Clément B and Théret N. Upregulation of the tumor suppressor gene menin in hepatocellular carcinomas and its significance in fibrogenesis. Hepatology 2006; 44: 1296-1307.

[20] Maderazo AB, Belk JP, He F and Jacobson A. Nonsense-containing mRNAs that accumulate in the absence of a functional nonsense-medi-

ated mRNA decay pathway are destabilized rapidly upon its restitution. Mol Cell Biol 2003; 23: 842-851.

[21] Chuang TW, Chang WL, Lee KM and Tarn WY. The RNA-binding protein Y14 inhibits mRNA decapping and modulates processing body formation. Mol Biol Cell 2013; 24: 1-13.

[22] Gehring NH, Neu-Yilik G, Schell T, Hentze MW and Kulozik AE. Y14 and hUpf3b form an NMD-activating complex. Mol Cell 2003; 11: 939-949.

[23] Hilleren P and Parker R. mRNA surveillance in eukaryotes: kinetic proofreading of proper translation termination as assessed by mRNP domain organization? RNA 1999; 5: 711-719.

[24] Ishigaki Y, Nakamura Y, Tatsuno T, Ma S and Tomosugi N. Phosphorylation status of human RNA-binding protein 8A in cells and its inhibitory regulation by Magoh. Exp Biol Med (Maywood) 2015; 240: 438-445.

[25] Noensie EN and Dietz HC. A strategy for disease gene identification through nonsense-mediated mRNA decay inhibition. Nat Biotechnol 2001; 19: 434-439.

[26] Hu YQ. To control the stability of the mRNA in higher eukaryotes. Guo Ji Yi Chuan Xue Za Zhi 1997; 44-45.

[27] Carrasco DR, Tonon G, Huang Y, Zhang Y, Sinha R, Feng B, Stewart JP, Zhan F, Khatry D, Protopopova M, Protopopov A, Sukhdeo K, Hanamura I, Stephens O, Barlogie B, Anderson KC, Chin L, Shaughnessy JD Jr, Brennan C and Depinho RA. High-resolution genomic profiles define distinct clinico-pathogenetic subgroups of multiple myeloma patients. Cancer Cell 2006; 9: 313-325.

[28] Kim TJ, Choi JJ, Kim WY, Choi CH, Lee JW, Bae DS, Son DS, Kim J, Park BK, Ahn G, Cho EY and Kim BG. Gene expression profiling for the prediction of lymph node metastasis in patients with cervical cancer. Cancer Sci 2008; 99: 31-38.

[29] Knuutila S, Aalto Y, Autio K, Björkqvist AM, El-Rifai W, Hemmer S, Huhta T, Kettunen E, Kiuru-Kuhlefelt S, Larramendy ML, Lushnikova T, Monni O, Pere H, Tapper J, Tarkkanen M, Varis A, Wasenius VM, Wolf M and Zhu Y. DNA copy number losses in human neoplasms. Am J Pathol 1999; 155: 683-694.

[30] Salicioni AM, Xi M, Vanderveer LA, Balsara B, Testa JR, Dunbrack RL Jr and Godwin AK. Identification and structural analysis of human RBM8A and RBM8B: two highly conserved RNA-binding motif proteins that interact with OVCA1, a candidate tumor suppressor. Genomics 2000; 69: 54-62.

[31] Kong LQ, Pu YH and Ren GS. Y14 expression in breast cancer tissue and significance. Zhong Guo Zhong Liu Lin Chuang 2010; 37: 1106-1108.

[32] Ishigaki Y, Nakamura Y, Tatsuno T, Hashimoto M, Iwabuchi K and Tomosugi N. RNA-binding protein RBM8A (Y14) and MAGOH localize to centrosome in human A549 cells. Histochem Cell Biol 2014; 141: 101-109.

[33] Arsura M and Cavin LG. Nuclear factor-kappaB and liver carcinogenesis. Cancer Lett 2005; 229: 157-169.

[34] Calvisi DF, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM and Thorgeirsson SS. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. Gastroenterology 2006; 130: 1117-1128.

[35] Wang C, Cigliano A, Delogu S, Armbruster J, Dombrowski F, Evert M, Chen X and Calvisi DF. Functional crosstalk between AKT/mTOR and Ras/MAPK pathways in hepatocarcinogenesis: implications for the treatment of human liver cancer. Cell Cycle 2013; 12: 1999-2010.

[36] Ohbayashi N, Taira N, Kawakami S, Togi S, Sato N, Ikeda O, Kamitani S, Muromoto R, Sekine Y and Matsuda T. An RNA biding protein, Y14 interacts with and modulates STAT3 activation. Biochem Biophys Res Commun 2008; 372: 475-479.

[37] Roignant JY and Treisman JE. Exon junction complex subunits are required to splice Drosophila MAP kinase, a large heterochromatic gene. Cell 2010; 143: 238-250.

[38] Togi S, Shiga K, Muromoto R, Kato M, Souma Y, Sekine Y, Kon S, Oritani K and Matsuda T. Y14 positively regulates TNF-alpha-induced NF-kappaB transcriptional activity via interacting RIP1 and TRADD beyond an exon junction complex protein. J Immunol 2013; 191: 1436-1444.

[39] Yang L and Baker NE. Cell cycle withdrawal, progression, and cell survival regulation by EGFR and its effectors in the differentiating Drosophila eye. Dev Cell 2003; 4: 359-369.

[40] Brechot C, Kremsdorf D, Soussan P, Pineau P, Dejean A, Paterlini-Brechot P and Tiollais P. Hepatitis B virus (HBV)-related hepatocellular carcinoma (HCC): molecular mechanisms and novel paradigms. Pathol Biol (Paris) 2010; 58: 278-287.

[41] Xu C, Zhou W, Wang Y and Qiao L. Hepatitis B virus-induced hepatocellular carcinoma. Cancer Lett 2014; 345: 216-222.

[42] Yan T, Zhao JJ, Bi XY, Zhao H, Huang Z, Li ZY, Zhou JG, Li Y, Li C, Cai JQ and Zhao P. [Prognosis of hepatocellular carcinoma: a study of 832 cases]. Zhonghua Zhong Liu Za Zhi 2013; 35: 54-58.