The Clinical Significance of RMI2 in Hepatocellular Carcinoma

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Abstract

Background: Hepatocellular carcinoma (HCC), which is the most common type of primary liver cancer, often presents at advanced stage with a dismal prognosis. Novel tumor biomarkers are needed to aid in HCC early detection and prognostication.

Methods: Immunohistochemical staining for RecQ-mediated genome instability protein 2 (RMI2) was performed in 330 surgically resected HCC specimens and 190 adjacent normal tissues. Univariate and multivariate regression analysis were applied to identify prognostic indicators of HCC outcomes. Patient’s survival was assessed with the Kaplan–Meier method.

Results: RMI2 in HCC tissue was significantly higher than that in adjacent normal tissues, and was positively correlated with HCC histological grade and stage (P < .05) but negatively correlated with the survival period. RIM2 was identified to be an independent prognostic indicator for HCC. Conclusion: The abnormal expression of RMI2 may be related to the occurrence and development of HCC. RIM2 could potentially serve as a novel tumor-specific biomarker for HCC diagnosis and prognosis prediction.

Keywords
RMI2, hepatocellular carcinoma, immunohistochemistry, bioinformatics, prognosis, survival

Abbreviations
HCC, hepatocellular carcinoma; CCNB2, cyclin B2; CDCA3, cell division cycle associated protein 3

Introduction

Liver cancer remains to be a global health challenge with staggering high morbidity and mortality.1 There will be more than 1 million new cases of liver cancer each year by 2025.2 The incidence of liver cancer ranks fourth in China among all types of cancer. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for approximately 90% of all cases.2 Although early stages of HCC can be treated with radiofrequency ablation or surgical resection,3,4 most patients with HCC presented to clinical attention at a relatively advanced stage with dismal prognosis.5,6 Thus, techniques like theranostics using a reliable and specific biomarker hold the promise of early detection and improved clinical outcome for HCC.

RecQ-mediated genome instability (RMI) protein 2 (RMI2), which is an important component of RMI complex, can form a stable subcomplex with RMI1 through an interaction similar to the OB folding domain in Replication Protein A (RPA). While RMI1 is present in most eukaryotes, RMI2 can only be found in plants and vertebrates, suggesting that RMI2 is essential in higher eukaryotes with complex genomes.7 The OB-fold

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domains of RMI1 and RMI2 heterodimerize, likely bringing RMI2 to the BLM-Topo IIIα-RMI1-RMI2 (BTR) complex. In previous in vitro studies, the BTR complex was shown to resolve double Holliday junctions. The BTR complex was further shown to be unstable in vitro without the RMI2 component, and RMI2-deficient cells under replication stress could not efficiently recruit BLM (the mutated helicase in Bloom syndrome) to chromatin and nuclear foci. These data suggest that RMI2 holds key functions in the BTR complex maintenance of chromosome stability. RMI1 gene variants expose people to a higher risk of tumorigenesis.\(^8\) The study reported by Xu et al\(^\text{7}\) indicated that RMI2 gene mutations or genetic variations may also lead to genome instability and increased risk of tumorigenesis,\(^9,10\) such as lung cancer.\(^11,12\) The survival rate of patients with lung adenocarcinoma with RMI2 high expression was significantly lower than that with RMI2 low expression. However, studies with regard to the correlation of RMI2 and HCC are scarce. Herein, we explored the expression of RMI2 in HCC and its correlations with patient clinical features and outcomes.

### Materials and Methods

#### Patient Population and Samples

Tissue samples from 330 HCC patients (268 men and 62 women with a median age of 56 years [range 30-85]) were collected from January 2006 to December 2012 at ZheJiang Provincial People’s Hospital. All samples were fixed with formaldehyde, embedded in paraffin, and fabricated into tissue microarrays that contained 330 HCC samples and 190 adjacent normal tissues. The clinicopathologic data, including patient age, sex, alpha-fetoprotein (AFP), tumor size, location, number, lymph node/distant metastasis and Edmondson grade, were collected from the electronic medical records. Survival time was defined as the interval from surgery to death or the last follow-up. The study was approved by the Ethics Committee of ZheJiang Provincial People’s Hospital (IRB:2021QT261). All patients signed an informed consent form for the advancement of clinical research.

#### Immunohistochemistry and Analysis

RMI2 expression was assessed by immunohistochemistry and Western blotting. Specifically, 4 mm thick sections were deparaffinized, dehydrated and rehydrated, and incubated with anti-RMI2 antibody (Delaware Avenue, CA, Santa Cruz Biotechnology Inc) mouse monoclonal antibody.

Two independent pathologists blind to the clinical data scored immunohistochemical slides according to the intensity and proportion of positive staining cells. Staining intensity standard: 0 = no staining, 1 = weak or light yellow staining, 2 = moderate or brownish yellow staining, 3 = strong or tan. The staining ratio scores are as follows: 0 points = cells stained less than 5%, 1 point = cells stained 6% to 26%, 2 points = cells stained 26% to 50%, 3 points = cells stained more than 50%. The final score, which was the result of positive cells multiplied by the staining intensity, was categorized as 0 to 4 points (low RMI2 expression) or ≥5 points (high RMI2 expression). Using RIPA buffer for protein extraction in tissues and perform Western blot analysis according to standard procedures. Primary antibodies were: RMI2 (Abcam, ab122685) the recommended dilution for IHC-P is 1:200, for WB is 1:1000, and GAPDH 1:10 000 (Abcam, EPR16891).

#### Statistical Analysis

The Student’s \(t\)-test and the chi-square test/Fisher’s exact test were used to analyze the measurement data and categorical data, respectively. The Kaplan–Meier method was applied to display the overall survival curve, and the difference between the curves was tested by Log-rank test. The Cox regression was used to perform multiple survival analysis on patient data to evaluate the relationship between RMI2 expression and prognosis. Two-sided \(P\) value less than .05 indicated statistical significance (Table 1).

#### Results

RMI2 is predominantly located at the cytoplasm of HCC cells. RMI2 positivity was found in 33 (17.4%) in the control specimens, which was significantly lower than that in the HCC tissues (17.4% vs 64.6%, \(P<.01\)). A typical example of RMI2 immunostaining is shown in Figure 1. Further verification with Western blotting also obtained similar results (Figure 2).

#### The Relationship Between RMI2 Expression and Pathological Data of HCC

The correlation between RMI2 expression and patient pathological parameters were tabulated in Table 2. The rate of RMI2 expression was significantly correlated with tumor size, number and microvascular invasion \((P<.05)\) but not with patient age \((P = .301)\), gender \((P = .241)\), presence of metastasis \((P = .124)\), Edmondson grade \((P = .506)\), liver cirrhosis \((P = .807)\), and hepatitis B virus (HBV) infection \((P = .806)\).

#### Survival Analysis

The median survival time of patients with high RMI2 expression was significantly shorter than that with low RMI2

### Table 1. Expression of RMI2 mRNA in HCC and Non-Carcinomatous Liver Tissues.

| Samples                 | RMI2 expression | Number | Negative | Positive | \(P\)  |
|-------------------------|-----------------|--------|----------|----------|------|
| HCC                     |                 | 330    | 117      | 213      | <.01 |
| Non-carcinomatous liver |                 | 190    | 157      | 33       |      |

Abbreviations: RMI2, RecQ-mediated genome instability protein 2; HCC, hepatocellular carcinoma.
protein expression (27.1 ± 1.4 months vs 34.5 ± 1.7 months, \( P < .05 \)). Kaplan–Meier analysis indicated that RMI2 high expression was related to reduced overall survival (\( P < .05 \), Figure 3). Univariate analysis of postoperative survival shows that tumor size (0.01), Edmondson grade (<0.01), Microvascular invasion (<0.01), AFP level (<0.01), RMI2 expression (0.04), and metastasis (\( P = .02 \)) were related to the postoperative survival. Nonetheless, gender, number of tumors, hbs antigen, and cirrhosis were of no prognostic value (Table 3).

**Discussion**

Hepatocellular carcinoma (HCC) is the sixth most common cancer in the world.\(^\text{13}\) The prognosis of advanced HCC is still unsatisfactory, and treatment options are limited.\(^\text{14}\) Surgical resection and liver transplantation are the two main treatment modalities for HCC, but the clinical outcomes remain unsatisfactory. A reliable and specific biomarker of HCC has the potential to assist in early detection of HCC. Several
Biomarkers, such as AFP and des-gamma-carboxy prothrombin (DCP), have already been approved for the early detection of HCC.\textsuperscript{15} However, the sensitivity of AFP for the diagnosis of HCC is only 60\% even if a low-level cut-off (10-20 ng/mL) is used.\textsuperscript{16} Furthermore, AFP is normal in 15\% to 30\% of patients with even advanced HCC.\textsuperscript{17} DCP is usually used in combination with AFP and serves as a supplementary indicator. It is still unclear whether the combination of AFP with DCP provides better clinical performance than AFP alone.\textsuperscript{18-20}

Oncogene and tumor suppressor gene mutations are involved in the tumorigenesis and progression of HCC. For instance, common carcinogens like HBV and HCV can cause the activation of oncogenes and the inactivation of tumor suppressor genes. The accumulation of genomic alterations will finally lead to the tumorigenesis.\textsuperscript{21} The identification of these genes, the understanding of their functions and true mechanisms provide specific targets for early theranostic technique.\textsuperscript{22}

RMI2 is a eukaryotic family of OB3, oligo-nucleotide-binding proteins. It is a dispensable component of the RMI complex and plays a vital part in the producing of homologous recombination intermediates in order to control DNA-crossover-formation in cells.\textsuperscript{23} RMI2 can interact with cyclin B2 (CCNB2) and cell division cycle-associated protein 3 (CDCA3), which function also as regulatory proteins and

| Table 2. Relationship Between RMI2 Expression and Clinicopathological Parameters of HCC Patients. |
|---------------------------------------------------------------|
| Clinical parameters                           | RMI2 expression | $c^2$   | $P$      |
| Gender                                       | Low  | High  |         |         |
| Male                                         | 1.376 | .241  |         |
| Female                                       | 1.071 | .301  |         |
| Age (years)                                  | 1.071 | .301  |         |
| $<55$                                        | 1.071 | .301  |         |
| $\geq 55$                                    | 1.071 | .301  |         |
| Size                                         | 11.216 | .001  |         |
| $<5$ cm                                      | 11.216 | .001  |         |
| $\geq 5$ cm                                  | 11.216 | .001  |         |
| Number of tumors                             | 9.394 | .002  |         |
| Single                                       | 9.394 | .002  |         |
| Multiple                                     | 9.394 | .002  |         |
| Metastasis                                   | 2.363 | .124  |         |
| M0                                           | 2.363 | .124  |         |
| M1                                           | 2.363 | .124  |         |
| Microvascular invasion                       | 7.671 | .006  |         |
| No                                           | 7.671 | .006  |         |
| Yes                                          | 7.671 | .006  |         |
| Edmondson grade                              | .443  | .506  |         |
| I + II                                       | .443  | .506  |         |
| III                                          | .443  | .506  |         |
| Cirrhosis                                    | 0.060 | 0.807 |         |
| Negative                                     | 0.060 | 0.807 |         |
| Positive                                     | 0.060 | 0.807 |         |
| HBV                                         | 0.060 | 0.807 |         |
| Absent                                       | 0.060 | 0.807 |         |
| Present                                      | 0.060 | 0.807 |         |

Abbreviations: RMI2, RecQ-mediated genome instability protein 2; HCC, hepatocellular carcinoma; HBV, hepatitis B virus.

The total number of cases is less than 330 because of incomplete pathological data.
Herein, the relative abundance of RMI2 protein in 330 cases of HCC tissues was quantitatively evaluated by immunostaining. Correlation analysis between clinicopathological characteristics and RMI2 staining showed that the proportion of stained tumor cells and microvascular invasion. Kaplan–Meier analysis was further applied to prove that the abundance of RIM2 can be an independent predictor of HCC prognosis. Analysis of the survival period of HCC patients with up-regulated RMI2 expression was significantly shortened. Thus, RIM2 can serve as an effective biomarker for predicting the progression of HCC.

Table 3. Multivariate Analysis of the Correlation Between Clinicopathological Parameters and Survival Time of Patients With HCC patients.

| Parameters               | Univariate analysis | Multivariate analysis |
|--------------------------|---------------------|-----------------------|
|                          | HR      | 95% CI | P  | HR      | 95% CI | P  |
| Age                      | 0.66    | 0.41 to 1.05 | .08 | NA      | NA     | NA |
| Gender                   | 1.55    | 0.91 to 2.64 | .11 | NA      | NA     | NA |
| Tumor size               | 1.96    | 1.22 to 3.31 | .01 | 1.56    | 0.75-3.23 | .24 |
| Number of tumors         | 1.25    | 0.69 to 2.29 | .46 | NA      | NA     | NA |
| Edmondson grade          | 2.75    | 1.72 to 4.41 | <.01 | 2.64    | 1.23-5.63 | .01 |
| Metastasis               | 4.83    | 2.56 to 9.14 | <.01 | 3.23    | 1.26-8.27 | .02 |
| Microvascular invasion   | 2.16    | 1.28 to 3.66 | <.01 | 1.15    | 0.54-2.45 | .72 |
| HBs antigen              | 1.16    | 0.65 to 2.09 | .61 | NA      | NA     | NA |
| Cirrhosis                | 1.14    | 0.69 to 1.89 | .60 | NA      | NA     | NA |
| AFP level                | 2.49    | 1.39 to 4.49 | <.01 | 1.61    | 0.77-3.36 | .21 |
| RMI2 expression          | 1.73    | 1.03 to 2.89 | .04 | 1.33    | 0.59-3.00 | .50 |

Abbreviations: HBs antigen, hepatitis B surface antigen; AFP, alpha-fetoprotein; HR, hazard ratio; CI, confidence interval.

Our study provides a novel tumor-specific target for HCC prognosis prediction. The clinical significance of RMI2 was proved in clinical samples, promising its reliability. Prospective multicenter studies are needed in the future to conclusively demonstrate the prognostic significance of RMI2. However, the value of our study might be discounted by the inherent defect of methodology due to the limited volume of collected samples and limited visualization under microscope.

Authors’ Note

WJX and LZH analyzed and interpreted the patient data regarding the bladder cancer. WH performed the histological examination of the tissue. ZB was a major contributor in writing the manuscript and ZDH, ZP edited the manuscript. All authors read and approved the final manuscript.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Supplemental Material

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