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Regulator of Chromosome Condensation 2 Expression Profile in Hepatocellular Carcinoma and Its Potential Link to Clinical Parameters

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ABSTRACT TD-60 is a heavily-conversed protein and its other name is Regulator of chromosome condensation 2 (RCC2). No studies so far have reported the role played by RCC2 in hepatocellular carcinoma. The prominent locations where RCC2 can be observed are nuclei in liver tumor and non-tumor tissues, during immunohistochemistry investigations. In this research, the authors used HCC tissue microarray to assess RCC2 expression. This study followed 2-step immunohistochemistry whereas the pathology score obtained from its results was analyzed further as per the standard protocol. According to the study results, RCC2 was inferred as a poor prognostic factor of HCC. In other terms, there was a positive correlation exist between the expression level of RCC2 and the pathological grading. It can be inferred that the patients with HCC and high amounts of RCC2 expression are prone to heavy risks due to high pathological grading. With these results as the basis, the current study speculates that the RCC2 may aggravate the oncogenic progression in HCC.

INTRODUCTION

The most commonly diagnosed cancer in the liver is Hepatocellular carcinoma (HCC), according to Villanueva (2019). Despite the reduced incidence rate of liver cancer for the past few decades, globally, China has the largest population suffering from liver cancer due to aging and tremendous growth in population (Jafri and Kamran 2019). According to Duan et al. (2019), even though one can prolong the survival time of HCC patients partially using chemotherapy or surgery, the mortality rate is still high due to recurrence or metastasis. When a set of new molecular markers is understood thoroughly, it may pave the way for new diagnostic and therapeutic strategies for HCC.

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According to Humphries et al. (2009), TD-60, also named as Regulator of Chromosome Condensation 2 (RCC2) is a heavily conversed protein. Grigera et al. (2012) identified the key role of RCC2 for the first time in mitotic cells. In the recent studies, various studies reported the crucial role played by RCC2 in the progression of cancer such as glioma (Yu et al. 2019), gastric cancer (Matsuo et al. 2013), colorectal cancer (Bruun et al. 2015), lung cancer (Pang et al. 2017; Lin et al. 2018), ovarian cancer (Buranjiang et al. 2019; Chen et al. 2019) and breast cancer (Chen et al. 2019). In gastric cancer, Matsuo et al. (2013) found that RCC2 was up-regulated and targeted by the tumor suppressor gene miR-29c. There was a reduction in the cell growth and modifications in the cell morphology observed in colorectal cancer during RCC2 knockdown (Bruun et al. 2015). The upregulated RCC2 was found to be promoting the metastasis of lung adenocarcinoma and it also increased the motility of the cell by triggering the epithelial-mesenchymal transition (Pang et al. 2017). The studies con-

ducted earlier confirmed the oncogenic role of RCC2. Further, few researchers (Yu et al. 2019; Wu et al. 2020) implicated the relationship between RCC2 and drug-sensitive or radio-resistance in tumors. However, there have been no reports published so far focusing on the potential function of RCC2 in hepatocellular carcinoma.

Objectives

The current study is aimed at validating the expression profile of RCC2 in hepatocellular carcinoma and to reveal the potential link between RCC2 and clinical parameters including overall survival and progression free survival.

MATERIAL AND METHODS

Clinical Materials

The study made use of HCC tissue microarray (HLiv-HCC180Sur-06) (Shanghai Outdo Biotech Co., Ltd. SOBC) to assess the expression of RCC2. In this microarray, a total of 90 HCC specimens was present along with paired adjacent carcinoma tissues (1.5 cm away from the carcinoma). Among the study population considered, that is, HCC patients, 74 were males while 16 patients were female. The sample population had a median age of 51.5 (ranged from 27 to 84). These patients reported to have tumor sized in the range of 1 cm to 15 cm. With regards to the stage of cancer, 61 patients were of stage I clinical-grade while 29 were stage-II. Table 1 shows the clinical characters in a detailed manner. These diagnosed HCC patients received no extra therapy before the surgery during February 2006 and May 2007. The patients were followed-up until February 2012. In this period, 49 patients were reported to be fatal due to HCC while 41 were still alive then. The median survival time was calculated to be 49.5 months.

Immunohistochemistry

The current study followed the two-step immunohistochemistry. After treating the tissue sections with retrieval antigen and EDTA buffer, the sections were kept under incubation at 4°C with primary antibody anti- RCC2 overnight. After this, the tissue sections were again incubated with secondary antibody (HRP-labeled anti-mouse antibody, DAKO). PBS was used to wash these sections which were then visualized using diaminobenzidine (DAB) and hematoxylin re-dying system. The immunohistochemistry results were given scores by the pathologists and the scoring pattern is as follows; '0' denotes negative, '+' for 1, '++' corresponds to 2 and '+++' denotes 3. Based on the proportion of positively stained cancer cells, the positive staining rate was defined that is, 'Negative' denotes 0, '1%-20%' corresponds to 1, '21%-40%' for 2, '41-60%' for 3, '61-80%' denotes 4 and '81-100%' for 5. The total score is calculated as a product of 'dyeing intensity score' and 'dyeing positive rate'.

Statistical Analysis

NPar test was utilized to assess the differential expression of RCC2 in HCC tissues as well as in adjacent tissues. Kaplan-Meier method as well as log-rank test were used to draw the survival curve on the basis of RCC2 expression and clinical characters. After this, the author used COX multivariate regression survival analysis with all the potential predictive factors. The correlation exists between few clinical immunohistochemical factors and RCC2 expression was evaluated using Spearman rank correlation coefficient. The same entity was also used for analyzing the correlation between TB, ALT, ALB, AFP, and GGT expression and RCC2 expression in HCC tissues. The authors used SPSS 22.0 software. $P < 0.05$ was considered significant.

RESULTS

Overexpression of RCC2 in Hepatocellular Carcinoma

The authors performed IHC staining for a total of 90 paired hepatocellular carcinoma tissues and adjacent non-tumor tissues in order to assess the expression profile of RCC2 in HCC. Figure 1 represents the IHC staining pictures of RCC2 on tumor as well as non-tumor tissues. It can be observed that the RCC2 got primarily localized in the nuclei of liver tissues of both tumor as well as non-tumor types. RCC2 was found to be up-regulated in tumor tissues

(2.00±2.41) while it was contradictory in paired non-tumor tissues (0.09±0.31, $p<0.001$).

RCC2 Correlated with Pathological Grading

The potential correlation that may exist between the expression of RCC2 and other clinical parameters such as clinical TNM, pathological grading, T, N, M, tumor size, gender and age was analyzed by the authors in order to understand the clinical correlation of RCC2 in HCC (Table 1). There was a positive correlation found in the results between RCC2 expression and pathological grading. The results further inferred no significant correlation between RCC2 and age, gender, T, N, M, tumor size, pathological grading or clinical TNM. The study also assessed the potential relationship between the expression of RCC2 and TB, ALT, ALB, AFP, GGT expression. There was a positive correlation between RCC2 and AFP (P=0.001, r=0.360), a significant HCC biomarker while there was no correlation found between other factors and RCC2

Table 1: Clinical pathological parameters of HCC patients

Clinical parameters	*No. patients*
Gender	
Male	74
Female	16
Age	
d”60	72
>60	18
Tumor Size	
d”5cm	48
>5cm	42
Pathological Grade	
I	1
II	57
III	32
T Stage	
T1	61
T2	29
cTNM Stage	
1	61
2	29

This table has all demographic information and pathological information

HCC Patients with High RCC2 Expression Have a Shorter Overall Survival

On the basis of different RCC2 expression levels such as low RCC2 expression level group and high RCC2 expression level group, the 90HCC patients were segregated into two groups. The authors further analyzed the correlation between different RCC2 expression levels with that of overall survival and disease-free survival rates. As shown in the Figure 2, the results infer that those patients with high RCC2 expression levels experience only a short overall survival time and disease-free survival time. Further, the poor prognosis factors were identified to be clinical TNM, large tumor size, and high T. Based on the COX regression analysis performed for all the prognostic factors, the results infer that tumor size was an independent prognostic factor.

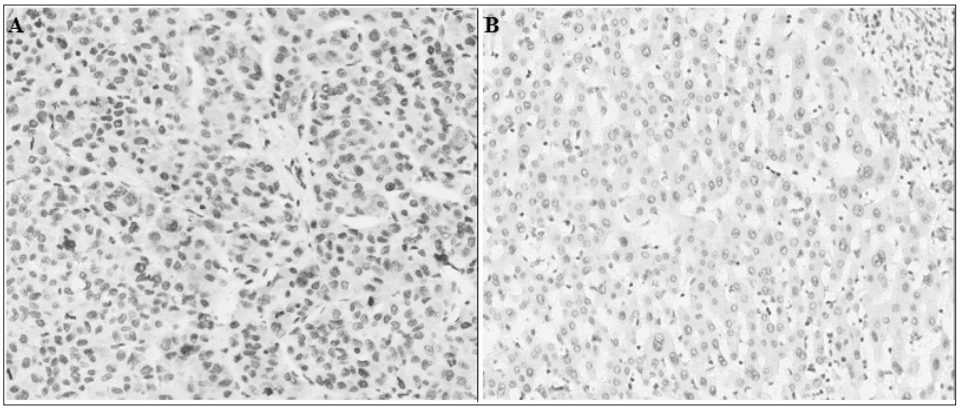

Fig. 1. Represent RCC2 immunohistochemical pictures of tumor (A) and non-tumor (B) live tissues
RCC2 got primarily localized in the nuclei of liver tissues of both tumor as well as non-tumor types. RCC2 was found to be up-regulated in tumor tissues

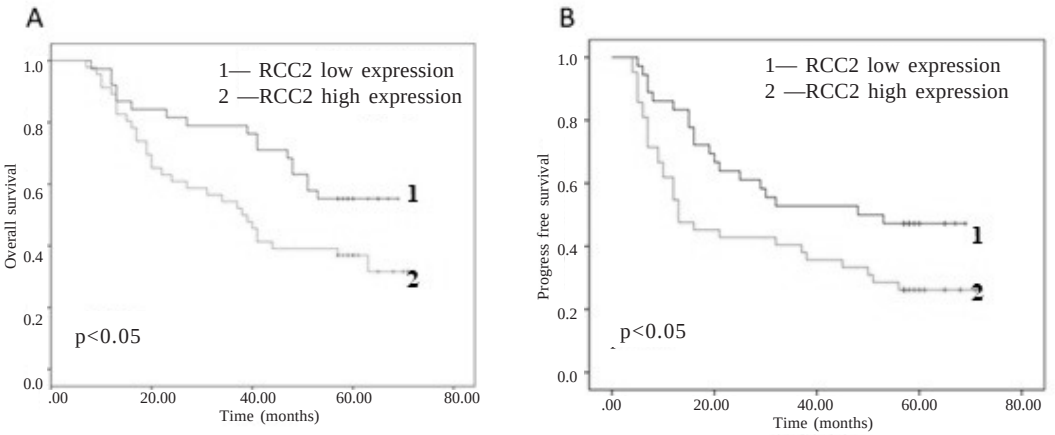

Fig. 2. Kaplan-Meier survival curves dependent on different RCC2 expression (A) Overall survival (B) Progress free suvival. *P* values were calculated with log-rank test

Patients with high RCC2 expression levels experience only a short overall survival time and disease-free survival time

DISCUSSION

According to researchers, RCC2 is predominantly localized in nuclei in hepatic tumor and non-tumor tissues during immunohistochemistry observations. The current study results inferred that RCC2 was a poor prognostic factor of HCC. The authors confirm that there is a positive correlation exist between RCC2 expression level and pathological grading. Those patients with HCC and high expression levels of RCC2 are prone to high-risks and high pathological grading. With results as a standard, the authors speculate that the RCC2 may enhance the oncogenic progression in hepatocellular carcinoma. The current study focused on those HCC patients in stage I and stage II. Future investigations must be conducted to analyze the potential role and activity of RCC2 in metastasis of HCC.

The researchers (Humphries et al. 2009; Chen et al. 2020; Zheng et al. 2020) conducted studies in RCC2 (Regulator of chromosome condensation 2) or TD-60, earlier inferred that this TD-60 is a highly-conversed protein. In the study conducted by Andreassen et al. (1991), RCC2 was identified with the help of human autoimmune antiserum at the spindle midzone in anaphase and telophase. Grigera et al. (2012) identified the important role played by RCC2 during mitosis process. Various studies conducted in the recent years established the role of RCC2 in carcinogenesis, in different cancer types such as breast cancer gastric cancer (Matsuo et al. 2013; Chen et al. 2019; Gong et al. 2019), colorectal cancer (Bruun et al. 2015; Chen et al. 2020; Wang et al. 2020; Zheng et al. 2020), glioma (Yu et al. 2019), lung cancer (Pang et al. 2017; Lin et al. 2018) and ovarian cancer (Buranjiang et al. 2019; Chen et al. 2019). RCC2 was identified to play a crucial role in LUAD metastasis in the study conducted by Pang et al. (2017). This study reported that RCC2 modulated the signaling pathways such as EMT and MAPK–JNK pathways. The report published by Bruun and colleagues mentioned that 50 UTR mutation of RCC2 diminishes the protein expression. This way it notably keeps the prognosis of microsatellite-stable (MSS) tumors under control (Bruun et al. 2015).

Matsuo et al. (2013) identified the role of RCC2 in the regulation and suppression of different microRNA in tumor cells. One of the finest examples of this phenomenon is miR-29c in gastric cancer. Bruun et al. (2015) reported that when RCC2 was absent in colorectal cancer, the cell growth got reduced while the cell morphology got altered significantly. In the study conducted by Pang et al. (2017), the upregulated RCC2 was shown to be promoting the metastasis of lung adenocarcinoma. It further triggered the epithelial-mesenchymal transition for enhanced cell motility. Even though RCC2's oncogenic role was confirmed earlier in different cancer types (Gong et al. 2019), there is no such

specific report published yet with regards to the functional role of RCC2 in HCC. Further, the potential correlation between RCC2 and teratocarcinoma is also not well documented yet (Chen et al. 2020; Wang et al. 2020; Zheng et al. 2020).

There is a dearth of studies that investigated the regulatory mechanism of RCC2 in tumors. RCC2 was found to have a relationship with epithelial-mesenchymal transition and cell autophagy (Wang et al. 2020; Zheng et al. 2020) in lung cancer. In the case of breast cancer, the oncogenic progression was induced by RCC2 via the regulation of Wnt signaling and promotion of EMT. These reports helped the authors to speculate that there exists a close relationship between RCC2 and EMT. Future investigations should be carried out to decipher the potential function of RCC2 on EMT related genes' expression in HCC or function. Song et al. (2018) mentioned that RCC2 was a target of p53, an established tumor suppressor. RCC2 expression was known to be regulated by microRNAs, including miR-29c and miR-331-3p (Matsuo et al. 2013; Buranjiang et al. 2019). Lin et al. (2018) reported that lncRNA LCPAT1 regulates RCC2 expression.

CONCLUSION

The study found a close relationship between HCC and RCC2. This finding was supported through the overexpression of RCC2 in HCC tissues. There was a positive correlation found between the expression level of RCC2 and pathological grading. So, high pathological grading may be allotted by those HCC patients with high RCC2 expression. So the current study speculates that RCC2 might improve the oncogenic progression in HCC. In addition to the above, the study also found a close correlation between the expression level of RCC2 and pathology grading.

RECOMMENDATIONS

The high expression levels of RCC2 among Hepatocellular carcinoma patients indicate the poor overall survival. These results infer that RCC2 might not be a favorable prognostic factor in HCC. Future investigations should be conducted focusing on the potential function of RCC2 on EMT related genes expression in HCC or function

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