

A pan-cancer analysis of RCC2 and its interaction with HMGA2 protein in an *in vitro* model of colorectal cancer cells

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Abstract. Regulator of chromosome condensation 2 (RCC2) is highly involved in the development of tumor malignancies. The underlying mechanisms remain to be elucidated. The present study aimed to explore the role of RCC2 in the development of tumor malignancies and explore the underlying mechanisms in colorectal cancer (CRC). RCC2 expression and survival analysis were performed in human pan-cancer. The results of searching its mRNA expression in The Cancer Genome Atlas (TCGA) database showed that RCC2 was highly expressed in different types of cancer. High RCC2 expression levels were significantly correlated with poor survival outcomes by the Kaplan-Meier analysis in the TCGA database. Immunohistochemistry revealed that RCC2 was higher expressed in 36 CRC tissues than in adjacent normal tissues. Co-immunoprecipitation revealed that RCC2 bound to high mobility group A2 (HMGA2). Ectopic expression of RCC2 promoted cell proliferation, migration and invasion, whereas knockdown of HMGA2 exerted the opposite effects. Collectively, the data provided a novel biomarker of RCC2 in various types of cancer. High RCC2 expression levels were correlated with poor prognosis in different types of cancer. In

addition, RCC2 may combine with HMGA2 to promote CRC malignancy.

Introduction

Regulator of chromosome condensation 2 (RCC2), also known as telophase disc-60 (TD60), was initially identified in the anaphase spindle midzone (1). RCC2 is an essential protein in the chromosomal passenger complex (2). It was defined according to the movements from centromeres during early mitosis to the spindle midzone (3,4). The RCC2 protein, encoded by the RCC2 gene, is a guanine exchange factor that activates Ras-related protein RalA, a small GTPase. The RCC2 and RalA proteins are both essential in kinetochore-microtubule functions in early mitotic stages (5).

Studies have documented that RCC2 facilitates tumorigenesis and enhances metastasis in different types of tumor. Matsuo *et al* (6) report that miR-29c downregulates RCC2 and inhibits the proliferation of gastric carcinoma. Micro (miR)-1247 targets RCC2 and suppresses the proliferation of pancreatic cancer (7). miR-331-3p suppresses ovarian cancer metastasis and proliferation by targeting RCC2 (8). RCC2 promotes breast cancer proliferation by regulating the Wnt signaling pathways (9). In addition, RCC2 is also been implicated in melanoma recurrence and overall survival outcomes (10). Studies have also revealed that RCC2 promotes the progression of CRC malignancies. Bruun *et al* (11) report high RCC2 expressions in patients with microsatellite instability (MSI). Impaired RCC2 levels affect clinical endpoints of CRC. High-risk patients with CRC and MSI were identified with cost-effective routine RCC2 assays. Song *et al* (5) reveal that p53 binds to a palindromic RCC2 motif to act as a transcriptional regulator. However, RCC2 mechanisms in CRC remain to be elucidated.

High mobility group A2 (HMGA2) is a small architectural transcription factor and contains three AT-hook DNA-binding motifs (12,13). Higher expression of HMGA2 leads to oncogenesis with increased cell proliferation and metastatic potential (14). Overexpression of HMGA2 promotes malignant progression in various types of tumors especially in CRC (15). The authors previously reported that HMGA2 promotes

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intestinal tumorigenesis by accelerating the degradation of p53 (16).

The present study aimed at determining the oncogenic role of RCC2 in various types of cancer by analyzing its expression levels in cancerous and normal tissues. The clinical overall and recurrence-free survival of RCC2 in various types of cancer were also determined. These cancers were stomach adenocarcinoma, CRC, liver cancer, prostate cancer, bladder urothelial carcinoma, renal clear cell carcinoma, head and neck squamous cell carcinoma, lung adenocarcinoma, endometrial cancer, sarcoma, mesothelioma, brain lower grade glioma, pancreatic adenocarcinoma, adrenocortical cancer and renal papillary cell carcinoma. The RCC2 expression levels in CRC and adjacent normal tissues were also determined. Finally, the relationships between RCC2 and HMGA2 were evaluated to ascertain the molecular mechanisms of RCC2 mediated CRC progression.

Materials and methods

Cell culture. Human CRC cell lines, including DLD1, HCT116, HCT8, HT29, LOVO, RKO, SW620 and SW480 cell lines, were maintained in Soochow University (Suzhou, China). The HT29 cell line was authenticated by STR identification. The cell lines were maintained at 37°C in RPMI-1640 supplemented with 10% fetal bovine serum, with the exception of HCT116, which was maintained in DMEM. 1% penicillin and streptomycin antibiotic and an atmosphere of 5% CO₂ was used for all cell lines.

Public dataset analysis. RCC2 gene expression levels in various types of cancer and correspondence clinical information were obtained from The Cancer Genome Atlas (TCGA) database. These included stomach adenocarcinoma, colorectal cancer, liver cancer, prostate cancer, bladder urothelial carcinoma, head and neck, squamous cell carcinoma, renal clear cell carcinoma, lung adenocarcinoma, endometrial cancer, sarcoma, mesothelioma, brain lower grade glioma, pancreatic adenocarcinoma, adrenocortical cancer and renal papillary cell carcinoma. The datasets were classified into two cohorts: The expression dataset in cancer tissues and in the adjacent normal tissues. A combination of receiver operating characteristic (ROC) curve analysis, specificity and sensitivity were used to choose a cutoff point. The RCC2 expression levels in patients with cancer were used to generate two groups: High and low expression groups. Based on the overall survival and recurrence-free survival times of patients derived from the clinical datasets, the differences between high and low RCC2 expression groups were analyzed and compared. The 10-year overall survival and recurrence-free survival rates was determined by the Kaplan-Meier analysis (17). The TIMER2 database (<http://timer.cistrome.org/>) was used to analyze the relationship between RCC2 expression and CD8⁺ T cells in digestive system tumors (18). Pearson's correlation analysis was performed to reveal the correlations between RCC2 and HMGA2 in digestive system tumors.

Western blot analysis. CRC cell pellets were lysed by the RIPA lysis buffer and a protease inhibitor cocktail (both Beyotime Institute of Biotechnology) was added. The protein

concentration of CRC cell lysates was determined with a BCA kit (Pierce; Thermo Fisher Scientific, Inc.). For western blotting 30 µg of cell lysate protein were analyzed by 6-18% SDS PAGE, transferred onto 0.45-µm PVDF membranes (MilliporeSigma). The PVDF membranes were blocked with 5% skimmed milk for 15 min at room temperature. Then the proteins were probed with primary antibodies against RCC2 (1:1,000; cat. no. 5104; CST Biological Reagents Co., Ltd.) and GAPDH (1:10,000; Clone 686613; R&D Systems Inc.) for 12 h at 4°C. The western blots were incubated with DyLight 680 or DyLight 800 conjugated secondary antibodies (Cell Signaling Technology, Inc.) for 1 h at room temperature and visualized by the Odyssey Imaging System (LI-COR Biosciences). Western blot images were normalized by Image Studio 3.1 software (LI-COR Biosciences).

Immunohistochemistry (IHC). The RCC2 expression levels in 36 cases of patients with CRC were assessed by immunohistochemistry of paraffin sections, which were from the First Affiliated Hospital of Soochow University (Suzhou, China). Primary RCC2 antibody was obtained from Abcam (cat. no. ab70788; rabbit RCC2 polyclonal antibodies, anti-RCC2; 1:200). This was performed as previously described (16). Briefly, immunohistochemistry was conducted on 2-µm sections using the BenchMark ULTRA automated stainer (Ventana Medical Systems, Inc.) in accordance with the manufacturer's instructions. RCC2 scoring was performed according to the proportion of positive cancer cells (1, 0-25%; 2, 25-50%; 3, 50-75% and 4, >75%) and the staining intensity of cancer cells (negative, 0; light yellow, 1; dark yellow, 2 and brown, 3). Slides were analyzed under bright field microscopy. The formula for obtaining the IHC staining score was: IHC staining score=percentage of positive cancer cells x staining intensity of the cancer cells. Scoring was carried out by two pathologists, independently. Approval for the present study was obtained from the Institutional Ethics Committee of Soochow University (authorization number ECSU-2019000212).

Plasmid construction and transfection. Full-length RCC2 coding sequences (CDSs) were subcloned into pcDNA3.1-FLAG plasmid while HMGA2 was subcloned into pcDNA3.1-Myc plasmid as previously described (16). Vectors with ligated sequences were confirmed by matched DNA sequencing. Lipofectamine[®] 2000 (Invitrogen; Thermo Fisher Scientific, Inc.) was used to transfect empty vector and pcDNA3.1-FLAG-RCC2 with or without pcDNA3.1-Myc-HMGA2 into 293T cells when the cell density was 70% according to the manufacturer's instructions. The transfection mixtures were pre-incubated for 15 min at room temperature before transfection. A total of 2 µg (1 µg each) of plasmid DNA was used in transfection of each well of a 6-well culture plate and the duration of transfection was 6 h. At 36 h post-transfection, the expression efficiency of RCC2 and HMGA2 was confirmed by western blot analysis.

Co-immunoprecipitation (Co-IP). 293T cells were divided into three subgroups. The first subgroup was transfected with pcDNA3.1 empty vector and pcDNA3.1-FLAG-RCC2, the second subgroup was transfected with pcDNA3.1 empty vector and pcDNA3.1-Myc-HMGA2, while the third

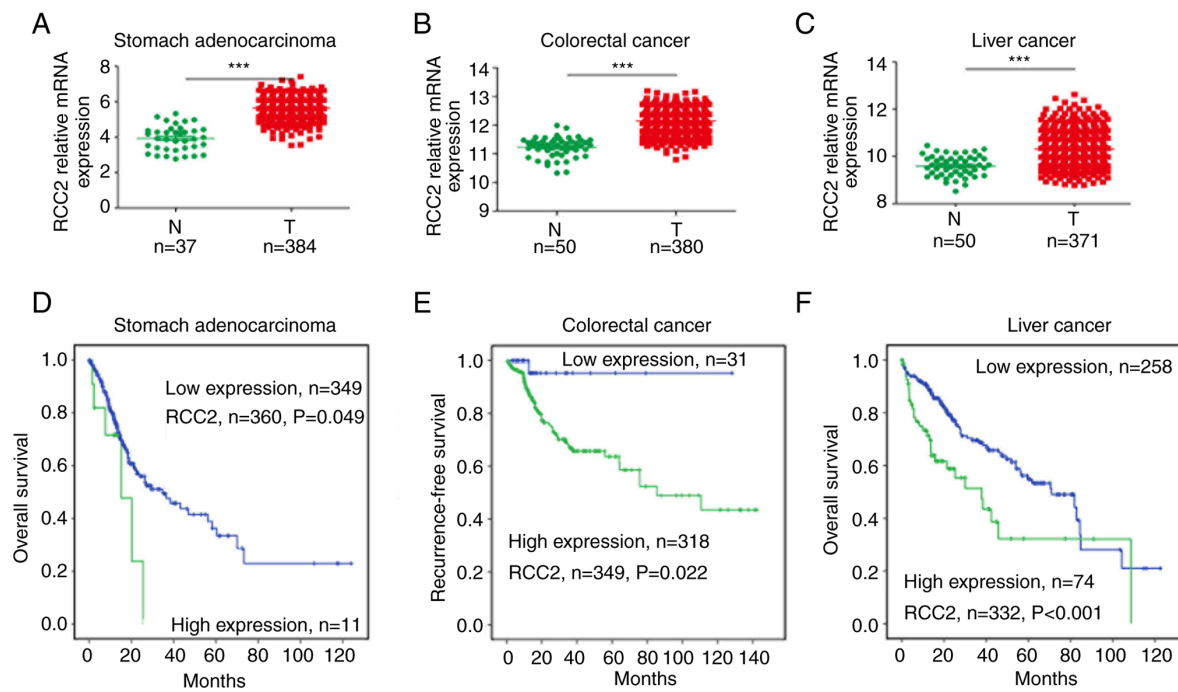


Figure 1. RCC2 mRNA expression levels are upregulated in digestive system tumors and were correlated with poor clinical prognosis. (A) Scatter plot of RCC2 in stomach adenocarcinoma and normal tissues. (B) Scatter plot of RCC2 in CRC and normal tissues. (C) Scatter plot of RCC2 in liver cancer and normal tissues. (D) Kaplan-Meier analysis of RCC2 expression levels in patients with stomach adenocarcinoma. (E) Kaplan-Meier analysis of RCC2 in patients with CRC. (F) Kaplan-Meier curve of RCC2 in patients with liver cancer (** $P < 0.001$). RCC2, regulator of chromosome condensation 2; CRC, colorectal cancer; N, normal; T, tumor.

subgroup was transfected with pcDNA3.1-FLAG-RCC2 and pcDNA3.1-Myc-HMGA2. A total of 4 μ g (2 μ g each) of plasmid DNA was transfected onto a 6-cm plate when the cell density was 50%. After 48 h, the cell pellets were lysed using lysis buffer [20 mM Tris-HCl (pH 7.4), 150 mM NaCl, 0.5% NP-40, 10% glycerol, 1 mM DTT and complete protease inhibitor cocktail] for 30 min on ice and centrifuged at 20,000 \times g for 15 min. Supernatants (300 μ g) were immunoprecipitated using M2-FLAG-magnetic beads (cat. no. M8823; MilliporeSigma) according to the manufacturer's instructions. Cell lysates were then analyzed by subjecting them to SDS-PAGE and immunoblotting with indicated antibodies.

Cell proliferation, migration, invasion assay and apoptosis assay. In 96-well plates, HCT116 cells were plated at 2,000 per well and cultured for 0, 24, 48, 72 and 96 h. Then, 10 μ l of the CCK-8 reagent (cat. no. C0039; Beyotime Institute of Biotechnology) was added into each well and the cells were incubated for another 2 h. The OD values at 450 nm were measured by microplate reader (Synergy 4 Hybrid Multi-Detection Reader; BioTek Instruments, Inc.). For migration and invasion assay, HCT116 cells (5×10^4 /well) resuspended in the serum-free RPMI-1640 were plated onto the upper chamber of a Transwell (cat. no. 3422, Corning, Inc.), with the upper chamber surface precoated with Matrigel for 1 h at 37°C (cat. no. 356234; BD Biosciences) for invasion assay. The bottom chamber contained RPMI-1640 with 30% FBS. After culturing for 24–48 h, cells transferred through the membrane were fixed with methanol for 30 min at room temperature and stained with 0.1% crystal violet for 20 min at room temperature (cat. no. C0121; Beyotime Institute of Biotechnology) and

the cells remaining in the upper chamber were wiped off. The images of three randomly selected fields of view were captured under a microscope at x20 magnification (Eclipse Ti-S; Nikon Corporation). To analyze the fraction of apoptotic cells, the HCT116 cells were detected by Annexin V-APC/7-AAD apoptosis detection kit (Nanjing KeyGen Biotech Co., Ltd.) according to the manufacturer's instructions. All these experiments were performed three times independently.

Statistical analysis. An analysis of the data was performed using the Statistical Package for Social Sciences (SPSS version 20.0; IBM Corp.). The experimental data are shown as the mean \pm standard deviation of triplicate independent sets of experiments. GraphPad Prism 7.0 (GraphPad Software Inc.) was used for graphs. For comparisons between two groups, data were analyzed using an unpaired Student's t-test and comparisons among multiple groups were performed using one-way analysis of variance followed by Tukey's multiple comparisons test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

RCC2 mRNA expression levels were upregulated in the digestive system tumors and were correlated with poor clinical outcomes. RCC2 was found to be significantly high expressed ($P < 0.001$) in digestive system tumors such as stomach adenocarcinoma (Fig. 1A), CRC (Fig. 1B), and liver cancer (Fig. 1C) when compared with the adjacent normal tissues. RCC2 was also found to be a biomarker for digestive system cancers. This was due to its expression being upregulated in cancer tissues

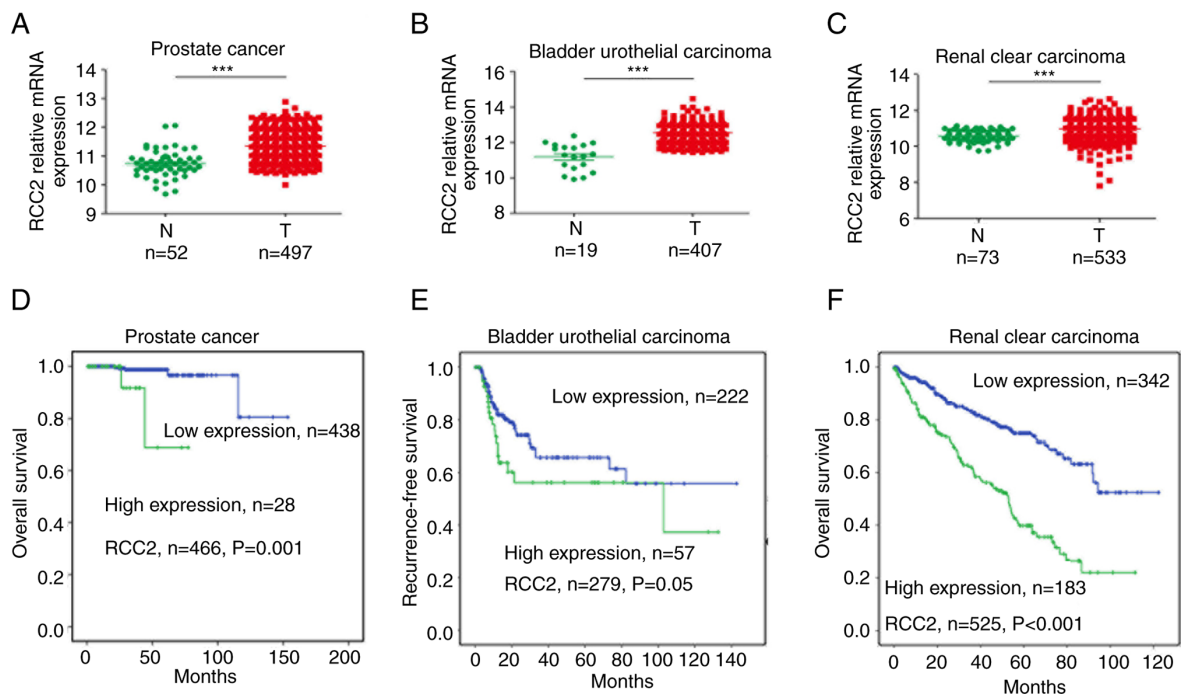


Figure 2. RCC2 expression levels are upregulated in the urogenital male reproductive system and were associated with poor clinical prognosis. (A) Scatter plot of RCC2 in prostate cancer and normal tissues. (B) Scatter plot of RCC2 in bladder urothelial carcinoma and normal tissues. (C) Scatter plot of RCC2 in renal clear cell carcinoma and normal tissues. RCC2 in (D) patients with prostate cancer, (E) patients with bladder urothelial carcinoma and (F) patients with kidney clear cell carcinoma data was analyzed by Kaplan-Meier analysis (**P<0.001). RCC2, regulator of chromosome condensation 2; N, normal; T, tumor.

and associated with poor overall survival outcomes. In 360 stomach adenocarcinoma patients, the high RCC2 expression group exhibited poor clinical overall survival outcomes (Fig. 1D, $P=0.049$). In 349 CRC (Fig. 1E) and 332 patients with liver cancer (Fig. 1F), a high RCC2 expression indicated poor recurrence-free survival/overall survival ($P=0.022$ and $P<0.001$, for each respective cancer). Furthermore, RCC2 expression was positively correlated with CD8⁺ T cells in colon adenocarcinoma (Fig. S1A), stomach adenocarcinoma (Fig. S1B) and liver hepatocellular carcinoma (Fig. S1C). RCC2 was positively correlation with cytotoxic T-lymphocyte associated protein 4 (CTLA4) in a number of tumors including colon adenocarcinoma (Fig. S1D), liver hepatocellular carcinoma (Fig. S1E) and stomach adenocarcinoma (Fig. S1F). RCC2 was also positively correlated with CD274 (PDL1) in liver hepatocellular carcinoma (Fig. S1E). Therefore, RCC2 may be a good biomarker for immune checkpoint inhibitor treatment of these tumors. Finally, the RCC2 expression levels was evaluated in different tumor stages including colon adenocarcinoma (Fig. S2A), liver hepatocellular carcinoma (Fig. S2B), and stomach adenocarcinoma (Fig. S2C). There was no difference between RCC2 expression level and tumor progression in the low RCC2 expression group. In the high RCC2 expression group, only in liver hepatocellular carcinoma did RCC2 expression level increase with tumor progression.

RCC2 expression levels are upregulated in the urogenital male reproductive system and were associated with poor clinical outcomes. The TCGA database exhibited similar results in the urogenital male reproductive system cancers such as prostate cancer (Fig. 2A), bladder urothelial carcinoma (Fig. 2B) and renal clear cell carcinoma (Fig. 2C). There were significantly

high RCC2 expression levels ($P<0.001$) in these cancer tissues compared with matched normal tissues. In 466 patients with prostate cancer (Fig. 2D), 279 patients with bladder urothelial carcinoma (Fig. 2E) and 525 renal clear cell carcinoma patients (Fig. 2F), high RCC2 expression levels ($P=0.001$, 0.05 and <0.001 , respectively) were correlated with poor prognosis for 10 year survival/recurrence-free survival. In prostate cancer, high RCC2 expression levels ($P=0.002$) were also associated with poor overall survival and recurrence-free survival (Fig. S3A).

High RCC2 expression levels are associated with head and neck squamous cell carcinoma and lung adenocarcinoma. The RCC2 mRNA expression levels were found to be significantly high ($P<0.001$) in head and neck squamous cell carcinoma (Fig. 3A) as well as in lung adenocarcinoma patients (Fig. 3B). Furthermore, the head and neck squamous cell carcinoma (Fig. 3C) and lung adenocarcinoma (Fig. 3D) with elevated RCC2 levels ($P=0.011$ and 0.001 , respectively) exhibited worse clinical outcomes in overall survival when compared with low expression levels. In addition, high RCC2 expression levels ($P=0.012$) were correlated with worse recurrence-free survival outcomes in 350 lung adenocarcinoma patients (Fig. S3B).

High RCC2 expression levels in endometrial cancer and sarcoma are correlated with worse clinical outcomes. RCC2 expression levels were found to be high in endometrial (Fig. 4A) and sarcoma cancer (Fig. 4B) tissues compared with correspondence normal tissues ($P<0.001$ and <0.05 , respectively). High expression levels of RCC2 were associated with worse recurrence-free survival in endometrial cancer (Fig. 4C) and worse overall survival in sarcoma (Fig. 4D; $P=0.066$ and 0.006 , respectively). A high expression was also correlated

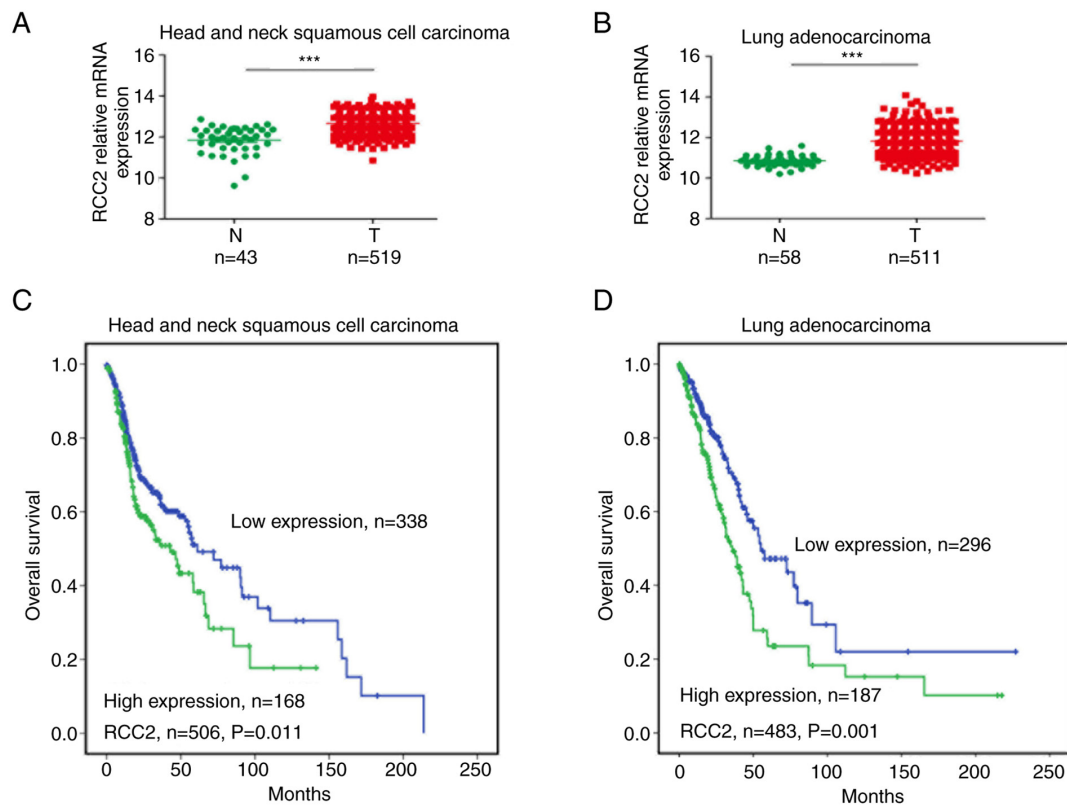


Figure 3. High expression levels of RCC2 in the head and neck squamous cell carcinoma and lung adenocarcinoma. Scatter plot of RCC2 in (A) head and neck squamous cell carcinoma and normal tissues and (B) lung adenocarcinoma and normal tissues. Kaplan-Meier analysis of RCC2 in (C) head and neck squamous cell carcinoma patients. (D) Kaplan-Meier analysis of RCC2 in lung adenocarcinoma patients (** $P < 0.001$). RCC2, regulator of chromosome condensation 2; N, normal; T, tumor.

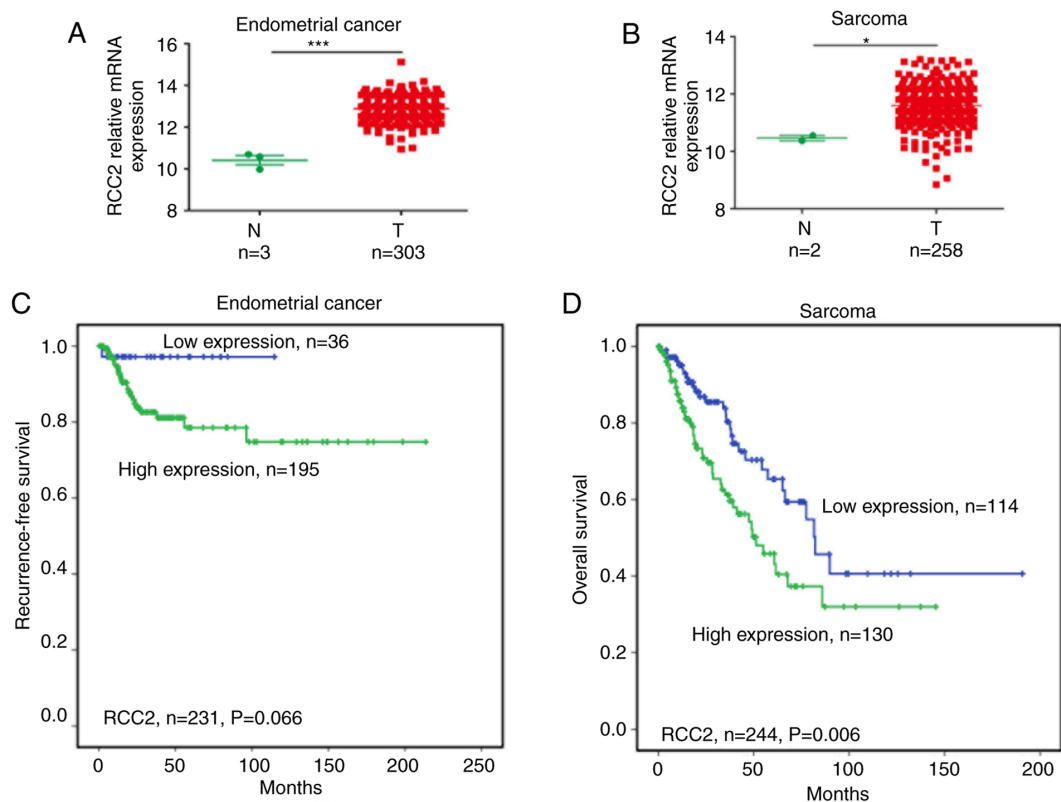


Figure 4. High expression levels of RCC2 in endometrial cancer and sarcoma are correlated with worse clinical outcomes. Scatter plot of RCC2 in (A) endometrial cancer and normal tissues and (B) sarcoma and normal tissues. Kaplan-Meier analysis of RCC2 in patients with (C) endometrial cancer and (D) sarcoma (* $P < 0.05$, *** $P < 0.001$). RCC2, regulator of chromosome condensation 2; N, normal; T, tumor.

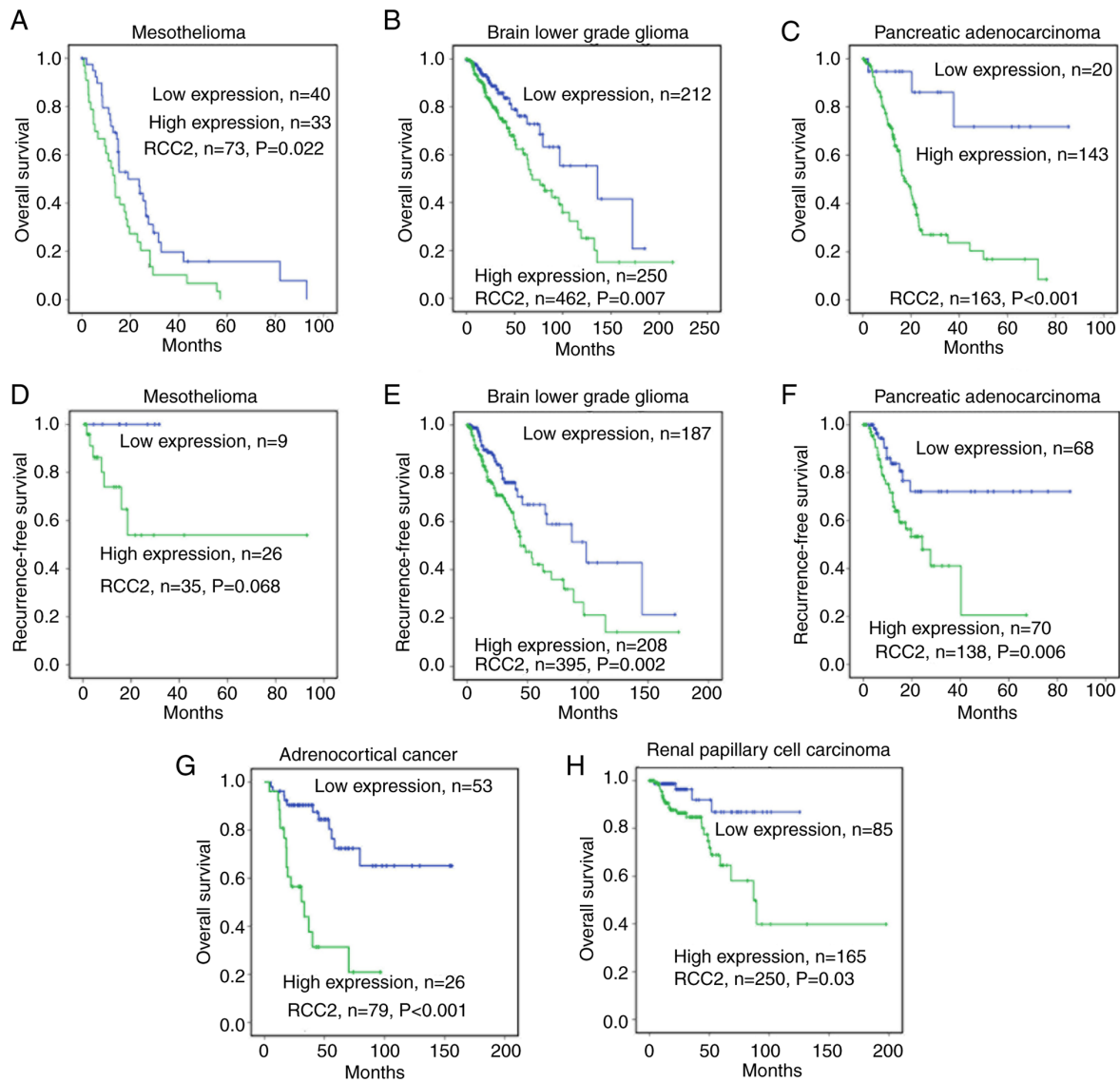


Figure 5. High expression levels of RCC2 in mesothelioma, brain lower grade glioma, pancreatic adenocarcinoma, adrenocortical cancer and renal papillary cell carcinoma are correlated with worse prognosis. Kaplan-Meier overall survival curve of RCC2 in (A) patients with mesothelioma, (B) patients with brain lower grade glioma and (C) patients with pancreatic adenocarcinoma. Kaplan-Meier analysis of RCC2 associated relapse-free survival in (D) patients with mesothelioma, (E) patients with brain lower grade glioma and (F) patients with pancreatic adenocarcinoma. Kaplan-Meier analysis of RCC2 associated overall survival in (G) patients with adrenocortical cancer and (H) patients with renal papillary cell carcinoma. RCC2, regulator of chromosome condensation 2.

with poor recurrence-free survival in sarcoma (Fig. S3C). In cholangiocarcinoma (Fig. S3D), breast cancer (Fig. S3E) and esophageal cancer (Fig. S3F), RCC2 was found to be highly expressed in cancer tissues ($P<0.001$) compared with the adjacent normal tissues. These findings show that RCC2 also serves as a biomarker and can predict clinical overall survival/recurrence-free survival time in endometrial cancer and sarcoma.

High RCC2 expression levels in mesothelioma, brain lower grade glioma, pancreatic adenocarcinoma, adrenocortical cancer and renal papillary cell carcinoma correlate with worse outcomes. In 73 patients with mesothelioma (Fig. 5A), 462 patients with brain lower grade glioma (Fig. 5B) and 163 patients with pancreatic adenocarcinoma (Fig. 5C), high RCC2 expression levels ($P=0.022$, 0.007 and <0.001 respectively) were correlated with poor overall survival. In the mesothelioma (Fig. 5D), brain lower grade glioma (Fig. 5E) and pancreatic

adenocarcinoma (Fig. 5F) cancers, the recurrence-free survival analysis revealed that high RCC2 expression levels were associated with worse 10-year recurrence-free survival for each of the above cancers ($P=0.068$, 0.002 , and 0.006 respectively). In 79 patients with adrenocortical cancer, elevated RCC2 expression was correlated with poor overall survival, ($P<0.001$; Fig. 5G). In 250 patients with renal papillary cell carcinoma, high RCC2 expression levels were correlated with poor overall survival ($P=0.03$; Fig. 5H).

RCC2 expression is elevated in CRC tissues and associated with HMG2 to promote malignancy. RCC2 is a novel cancer biomarker and can be used as a predictor for poor clinical prognosis. To uncover the role of RCC2 in CRC, immunohistochemical staining in 36 paired CRC tissues and adjacent normal tissues were performed. It revealed that RCC2 was highly expressed in CRC tissues (Fig. 6A). The IHC staining

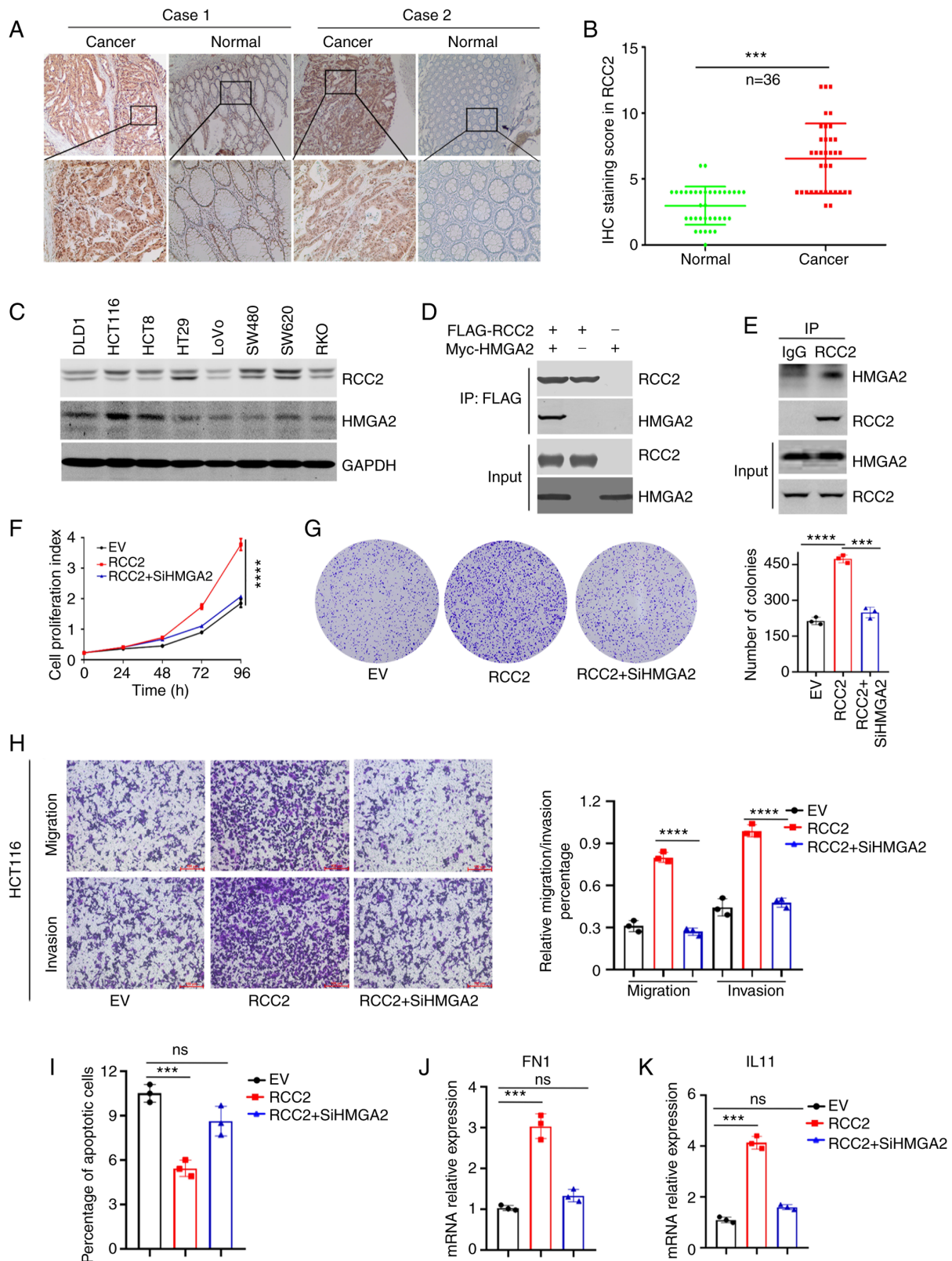


Figure 6. RCC2 is highly expressed in CRC tissues and associates with HMGA2 to promote malignant CRC progression. (A) Representative immunohistochemical staining of RCC2 in CRC tissues and corresponding normal tissues (magnifications x25 and inset, x100). (B) Immunohistochemical staining score of RCC2 in 36 paired CRC tissues. (C) Western blot analysis of RCC2 and HMGA2 in CRC cell lines. (D) Immunoprecipitation of the Myc-HMGA2 by an anti-FLAG antibody in 293 cells transfected with pcDNA3.1-FLAG-RCC2 and/or pcDNA3.1-Myc-HMGA2 as indicated. (E) Endogenous RCC2 was coprecipitated with endogenous HMGA2. (F) CCK8 analysis was conducted to detect the cells proliferation. (G) Colony formation assays were carried out to explore the effect of RCC2 and HMGA2 on the proliferation of HCT116 cells. Left panel: representative images, right panel: quantification analysis. (H) Transwell migration and invasion assays were performed (left panel) and calculation of the rate of migration/invasion in corresponding HCT116 cells (right panel) (red scale bar, 100 μ m). (I) Apoptosis was detected by 7AAD/Annexin-V labeling, quantitation of data is shown. (J) FN1 and (K) IL11 mRNA expressions were measured by reverse transcription-quantitative PCR. Data are shown as the mean \pm standard deviation of triplicate independent sets of experiments (*** P <0.001, **** P <0.0001, ns, non-significant). RCC2, regulator of chromosome condensation 2; CRC, colorectal cancer; HMGA2, high mobility group A2; FN1, fibronectin 1.

score showed that RCC2 expression was significantly high in CRC compared with the adjacent normal tissue ($P < 0.001$; Fig. 6B). It has been documented that HMGA2 promotes CRC malignancy by regulating the translation of fibronectin 1 (FN1) and IL11 (19). To promote CRC tumorigenesis, HMGA2 also enhances the degradation of P53 (16). The present study revealed that RCC2 and HMGA2 were highly expressed in HCT116, HCT8 and SW620 cell lines (Fig. 6C). In addition, RCC2 was shown to interact with HMGA2 in 293 cells that had been transiently transfected with pcDNA3.1-vector, pcDNA3.1-FLAG-RCC2, pcDNA3.1-Myc-HMGA2 expression plasmids, followed by dual Co-IP assays with an anti-FLAG antibody. The RCC2 and HMGA2 proteins Co-IP reciprocally in these cells (Fig. 6D). The endogenous Co-IP assay also confirmed the interaction between RCC2 and HMGA2 (Fig. 6E). In addition, RCC2 was positively related to HMGA2 in liver hepatocellular carcinoma (Fig. S4B) and stomach adenocarcinoma (Fig. S4C). However, there was no correlation between RCC2 and HMGA2 in colon adenocarcinoma (Fig. S4A). To test whether RCC2 expression in CRC cells affected their proliferation or tumorigenicity through HMGA2, RCC2-WT was ectopically expressed in HCT116 cells using lentiviral constructs and knockdown of HMGA2. *In vitro* growth kinetics assay revealed that overexpression of RCC2 significantly promoted cell proliferation of HCT116 and knockdown of HMGA2 reduced the proliferation rate of HCT116 (Fig. 6F). Colony formation assay also showed a similar result (Fig. 6G). Migration and invasion experiments were conducted to determine whether RCC2 and HMGA2 contribute to the migratory and invasive characteristics of CRC cells. The results revealed that overexpression of RCC2 significantly increased the migration and invasion of HCT116 cells and this effect was eliminated with downregulation of HMGA2 (Fig. 6H). Overexpression of RCC2 blocked the spontaneous apoptosis of HCT116 cells and this effect was mediated by HMGA2 (Fig. 6I). To test whether RCC2 could regulate the activity of HMGA2, which contributes to colorectal carcinogenesis, the HMGA2 downstream target genes FN1 (Fig. 6J) and IL11 (Fig. 6K) were detected. The results showed that RCC2 could promote the transcriptional activation of HMGA2. Overall, these results suggested that RCC2 promotes CRC malignancy by associating with HMGA2.

Discussion

RCC2 was first identified as a nuclear protein located at the chromosomal centromeres essential for cell division (1). The role of RCC2 in the establishment and progression of tumors has been studied extensively in recent years. RCC2, as an oncogene, is involved in cancer tumorigenesis and metastasis. RCC2 overexpression promotes cancer malignant progression (20). In breast cancer, RCC2 promotes malignant progression by regulating the Wnt signaling pathways and epithelial-mesenchymal transition (EMT) (9). In lung cancer, RCC2 mediates the effect of long non-coding RNA LCPAT1 on migration, invasion, cell autophagy and EMT (21,22). Conversely, the downregulation of RCC2 mRNA expression leads to opposite effects; miRNAs such as miR-29c, miR-1247 and miR-331-3p inhibit cancer malignancy by

targeting RCC2 (6-8). The sarcomas are a group of tumors with a wide variety of localization and the survival rate depends on the affected organ. For example, in head and neck sarcomas the survival rate is influenced by the surgical removal (23). The present study evaluated the role of RCC2 in different types of tumor including stomach adenocarcinoma, CRC, liver cancer, prostate cancer, bladder urothelial carcinoma, renal clear cell carcinoma, head and neck squamous cell carcinoma, lung adenocarcinoma, endometrial cancer, sarcoma, mesothelioma, brain lower grade glioma, pancreatic adenocarcinoma, adrenocortical cancer and renal papillary cell carcinoma. The results showed that RCC2 was upregulated in these types of cancer. Moreover, patients with highly expressed RCC2 exhibited a short overall and recurrence survival rate.

To further understand the mechanistic role of RCC2 in CRC. Immunohistochemical staining revealed that RCC2 was highly expressed in patients with CRC. Whole-genome sequencing reveals that RCC2 is one of the commonly mutated genes in CRC (24). RCC2 acts as an oncogene in microsatellite instable tumors, and low level of RCC2 protein expression is associated with poor prognosis of microsatellite stable tumors. One reason is that RCC2 inhibits cancer cell metastasis by regulating integrin $\alpha 5 \beta 1$ -fibronectin signaling pathway (11,25). Our knowledge of the different roles of RCC2 serves in various phases of tumor progression and metastasis is limited.

The present study found that RCC2 and HMGA2 were highly expressed in HCT116, HCT8 and SW620 CRC cell lines. Co-IP assays demonstrated that RCC2 interacted with HMGA2. RCC2 promotes tumor metastasis by interacting with Rac1 and Arf6 (26,27). The present study identified a new RCC2 binding protein that provided new insights into RCC2-mediated tumor progression. It was also found that RCC2 expression is positively related to HMGA2 in liver hepatocellular carcinoma and stomach adenocarcinoma. Although HMGA2, as an architectural transcription factor, has no intrinsic transcriptional activity, it can induce gene transcription by changing chromatin architecture (28,29). It was hypothesized that the interaction between RCC2 and HMGA2 promotes architectural changes in promoter regions of some genes. Ectopic expression of RCC2 promoted cell proliferation, migration and invasion *in vitro*, whereas knockdown of HMGA2 exerted the opposite effects. In addition, RCC2 promoted the transcriptional activation of HMGA2. Further studies characterizing RCC2 coordination with upstream and downstream functional pathways and functional target proteins are required. Taken together, the results of the present study suggested that RCC2 could be a novel biomarker in human cancer and provide new insights into the mechanisms of RCC2 in CRC progression.

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Availability of data and materials

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

Authors' contributions

LH and SH designed the experiments. YW and LH wrote and revised the manuscript. GG, YS, YM and HY developed the methodology. YW and SH analyzed the data. LH and SH supervised the study. LH and SH confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethical approval and consent to participate

All cancer tissues were obtained with the approval of the First Affiliated Hospital of Soochow University's Institutional Ethics Committee (authorization number ECSU-2019000212).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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