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ORIGINAL RESEARCH

RETRACTED ARTICLE: Effects of LncRNA HCP5/miR-214-3p/MAPK1 Molecular Network on Renal Cell Carcinoma Cells

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Jun-Feng Hao[1,*]
Pei Chen[2,*]
He-Yi Li[3]
Ya-Jing Li[1]
Yu-Ling Zhang[2]

[1]Department of Nephrology and Blood Purification Center, Jin Qiu Hospital of Liaoning Province (Geriatric Hospital of Liaoning Province), Shenyang City, Liaoning Province 110000, People's Republic of China; [2]Department of Basic Medical Sciences, Jiangsu College of Nursing, Huai'an, Jiangsu Province 223000, People's Republic of China; [3]Department of Ophthalmology, Jin Qiu Hospital of Liaoning Province (Geriatric Hospital of Liaoning Province), Shenyang City, Liaoning Province 110000, People's Republic of China

*These authors contributed equally to this work

Background: Recent researches have shown that long non-coding RNA (LncRNA) is often disordered and acts in many carcinomas. Clear cell renal cell carcinoma (ccRCC) is the main reason for carcinoma-related deaths, which are mainly caused by the metastasis. HCP5 is a newly discovered LcnRNA. Early studies have found that HCP5 acts in neoplasm metastasis, but the mechanism of HCP5 in ccRCC is still unclear.

Methods: The expression of HCP5 in human renal cell carcinoma (RCC) was detected by real-time quantitative PCR. The biological effect of LncRNAs in proliferation, migration, invasion and metastasis of RCC cells was explored by gain-of-function and loss-of-function tests. The molecular mechanism of LncRNAs was explored by RNA immunoprecipitation and Western blot.

Results: qRT-PCR revealed that HCP5 was enhanced in neoplasm tissues of ccRCC patients and correlated with the metastatic characteristics of RCC. Over-expression of HCP5 promoted the proliferation, migration and invasion of renal carcinoma cells. The deletion of HCP5 inhibited the proliferation, migration and invasion of RCC in vitro and the metastasis of RCC in vivo. Mechanically, HCP5 inhibited the growth and metastasis of ccRCC cells by regulating miR-214-3p/MAPK1 axis.

Conclusion: HCP5, as a key LncRNA, can promote ccRCC metastasis by regulating miR-214-3p/MAPK1 axis and may be a biomarker and be helpful for judging the prognosis of ccRCC.

Keywords: HCP5, miR-214-3p, MAPK1, clear cell renal cell carcinoma

Introduction

Renal carcinoma is a malignant neoplasm of urinary system and is also the main reason for death related to malignant neoplasm of urinary system. Statistics show that more than 80% of renal carcinoma patients are clear cell renal cell carcinoma (ccRCC).[1] Compared with other renal cell carcinoma (RCC) subtypes, ccRCC has high metastasis and recurrence. In the early stage of renal carcinoma, it is mainly treated by surgical resection, and the prognosis of patients is ideal.[2] However, once a patient has metastasized, it is difficult to carry out routine treatment, and it can only be controlled by targeted drugs. Long-term medication may also lead to drug resistance, resulting in poor prognosis of patients.[3] Data show that the 5-year survival of metastatic renal carcinoma patients is less than 10%, while that of non-metastatic renal carcinoma patients is more than 30%.[4] At present, there is a lack of predictive indicators for renal carcinoma metastasis. Therefore, it is particularly important to find more reliable biomarkers to predict the development and prognosis of renal carcinoma and control the development of renal carcinoma.

Correspondence: Yu-Ling Zhang
Tel +86-15162920912
Email yulingzhang2009@163.com

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Non-coding RNA (ncRNA) is considered as an important participant in epigenetic regulation.[5] Among them, long-chain non-coding RNA (LncRNA), as a hot research field in recent years, is considered as an important regulatory gene in biological process.[6,7] LncRNA is a kind of ncRNA with more than 200 nucleotides, and many studies have found that LncRNA is closely related to the development of ccRCC.[8–10] HLA complex P5 (HCP5) is located on human 6p21.33 chromosome.[11] Early researches have found that HCP5 is enhanced in both oral carcinoma and colorectal carcinoma, and it can inhibit neoplasm invasion and migration by regulating HCP5.[12,13] However, there are few studies on the regulatory effect of HCP5 and ccRCC. In this research, we analyzed The Carcinoma Genome Atlas (TCGA) and found that HCP5 was enhanced in ccRCC, suggesting that HCP5 might be involved in the development of ccRCC.

Competing endogenous RNAs (ceRNA) are a new mechanism of RNA interaction.[14] LncRNA or ceRNA regulates gene expression by competitively binding microRNA (miR).[15] We predicted the latent specific binding of HCP5 to miR to further explore the mechanism of HCP5 and found that miR-214-3p had specific binding with HCP5. Previous studies have revealed that miR-214-3p acts in neopla[illegible] invasion,[16] so we speculate that there might b[illegible] a regulatory relationship between HCP5 and miR-214-3p.

This research intended to explore the la[illegible]nt va[illegible]e of HCP5 in RCC and give latent reference [illegible] clinica[illegible] treatment and new drug development.

Methods and Data

HCP5 Analysis in TCGA Datab[illegible]se

TCGA database is [illegible]very i[illegible]portant cancer database, which mainly contains [illegible]i[illegible]al data, genome variation, mRNA expres[illegible]on, [illegible]iRNA [illegible]xpre[illegible]ion, methylation and other data [illegible] various human ca[illegible]ers (including subtypes), and is an imp[illegible]tan[illegible] data so[illegible]e for cancer researchers.[17] In this study, we u[illegible] GEPIA2 (http://gepia2.carcinoma-pku.cn/#index)[17] to ana[illegible]e the relative expression of HCP5 in ccRCC tissue in TCGA data, and visualized a box-plot.

Clinical Data

In this research, 66 ccRCC neoplasm tissues and adjacent tissues were selected in Jinqiu Hospital of Liaoning Province from January 2012 to January 2014. The collected tissues were transported by liquid nitrogen and then sent to the laboratory for testing. All patients have signed informed consent and had not received any treatment before. The experiment was classified according to Fuhrman neoplasm grade,[18] approved by the Medical Ethics Committee, and conformed to the Declaration of Helsinki.[19]

Cell Line Culture and Transfection

A498 (HTB-44) ccRCC was purchased from American Type Culture Collection. Caki-1 (SCSP-5064), 786–0 (TCHu186), and 769-P (TCHu215) ccRCC and 293T (SCSP-502) were obtained from the cell bank of Chinese Academy of Sciences. The purcha[illegible]d cells [illegible]re cultured in culture medium, among whic[illegible] Caki-1 cells [illegible]were cultured in 5A medium of Mc[illegible]oy. A4[illegible], 786–0 [illegible]nd 769-P cells were cultured in [illegible]swell Park M[illegible]al Institute-1640 (RPMI-1640, Gi[illegible], Wa[illegible]am, MA, USA) medium. The 293t cells we[illegible] culture[illegible]n Dulbe[illegible]co's Modified Eagle Medium (DM[illegible] Invitroge[illegible], Carlsbad, CA, USA). All the media contained [illegible]0% fetal bovine serum (FBS, Gibco, Waltha[illegible]A, USA), [illegible]0U/mL penicillin, 100g/mL streptomy[illegible]in, and all the cells were cultured at 37°C with 5% [illegible]O2.

T[illegible]nsfection [illegible]f cells: siRNA targeting HCP5 (si-HCP5) a[illegible]RNA control (si-NC), miR-214-3p mimetic, mi[illegible]A control (miR-NC), miR-214-3p inhibitor (miR-214-3p-inhibit) and anti-miR-NC control were prepared by GenePharma Co., Ltd (Shanghai, China). To construct the over-expression plasmid of HCP5, the full-length sequence of HCP5 was amplified by PCR, and then subcloned into pcDNA3.1 vector (Thermo Fisher Scientific), named pcDNA3.1-HCP5 (HCP5). According to the manufacturer's instructions, all these plasmids and oligonucleotides were transfected into ccRCC cells by Lipofectamine 2000 reagent (Thermo Fisher Scientific).

qRT-PCR Detection

Total RNA was extracted from tissue samples and cell groups using TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. The quality and concentration of RNA were detected by NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Inc.). Complementary DNA (cDNA) was reverse transcribed from total RNA (1μg) using PrimeScript RT kit (TaKaRa, JPN). The real-time PCR amplification was performed with ABI 7500 (Applied Biosystems, Foster, CA, USA) and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster, CA, USA). Upstream primer sequence of HCP5: 5′-GACTCTCCTA CTGGTGCTTGGT-3′, downstream primer: 5′-CACTGCC

TGGTGAGCCTGTT-3′; upstream primer of miR-214-3p: 5′-GCACAGCAGGCACAGACA-3′, downstream primer: 5′-CAGAGCAGGGTCAGCGGTA-3′; upstream primer of MAPK1: 5′-CAGTTCTTGACCCCTGGTCC-3′, downstream primer: 5′-TACATACTGCCGCAGGTCAC-3′; upstream primer of GAPDH: 5′-GCACCGTCAAGGCTGAGAAC-3′, downstream primer: 5′-TGGTGAAGACGCCAGTGGA-3′; upstream primer of U6: 5′-CTCGCTTCGGCAGCAGCACATATA-3′; downstream primer: 5′-AAATATGGAACGCTTCACGA-3′. The $2^{-\Delta\Delta Ct}$ method was used to calculate each expression level as a threshold cycle (Ct),[20] and each group of data was repeated three times.

Detection of Proliferation

CCK8 (Dojindo, Japan) was applied to measure cell proliferation. The transfected cells were collected and cultivated in a 96-well plate (5.0×10^3 cells per well) for 24h, 48h, 72h, 96h. Then, CCK8 reagent was put in and cultivated at 450nm for 1h, and multi-functional microplate reader was applied to detect absorbance.

Detection of Invasion

Twelve hours before the experiment, Matrigel (200 mg/mL) was put into the Transwell compartment (C[illegible] NY, USA). The transfected cells were collected and [illegible]gulated to 1.0×10^4 cells. Cells were cultivated in serum-[illegible] medium for 24h, and then 600μL of [illegible]edium [illegible]ncluding 10% FBS was put into the lower cham[illegible]er. The [illegible]lls on the upper surface was removed with [illegible]otton [illegible]os. The cells invading the membrane were f[illegible]d with 4% [illegible]araformaldehyde for 20min and staine[illegible] with [illegible]1% crystal violet for 30min. The number [illegible] stained cel[illegible] was tested using a phase contrast m[illegible]roscope with a magnification of 20 times.

Detection of Apoptosis

The ap[illegible]ptosis w[illegible] detected by Annexin V-FITC/PI apoptosis dete[illegible] kit (KeyGEN Biotech, Nanjing, China). The transfect[illegible] cells were suspended in Annexin binding buffer. Then, the cells were stained with Annexin V/FITC and PI solution and analyzed by flow cytometry. FACSAN flow cytometer (BD Biosciences, San Jose, California, USA) was used to detect apoptosis.

Western Blot Analysis

Total protein was extracted from cells by RIPA lysis containing protease inhibitor (Beyotime, China) and phenylmethylsulfonyl fluoride (PMSF). Protein concentration was detected by BCA kit (Merck). Total protein (50μg) was added to 10% SDS-page and transferred to PVDF membrane (Millipore Corporation, USA). Then, 5% skim milk was used to seal the membrane at room temperature for 2h. Then, primary antibody (MAPK1, 1: 1000, GADPH, 1: 2000, Cell Signaling Technology) was added, washed with 1×TBST (pH7), and incubated overnight at 4°C. After that, the membrane was cultivated with the secondary antibody for 1h. The bands were visualized by ECL chemiluminescence detection system (Thermo Fisher).

Double Luciferase Re[illegible]orter G[illegible]ne Detection

The 293 T were [illegible]ultivated [illegible]on a [illegible] well plate. Partial sequence of L[illegible]cRI[illegible]-H[illegible]P5 incl[illegible]ding binding sites of WT and M[illegible] of miR-[illegible]4-3p [illegible]as synthesized and subcloned i[illegible]o pr[illegible]GLO lucif[illegible]se reporter vector. Then, 293 T were transfecte[illegible]with luciferase reporter gene construct w[illegible] miR-214-3p [illegible]mimetic or NC mimetic of [illegible]ipofectamin[illegible] 2000. After incubation for 48h, the cells [illegible]ere obtaine[illegible] and analyzed for luciferase activity. miR-21[illegible]3p an[illegible]itogen-activated protein kinase 1 (MAPK1) were predicted in the same way as above.

RNA Immunoprecipitation (RIP)

RIP was performed by EZ-Magna RIP RNA binding protein immunoprecipitation kit (Millipore). Cells were lysed into complete RIP lysis buffer. A total of 100 μ cell lysate were cultivated with RIP buffer including human anti-Ago2 antibody (with the dilution of 1:50, Millipore) and negative control normal mouse IgG conjugated magnetic beads. The samples were cultivated with proteinase K buffer, and then the target RNA was obtained for further study.

Metastatic Neoplasm Experiment in vivo

Male BALB/C thymic nude mice (5 weeks old, Charles River, Beijing, China). The environment was adjusted one week before the experiment. This study was approved by the Animal Ethics Committee and conformed to the Laboratory Animal Guideline. A total of 4×10^6 769-P cells stably transfected by sh-HCP5 or sh-NC were subcutaneously inoculated to the right dorsal side of each mouse for further constructing Rcc xenotransplantation model. The neoplasm volume of nude mice was detected every 7 days. (V= (shortest diameter)^2 × (longest

diameter) × 0.5 to calculate neoplasm volume (mm^3)). Neoplasm tissue was resected on the 28th day for further study. This study was approved by the Medical Ethics Committee of Jinqiu Hospital of Liaoning Province. This study was conducted according to the "Laboratory animal-Guideline for ethical review of animal welfare" issued by China in 2018.

Statistical Methods

In this study, GraphPad 7 was used to visualize required pictures and analyze data. SPSS20.0 was used to analyze independent prognostic factors of patients. Independent sample *t* test was used for comparison between groups. Counting data were expressed by percentage (%) and analyzed by chi-square test, which was expressed as X2. One-way analysis of variance was used for comparison among groups, and LSD-*t* test was used for comparison after the event, and repeated measurement analysis of variance was used for analyzing multiple time points expression, which was expressed as F. Bonferroni was used in back testing, Pearson test was used to analyze the correlation of genes, K-M survival curve was used to visualize the total survival situation of patients, Log-rank test was used to analyze the prognosis of patients, and multivariate Cox regression analysis showed that there were statistical differences when $P < 0.05$.

Results

Expression of HCP5 in ccRCC Was Up-Regulated and the Prognosis Was Poor

TCGA analysis showed that HCP5 increased in Kidney renal clear cell carcinoma (Figure 1A), and qRT-PCR showed that HCP5 was enhanced in neoplasm tissues of

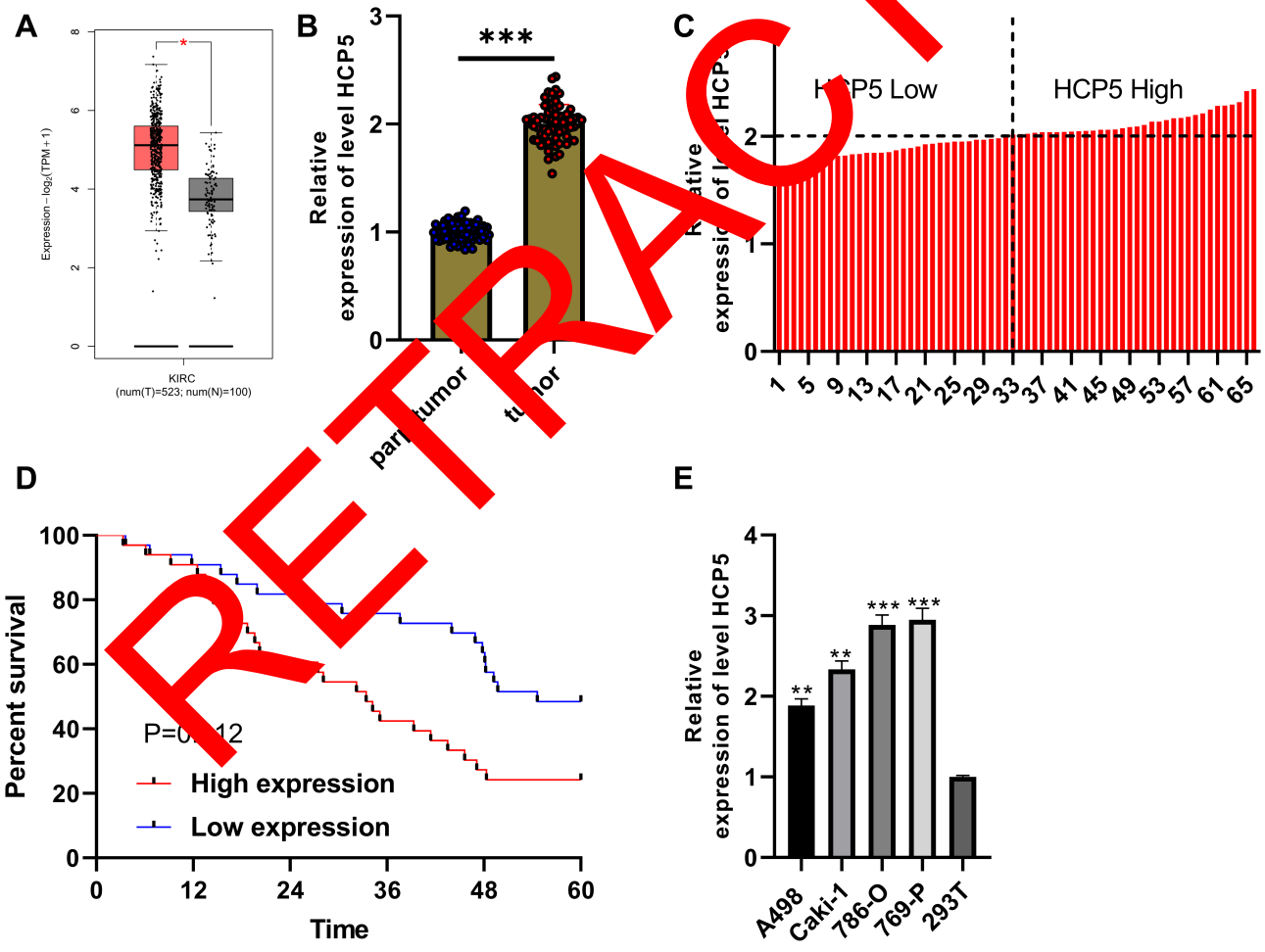

Figure 1 Expression and survival analysis of HCP5 in ccRCC patients. (**A**) GEPIA2 online software analyzed the expression of HCP5 in the TCGA database. (**B**) qRT-PCR was applied to detect the relative expression level of HCP5 in neoplasm tissues of ccRCC patients. (**C**) Median expression of HCP5 in patients with ccRCC. (**D**) K-M survival analysis 5-year survival of patients with high and low expression of HCP5. (**E**) Detection of relative expression of HCP5 in renal cell carcinoma cell line by qRT-PCR. * indicates P<0.05; ** indicates P<0.01; *** indicates P<0.001.

ccRCC patients (Figure 1B). To further observe the relationship between HCP5 and clinical data of patients, we divided patients into high and low expression groups according to the median value (Figure 1C). Through analysis, it was revealed that HCP5 had no correlation with the age, sex and neoplasm size of patients, but was related to Fuhrman neoplasm grade and lymphatic metastasis (Table 1). Further analysis also revealed that the 5-year survival of high HCP5 decreased evidently (Figure 1D). In addition, qRT-PCR detection also revealed that HCP5 in RCC lines was also increased (Figure 1E). Therefore, HCP5 may be a latent prognostic indicator of ccRCC.

Down-Regulating HCP5 Could Hinder the Growth of RCC

In order to further determine the mechanism of HCP5 in RCC, we established si-HCP5#1 vector (Figure 2A) and transfected it into 786-O and 769-P cells. The result revealed that HCP5 in cell lines was inhibited (Figure 2B), indicating that the construction was successful. Then we tested the proliferation, invasion and apoptosis by CCK-8, Transwell and FACS experiments. The experimental results revealed that after transfection of si-HCP5#1, cell proliferation (Figure 2C) and invasion ability was inhibited (Figure 2D), while the apoptosis was induced (Figure 2E). In addition, we also established a nude mouse model. It was found that the neoplasm volume of nude mice intervened by sh-HCP5 was evidently lower than that of sh-NC within 28 days, and the neoplasm mass also decreased evidently (Figure 2F). Therefore, HCP5 is involved in the development of renal cells and is expected to be a latent therapeutic target.

HCP5 Could Target miR-214-3p

ceRNA promoted the research of LncRNA and miR in diseases. We predicted the latent binding of HCP5, and found that there was a targeting relationship of miR-214-3p with HCP5 (Figure 3A), and miR-214-3p was enhanced in transfected cells by qRT-PCR (Figure 3B). Furthermore, the targeting correlation of HCP5 with miR-214-3p was verified by double luciferase and RIP experiments (Figure 3C-D). Furthermore, we found that miR-214-3p was low expressed in neoplasm tissues of patients with ccRCC by qRT-PCR (Figure 3E) and was negatively correlated with HCP5 (Figure 3F). These experiments confirmed that HCP5 can regulate miR-214-3p.

miR-214-3p Mediated by HCP5 Regulated the Expression of MAPK1

MAPK1 is predicted to be a latent target of miR-214-3p through TargetScan, starbase, Tarbase and miRDB

Table 1 Relationship Between HCP5 and Clinical Data of ccRCC Patients

Factor		Relative Expression of HCP5		χ^2value	P value
		Low Expression (n=33)	High Expression (n=33)		
Age	≥60 years old (n=29)	15	14	0.061	0.804
	< 60 years old (n=37)	18	19		
Gender	Male (n=44)	20	24	1.091	0.296
	Female (n=22)	13	9		
neoplasm size	≥7cm (n=30)	18	12	2.200	0.138
	<7cm (n=36)	15	21		
Fuhrman neoplasm grade	I+II (n=38)	25	13	8.932	0.0028
	III+IV (n=28)	8	20		
Lymphatic metastasis	Transfer (n=25)	6	19	10.882	0.001
	Non transfer (n=41)	27	14		

Abbreviation: cRCC, clear cell renal cell carcinoma.

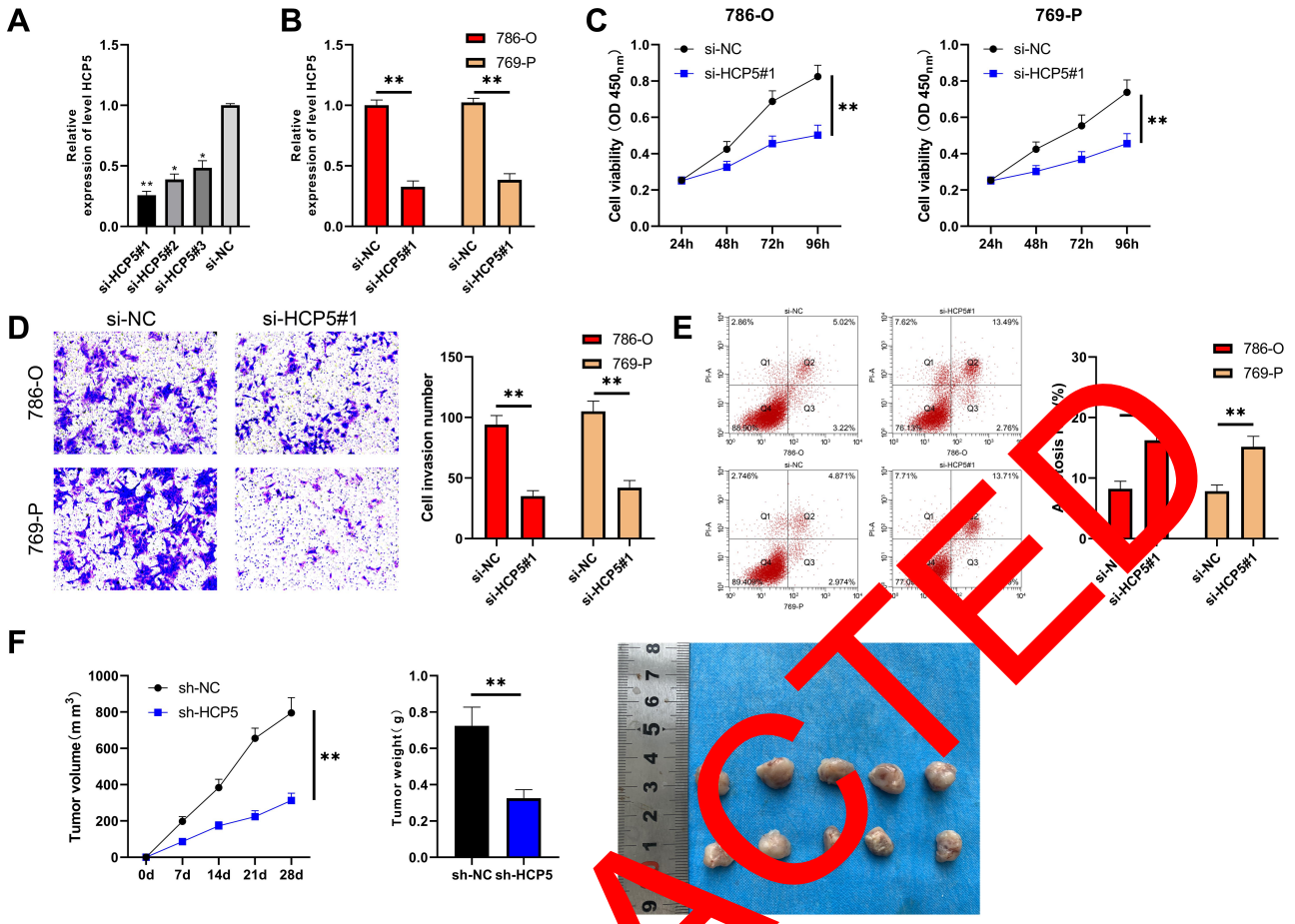

Figure 2 HCP5 could inhibit the growth of renal cell carcinoma. (**A**) qRT-PCR was applied to detect the relative expression of HCP5 after si-HCP5 construction. (**B**) qRT-PCR was applied to detect the relative expression level of HCP5 in cells transfected with si-HCP5#1 of 786–0 and 769-P. (**C**) CCK-8 test was applied to detect the changes of cell proliferation ability after transfection of si-HCP5#1. (**D**) Transwell test was applied to detect the changes of cell invasion number after transfection of si-HCP5#1. (**E**) FACS detection of apoptosis rate of si-HCP5#1 cells. (**F**) Changes of neoplasm volume in nude mice with and neoplasm mass after execution.* indicates P<0.05;** indicates P<0.01.

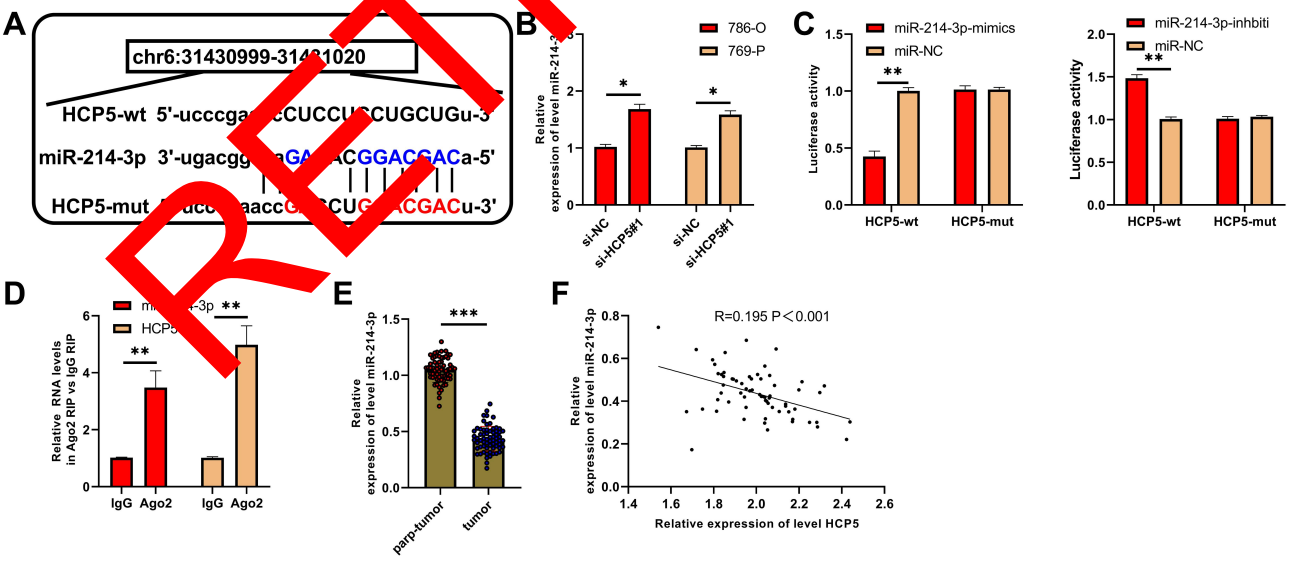

Figure 3 HCP5 could target miR-214-3p. (**A**) Online prediction of target binding sites and mutation sites of HCP5 and miR-214-3p. (**B**) qRT-PCR was applied to detect the relative expression of miR-214-3p in renal carcinoma cell lines transfected with si-HCP5#1. (**C**) Double luciferase report analysis of targeted binding of HCP5 with miR-214-3p. (**D**) RIP experiment analysis of targeted binding between HCP5 and miR-214-3p. (**E**) qRT-PCR detection of miR-214-3p relative expression in neoplasm tissues of ccRCC patients. (**F**) Pearson test was applied to analyze the relative expression of miR-214-3p and HCP5 in neoplasm tissues of ccRCC patients. * indicates P<0.05; ** indicates P<0.01; *** indicates P<0.001.

websites. In order to verify the targeting relationship between them (Figure 4A), it was proved by double luciferase report experiment that miR-214-3p-mimics could inhibit the fluorescence activity of MAPK1-wt (Figure 4B). qRT-PCR revealed that MAPK1 expression increased in neoplasm tissues of ccRCC patients (Figure 4C), and correlation exploration revealed that MAPK1 mRNA expression in neoplasm tissues of ccRCC patients had a negative correlation with miR-214-3p, but had a positive correlation with HCP5 (Figure 4D). Co-transfection experiments were carried out to confirm that HCP5 could regulate miR-214-3p to change MAPK1. The experiment revealed that transfection of pcDNA-3.1-HCP5 inhibited the inhibitory role of miR-214-3p-mimics on MAPK1 mRNA and protein in RCC cells, while transfection of si-HCP5#1 inhibited the promotion role of miR-214-3p-inhibit on MAPK1 mRNA and protein in RCC cells (Figure 4E-F). Therefore, HCP5 can mediate miR-214-3p to affect MAPK1.

HCP5 Could Regulate MAPK1 to Change the Growth of RCC Through miR-214-3p

At the end of the study, in order to find out whether HCP5 is participated in the occurrence of RCC by regulating miR-214-3p/MAPK1 axis, we detected the proliferation, invasion and apoptosis after co-transfection. The experiment found that pcDNA-3.1-HCP5 inhibited the proliferation and invasion of RCC cells by miR-214-3p-mimics (Figure 5A-B), and reduced apoptosis (Figure 5C). While si-HCP5#1 inhibited miR-214-3p-inhibit from promoting proliferation and invasion and promoted apoptosis. These experiments can prove that HCP5 can regulate MAPK1 to

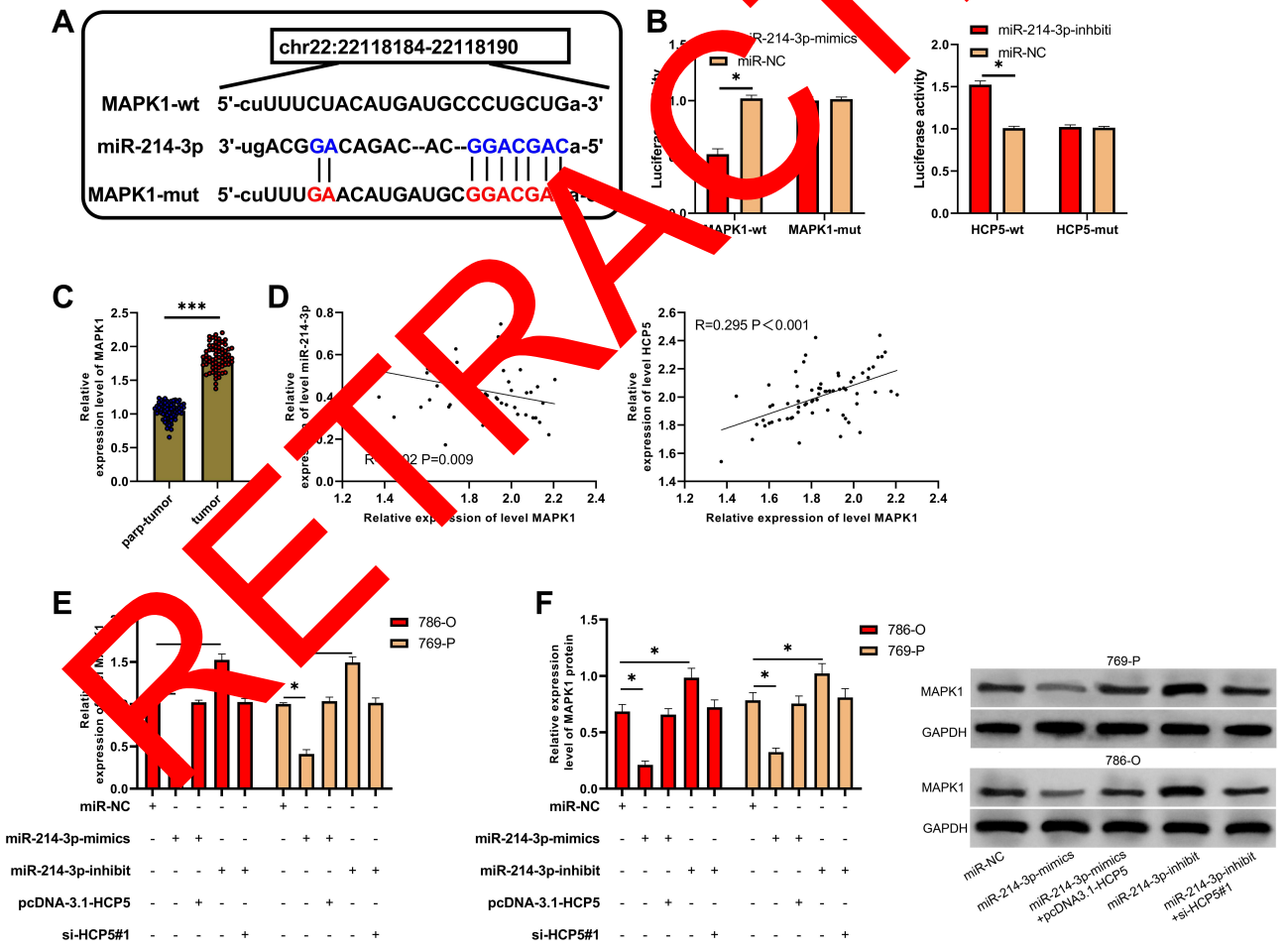

Figure 4 miR-214-3p mediated by HCP5 regulated the expression of MAPK1. (**A**) Targeted binding sites and mutation sites of miR-214-3p and MAPK1. (**B**) Double luciferase report confirmed that miR-214-3p binded to MAPK1. (**C**) qRT-PCR was applied to detect the relative expression of MAPK1 mRNA in neoplasm tissues of ccRCC patients. (**D**) Pearson test was applied to analyze the correlation between MAPK1 and HCP5 or miR-214-3p in neoplasm tissues of ccRCC patients. (**E**) qRT-PCR was applied to detect the relative expression of MAPK1 mRNA in co-transfected cell lines. (**F**) WB was applied to detect the relative expression of MAPK1 protein in co-transfected cell lines. * indicates P<0.05; *** indicates P<0.001.

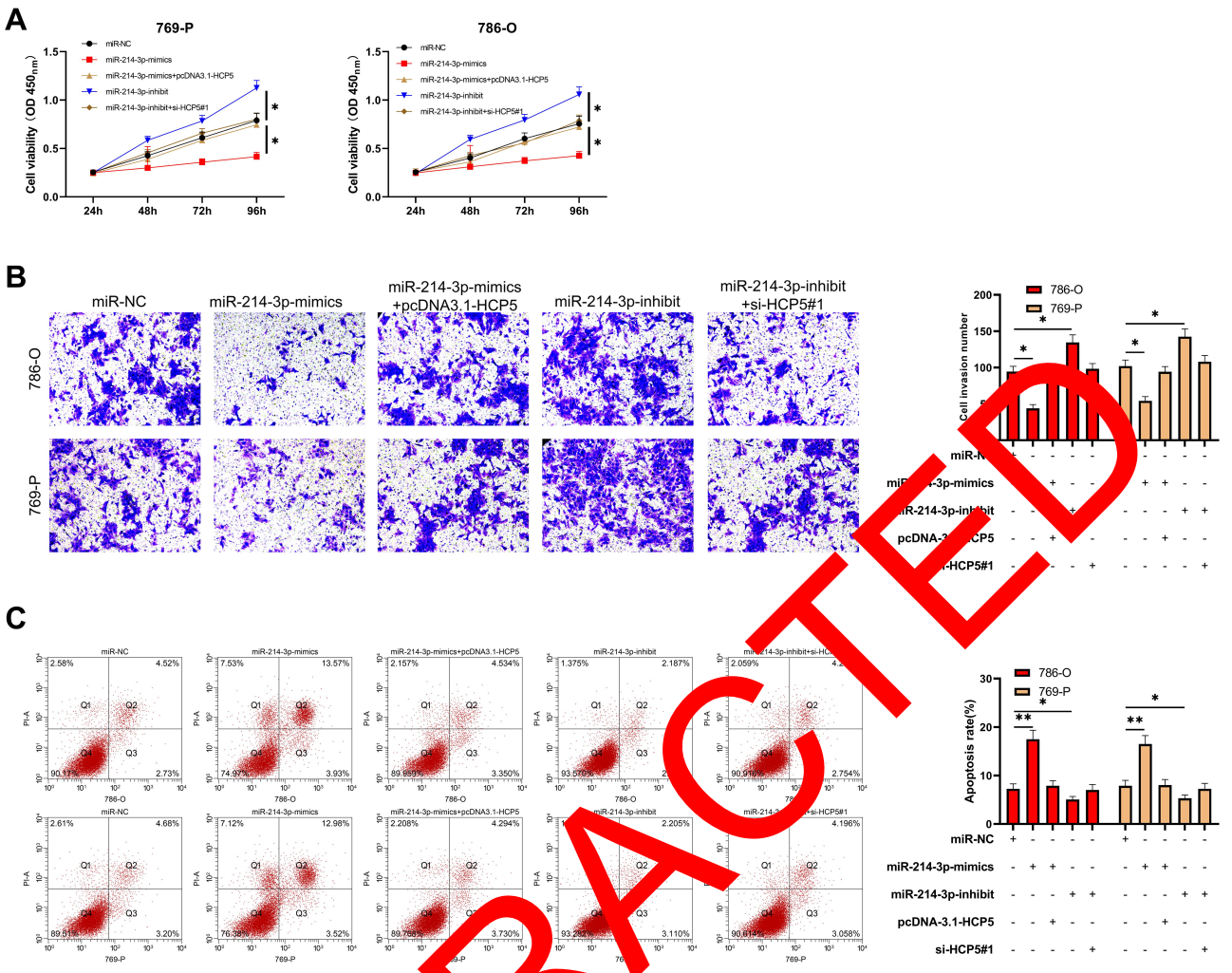

Figure 5 HCP5 could regulate MAPK1 to change the [illegible]owth o[illegible]cell carcinoma through miR-214-3p. (**A**) CCK-8 experiment was applied to detect the changes of cell proliferation ability after co-transfection. (**B**) Trans[illegible] test was app[illegible] to detect the changes of cell invasion number after co-transfection. (**C**) FACS was applied to detect the change of apoptosis rate after co-transfecti[illegible]dicates P<0.05, [illegible]ndicates P<0.01.

change the progression [illegible]d metastasis of [illegible]CC through miR-214-3p.

Discussion

Renal cell [illegible]arcino[illegible] has a close correlation with the carcinoma dea[illegible]but it is considered that a better solution is explored at pr[illegible]nt.[21] In this research, we found that HCP5 was enhanced in ccRCC, and in vitro studies revealed that HCP5 could induce apoptosis of RCC and inhibit neoplasm growth. Further studies have shown that HCP5 plays a role in RCC by targeting miR-214-3p/MAPK1 axis.

More and more studies have found that LncRNAs can regulate many biological pathways.[22] It can also promote or inhibit the growth and metastasis of ccRCC.[23] As an early LncRNA, HCP5 has a correlation with the poor prognosis of lung carcinoma and gastric carcinoma.[24,25] In our study, we found that the expression of HCP5 in ccRCC was decreased, which was the same as the result of Zhang et al.[26] However, the effect of HCP5 in the progression of ccRCC needs further exploration. Later, we found that HCP5 can hinder the progression of RCC in vitro, and FACS detection also found that the apoptosis rate increased after knocking down HCP5. It is suggested that HCP5 may be applied as a target for RCC.

ceRNA theory promoted the exploration of LncRNA in many diseases such as neoplasm.[27] And we have also found that LncRNA can participate in the progression of neoplasms by regulating knotted miR.[28–30] In this research, it was revealed that miR-214-3p and HCP5 have targeted binding sites by analyzing the binding of HCP5 to miR. miR-214-3p is a short-chain non-coding

RNA discovered in the early stage, which has low expression in various neoplasms such as lung carcinoma[32] and colon carcinoma,[32] and is a neoplasm suppressor gene. In the research of Das et al,[33] it was found that microRNA-214 reduced the expression of insulin-like growth factor −1 (IGF-1) receptor and mTORC1 downstream signal in RCC cells, thus inhibiting the growth of RCC. We also found that miR-214-3p was reduced in ccRCC neoplasms, which verified each other. In addition, there was a correlation of miR-214-3p with HCP5 in ccRCC by correlation analysis. This indicated that there was a latent targeting correlation of miR-214-3p with HCP5, and then we confirmed the targeting relationship between miR-214-3p and HCP5 by RIP and double luciferase report analysis.

In order to further determine the in-depth mechanism of HCP5, we predicted the target gene of miR-214-3p, and found that there was targeted binding of MAPK1 with miR-214-3p. MAPK1, as a member of MAP kinase family, is the binding point of many biochemical signals, and participates in many cellular processes. As a transcription inhibitor, MAPK1 plays a role independent of its kinase activity.[34,35] miR-214-3p-mimics/inhibit can inhibit or promote the fluorescence activity of MAP[illegible], indicating that miR-214-3p can regulate MAPK[illegible] in a targeted way. In addition, we also carried out res[illegible] experiments. After co-transfection, we found that mi[illegible] 214-3p-mimics inhibited MAPK1 m[illegible]NA and protein in RCC cells, while transfection of [illegible]-HCP[illegible] inhibited the promotion of miR-214-3p-inhi[illegible] on MAPK[illegible] mRNA and protein in RCC cells. In addition, cell function experiments also revealed that the proliferation, invasion and apoptosis were reversed after pcDNA-3.1-HCP5 and si-HCP5#1 were co-transfected with miR-214-3p-mimics /miR-214-3p-inhibit, respectively. Therefore, HCP5 could regulate MAPK1 to change the growth of RCC through miR-214-3p.

However, there are still some shortcomings in this study. First of all, our study did not further detect other related proteins of MAPK signaling pathway, and the way regulating the metastasis of RCC needs further exploration. Secondly, there are few healthy control samples collected in this study, which may lead to bias in the results of this study, so we cannot judge whether HCP5 can be used as a potential diagnostic indicator of ccRCC. Therefore, we hope to supplement our research experiments and carry out more basic research in future, so as to improve our conclusions.

Conclusion

To sum up, HCP5 is enhanced in ccRCC, which can inhibit the occurrence of RCC by mediating miR-214-3p/MAPK1 axis, and is a potential therapeutic target of ccRCC.

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Disclosure

The authors report no conflicts of interest in this work.

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