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Original Article

Aberrant expression of MEK5 in human hepatocellular carcinoma and its clinical significance

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Abstract: Mitogen/extracellular signal-regulated kinase kinase-5 [MEK5] has been confirmed to play a significant role in tumor carcinogenesis and progression. However, few studies have investigated the expression of MEK5 in hepatocellular carcinoma [HCC]. Meanwhile, the association between MEK5 expression and the clinical outcome of the disease remains poorly understood. In this present study, MEK5 mRNA and protein expression in HCC tissues were analyzed using the QRT-PCR, Western blot and immunohistochemistry methods. The association between MEK5 expression and the clinicopathological features and prognosis of patients was determined by statistical analysis. The results indicated that MEK5 was highly expressed in HCC tissues, and the MEK5 overexpression was associated with tumor size, tumor stage, and histological differentiation. Furthermore, shRNA-mediated knockdown of MEK5 in HCC cell lines decreased the cell proliferation rate *in vitro*, which implies the MEK5 expression might play a pivotal role in HCC development. The Kaplan-Meier survival and Cox regression analysis revealed that MEK5 overexpression was an independent factor for poor prognosis of HCC patients. These results indicate the MEK5 overexpression in HCC was correlated with the poor outcome of HCC patients and might be a potential molecular target for the HCC treatment.

Keywords: MEK5, hepatocellular carcinoma, shRNA, biomarker, prognosis

Introduction

Hepatocellular carcinoma (HCC) is one of the most malignance cancers, the fifth most common cancer, and the third cancer-related death worldwide [1]. HCC is relatively uncommon in the United Status and many other developed countries [2]. Meanwhile, most of the HCC patients were found in the developing countries where hepatitis B or hepatitis C infections were common, especially in China. Even though the cancer incidence and mortality for HCC has been decreasing in recently years according to the newly published cancer epidemiology statistics in China [3]. The newly diagnosed cases are still in a large and rising number in 2015 due to the population growth and ageing in China [3]. At present, surgical resection and liver transplantation are still the most promising treatment manners for early-stage HCC; however, the prognosis of HCC remains poor for late-stage HCC due to the high level of tumor invasiveness, extrahepatic metastasis, and resistance to chemotherapy [4]. For this reason, facilitating the earlier diagnosis of HCC and indentifying new biomarkers for the treatment of HCC may hold the greatest potential to have a more immediate impact on the existing burden of cancer in China.

Mitogen-activated protein kinase kinases (MEKs/MAPKKs) represent a family of protein kinases in the upstream of the MAP kinases [5]. There are mounting literatures revealed that MAP kinases play an important role in cancer development and response to therapeutics through regulating cell proliferation, angiogenesis, apoptosis, and hormone-independence [6, 7]. Mitogen/extracellular signal regulated kinase kinase-5 (MEK5) locates in human chromosome 15q23 and encodes a 444-amino-acid protein, is a key kinase in the MEK5-ERK5 signal pathway [8, 9]. Both MEK5 and ERK5 are functionally and structurally distinct from other MAPKs. It has been well established that MEK5 has a novel docking site on the N-terminus con-

Table 1. Correlations of MEK5 expression with the clinicopathological features of HCC

Variable	No. of cases	MEK5 expression		*P* value
		High	Low	
Gender				
Male	68	39	29	NS
Female	56	37	19	
Age (years)				
> 50	79	48	31	NS
< 50	45	28	17	
Tumor size (cm)				
≥ 5	83	57	26	0.016
< 5	41	19	22	
Smoking status				
Yes	53	36	17	NS
No	71	40	31	
HBsAg				
Positive	54	48	16	NS
Negative	70	49	21	
Differentiation grade				
Well/Moderate	56	29	27	0.049
Poorly	68	47	21	
TNM stage				
I-II	67	49	18	0.018
III-IV	57	30	27	

NS: Not significant; MEK-5: Mitogen/extracellular signal regulated kinase kinase-5; HBsAg, hepatitis B virus surface antigen.

taining a different consensus motif compared with other MEKs [10]. Meanwhile, ERK5 has a larger C-terminus than other MAPKs which was speculated to allow for specific targeting by inhibitors without affecting other kinases in the pathway [11]. To transduce cellular signal, MEK5 is phosphorylated in Serine 311 and Threonine 315 and thus convert to its active form which process is mediated by MEKK2,3/Tpl2. Then, the activated MEK5 (pMEK5) will specifically activate ERK5, and the activated ERK5 will then phosphorylate the downstream oncogenes including c-MYC, Sap1, c-FOS, MEF2, and NF-κB [10]. A number of published reports have demonstrated the overexpression or activation of MEK5-ERK5 in a variety of human cancers, such as prostate cancer [12], breast cancer [13], malignant mesotheliomas [14], leukemia [15] and colorectal cancer [16, 17]. Meanwhile, the overexpression of MEK5 has been proved to be associated with the increased activation of STAT3, which has been well-established to be associated with cell proliferation and metastasis [18]. These researches also revealed that MEK5 is an indicator for poor prognosis of cancer patients.

Previous study has demonstrated that MEK5/ERK5 pathway is associated with the development of multidrug-resistant hepatocellular carcinoma though the clearly mechanism is still unknown [19]. However, the prognosis value of MEK5 in HCC remains unclear. Therefore, in this study, we analyzed the expression of MEK5 in HCC patients using Real-time quantitative PCR (QRT-PCR), western blot and immunohistochemisty methods. Then, we explored the possible correlations between MEK5 expression and tumor progression, to investigate its role in tumor development and prognosis.

Material and methods

Clinical patients and tissue specimens

A total of 124 patients who diagnosed as HCC and went through surgical treatment in the Second people's Hospital of Tianjin between February 2005 and August 2010 were recruited in this study. The cohort included 68 males and 56 females with the mean age of 59.2 ages. Meanwhile, these patients did not receive any types of anti-cancer treatments before, including chemotherapy, radiation and biological immune therapy. The cancer tissues were surgically removed from HCC patients, the matched non-cancerous tissues were obtained from the distal edge of the resection at least 5 cm from the tumor. These specimens were frozen and stored at -80°C for further QRT-PCR, western blot and immunohistochemisty analyses. The cliniopatholigcal features, including gender, age, tumor size, differentiation grade, HBsAg, smoking status, tumor-mode-metastasis (TNM) stage and follow-up 5-years survival rate, were obtained from all the enrolled patients and presented in **Table 1**. The overall survival time was calculated as the time between surgery date and mortality date. The patients were classified into stage I/II or III/IV groups according to the TNM staging system. All patients were signed the written informed consent. The Ethics Committee of the Second people's Hospital of Tianjin approved all the protocols according to Declaration of Helsinki

and the whole study the performed under the monitor of Ethic Committee.

Cell lines and cell culture

Two HCC cell lines (HepG2 and HuH7) and one normal liver cell line (L02) were obtained from American Type Culture Collection (ATCC, Mannasas, VA, USA). These cells were incubated in Dulbecco's modified Eagle's medium (DMEM, Invitorgen, Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS, Invitorgen, Thermo Fisher Scientific, Inc., Waltham, MA, USA). All the cells were cultured in a humidified 37°C incubator supplemented with 5% CO_2.

ShRNA transfection to knockdown the expression of MEK5

To down-regulate the expression of MEK5 in HCC cell lines and normal liver cell line, MEK5-specific shRNA and non-targeting shRNA was designed according the previous published research [18]. These shRNAs were cloned into the lentiviral vector by Cyagen Biosciences Inc. (Suzhou, China). The MEK5-specific shRNA sequences used in this study were as follows: MEK5sh1, 5'-CCGTTCATCGTGCAGTTCAAT-3'; and MEK5sh2, 5'-GAGAACCAGGTGCTGGTAATT-3'. The HCC cell lines HepG2 and HuH7 were seeded in six-well plates at a density of 5×10^5 cells/well and cultured overnight until 70-80% confluence was achieved to obtain maximum transfection efficiency. The transfection was conducted using lipofectamine 2000 (Invotrogen, Carlsbad, CA, USA) according to the supplier's protocol followed by puromycin selection (1 μg/mL, Sigma-Aldrich, St. Louis, MO, USA) for 6 days. Cells were divided into three groups as follows: the knockdown cells were transfected with MEK5 shRNA lentivirus; the negative control cells were transfected with non-targeting shRNA lentivirus and the blank control cells were not transfected. The silencing efficiency of MEK5 was measured by QRT-PCR and western blot at 48 h post-transfection. And the effect of MEK5 down-regulation on the HCC cell proliferation was assessed by MTT assay.

Cell proliferation analysis

The cell proliferation rate of cell lines from the knockdown group, negative control group, and blank group was measured by the classical MTT assay. In brief, the cell lines were seeded into 96 well plates at a density of 5,000 cells/well. The cell proliferation rate was evaluated by adding 20 μL of 5 mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA). After incubation at 37°C for 4 h, 150 μL of DMSO was added to each well to dissolve the precipitates of the reduced MTT and then measure the absorbance value at 490 nm using the microplate reader (FLUOstar OPTIMA spectrofluorometer, BioTek Synergy HT, Winooski, VT, USA). The measurements were acquired at 12 h, 24 h, 48 h and 72 h. The results are presented as the mean ± standard deviation of three independent experiments.

Total RNA isolation and cDNA synthesis

Total RNA was extracted from all the 124 fresh tissue specimens and cultured cell lines using the Promega SV Total RNA isolation kit (Madison, Wisconsin, USA) according to the manufacturer's instructions. Briefly, the homogenized samples were mixed with the RNA lysis buffer at first and the RNA dilution buffer was added to the mixture, followed by centrifugation. After that, the supernatant was transferred to a fresh tube and 95% ethanol was added. At last, the solution was transferred to RNA extraction Spin column. The total RNA was eluted from the column using RNA wash solution and stored at -80°C for further use. The RNA was quantified using the DNA/RNA concentration determination equipment (NanoDrop 3000, ThermoFisher Scientific, MA, USA) and then reverse-transcribed to cDNA using the Reverse Transcription System (Promega, Madison, WI, USA) according to the recommendations provided by the manufacturer.

Real-time quantitative PCR

QRT-PCR was performed to measure the mRNA expression of MEK5 in all the fresh tissue specimens and cultured cell lines. The QRT-PCR was conducted in the ABI 7300 real-time PCR thermal cycle instrument (ABI, MA, USA) using the Platinum SYBR Green QPCR SuperMix UDG with ROX kit (Invitrogen, Waltham, MA, USA) according to the protocols provided by the suppliers. The optimized conditions for QRT-PCR is as follows: 95°C for 10 minutes followed by 40 cycles at 95°C for 15 seconds, and 60°C for 1

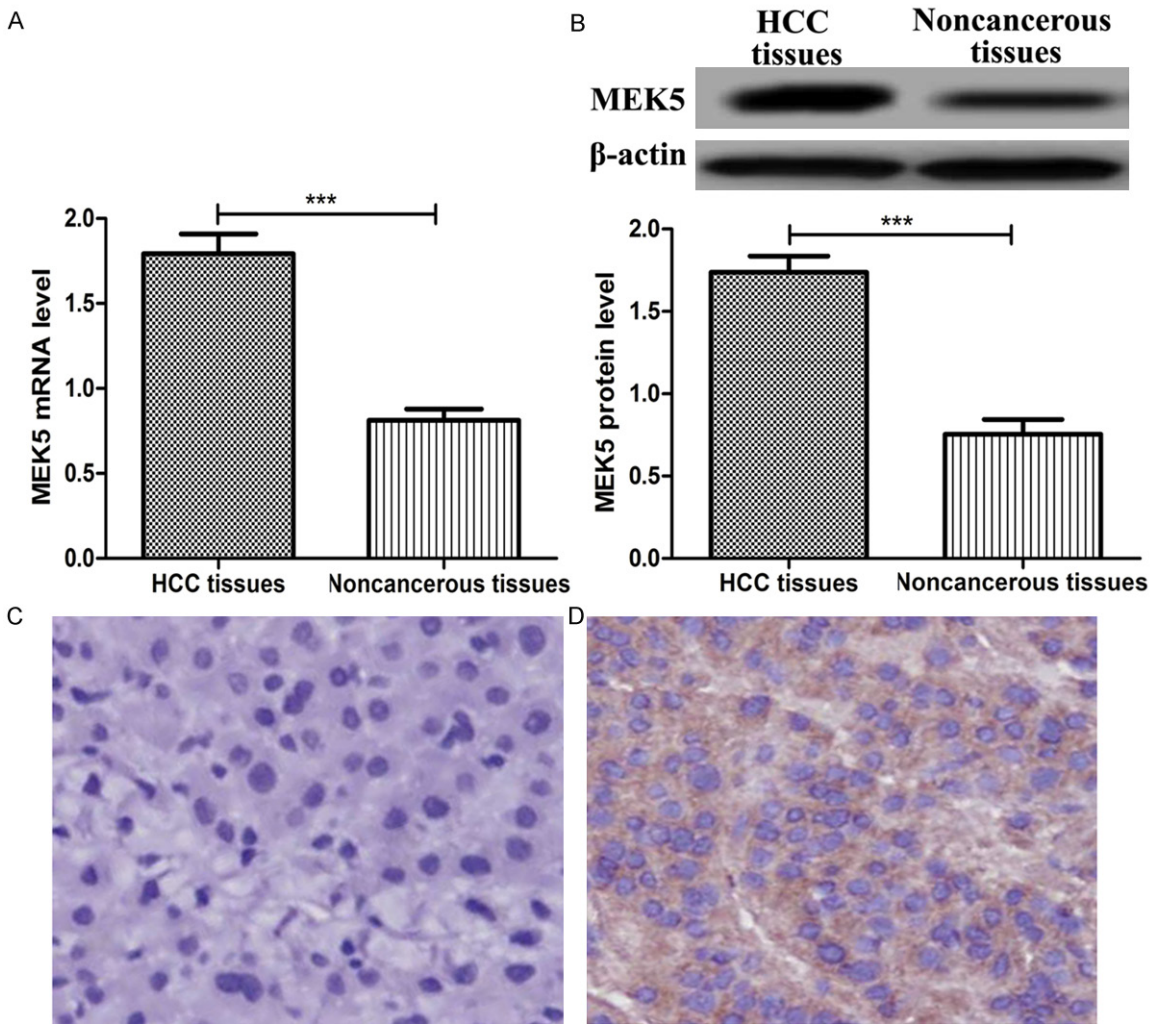

Figure 1. MEK5 was upregulated in the HCC tissues. A. QRT-PCR analyzes MEK5 expression in HCC tissues and noncancerous liver tissues. B. Western blot analyzes MEK5 expression in HCC tissues and noncancerous liver tissues. C. IHC analyzes the MEK5 expression in noncancerous liver tissue. D. IHC analyzes the MEK5 expression in HCC tissue (× 200 magnification, NS: Not significant, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

minute. The primers for QRT-PCR were: MEK5, Forward, CTTTAATGCCTCTCCAGCTTCT; Reverse, CCATCATTGAACTGCACGAT; GAPDH, Forward, GGGAAACTGTGGCGTGAT; Reverse, GAGTGGGTGTCGCTGTTGA. The relative expression level of STK33 was calculated using the threshold cycle (ΔΔCT) method and normalized to expression of endogenous GAPDH. Each sample was done in triplicate and the average was calculated.

Western bolt analysis

The fresh 124 tumor tissues, 124 non-cancerous tissues, cell lines selected and built in this study were subject to western blot analysis. The total protein was firstly extracted from the tissues and cell lines using the Tissue or Cell Total Protein Extraction Kit (Sangon Biotech Co., Ltd., Shanghai, China). The protein concentration was determined by Pierce BCA Protein Assay Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA). Equal amounts of protein were separated by electrophoresis on a 12% SDS polyacrylamide gel using Bio-Rad Mini-Protein III system (100 V for 2 h, Hercules, CA, USA) and then electro-transferred on a PVDF membranes (GE Healthcare Life Sciences, Marlborough, MA, USA) in 200 mA for 50 min in transfer buffer. The membrane was blocked with 5% Bovine Serum Albumin [BSA, Sigma-Aldrich, St. Louis, MO, USA) at room tempera-

ture for 1 h. Then, the membrane was incubated with primary monoclonal antibody against MEK5 (1:1000, 610957, BD Transduction Laboratories, San Diego, CA, USA) and β-actin (1:1000, SC47778, Santa Cruz Biotechnology Inc., Dallas, TX, USA). Horseradish peroxidase-conjugated secondary antibodies were applied at a dilution of 1:500 (SC516087, Santa Cruz Biotechnology Inc., Dallas, TX, USA) and detected using enhanced chemiluminescence detection kit (ECL, GE Healthcare Life Sciences, Marlborough, MA, USA). All the above procedures were conducted in accordance with the manufacturer's instructions. The protein expression level of MEK5 was normalized to that of β-actin.

Immunohistochemisty

We applied immunohistochemistry analysis to determine the MEK5 expression status in HCC tissues. The fresh paraffinized tissues were firstly deparaffinized by xylene and rehydrated by graded ethanol, followed with 3% hydrogen peroxide treatment to block the endogenous peroxidase activity. Then, the slides were retrieved in citrate buffer (pH6.0) at 121°C for 1.5 h. After that, the slides were incubated overnight with the primary antibody against MEK5 (1:300, 610957, BD Transduction Laboratories, San Diego, CA, USA). The slides were sequentially incubated with the horseradish peroxidase-conjugated secondary antibody (SC516087, Santa Cruz Biotechnology Inc., Dallas, TX, USA) for 30 min at room temperature. The slides were stained with 3,3-diaminobenzidine (DAB) liquid for 1 min. Finally, the slides were counterstained with hematoxylin, dehydrated and mounted. All slides were independently assessed by two pathologists, who were blinded to the cases.

Statistical analysis

All of the experimental data were analyzed using the SPSS v.17.0 (SPSS, Inc., Chicago, IL, USA). Data were present as the mean ± standard deviation. The Chi-square and Fishers' exact tests were used to analyze the relationship between the MEK5 overexpression and the collected clinicopathological features. The overall survival rates were calculated using the Kaplan-Meier method and differences between the groups were determined with log-rank test. Univariate Cox proportional hazards regressions were applied to estimate the individual hazard ratios (HR) for overall survival. The significant variables in the univariate analysis ($P < 0.05$) were then put into the multivariate analysis. The HR with 95% confidence interval (CI) was measured to estimate the hazard risk of individual factors. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

MEK5 expression in HCC tissues and adjacent normal tissues

We firstly examined the expression of MEK5 in HCC tissues and adjacent normal tissues using QRT-PCR and Western blot. The QRT-PCR results presented in **Figure 1A** demonstrated the mRNA expression levels were approximately two-folds higher in HCC tissues compared to the adjacent normal tissues. Moreover, the MEK5 protein expression levels in HCC tissues and adjacent normal tissues were analyzed using western blot. The results shown in **Figure 1B** indicate that the MEK5 is up-regulated in HCC tissues than in adjacent normal tissues and statistical significance, which is the same as the observations in QRT-PCT analysis. Among the 124 patients enrolled in this study, 76 of them (61.29%) were found to be MEK5 overexpressed and thus classified as high-MEK5 expression group, while the rests of them were classified as low-MEK5 expression group.

To confirm the overexpression status of MEK5 in HCC tissues seen by QRT-PCR and western blot, we next examined the expression of MEK5 in the archived HCC tissues and adjacent normal tissues using the immunohistochemistry method. According to the MEK5 immunoreactive intensity, we confirmed the tissues from the high-MEK5 expression group showed an upregulated MEK5 expression status (**Figure 1C**, **1D**). In the meantime, the MEK5 expression was proved to be down-regulated in the low-MEK5 expression group. These results implied that MEK5 expression was markedly higher in HCC tissues than in adjacent normal tissues and might plays an important role in HCC.

Knockdown the expression of MEK5 in HCC cell lines could inhibit cell proliferation

We further studied the expression of MEK5 in HCC cell lines and its role on cell proliferation to

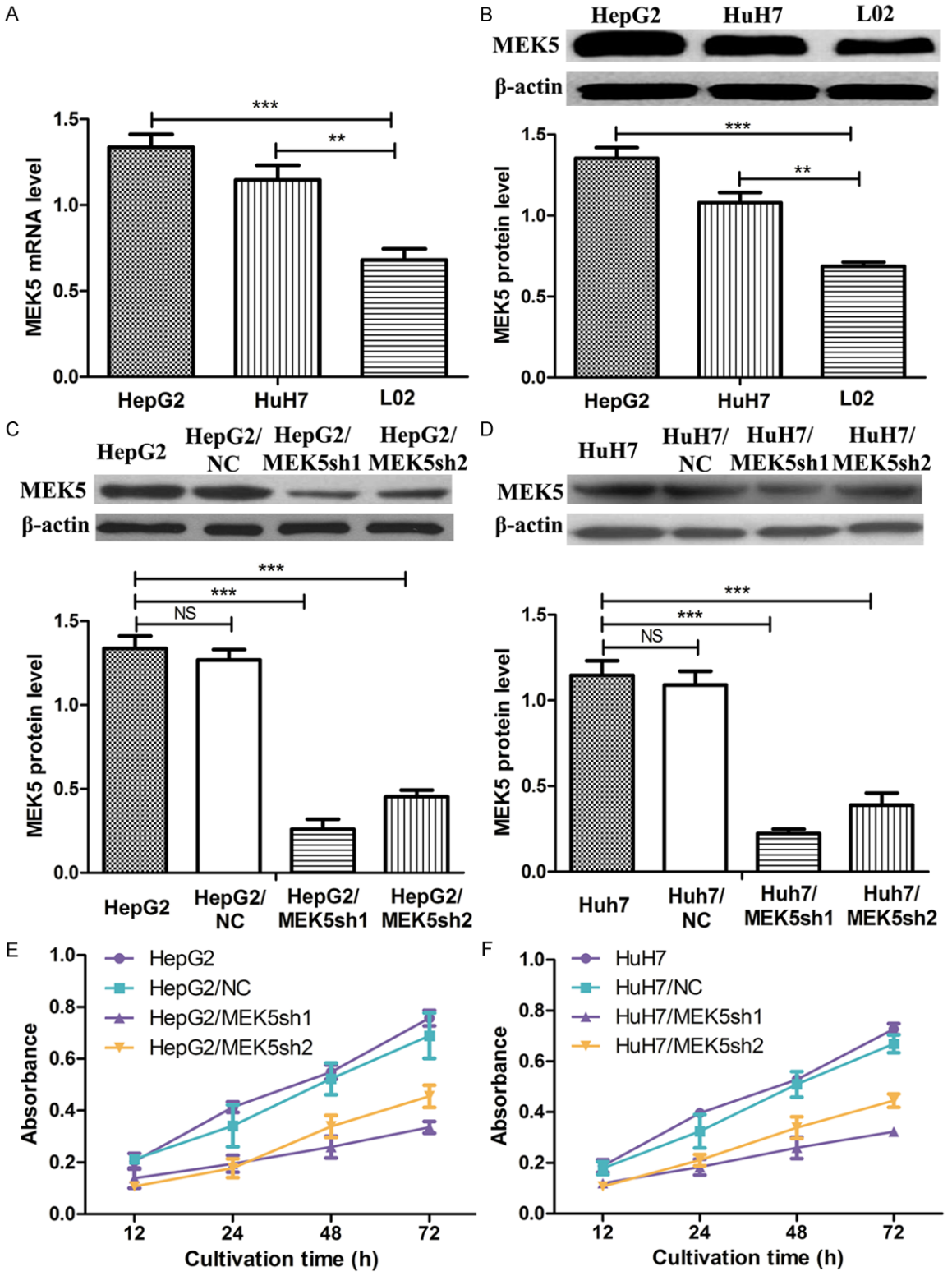

Figure 2. MEK5 promotes HCC cell proliferation. A. QRT-PCR analyzes MEK5 expression in HCC cell lines HepG2 and HuH7. B. Western blot analyzes MEK5 expression in HCC cell lines HepG2 and HuH7. C. Western blot analyzes MEK5 expression in HepG2 cell lines transfected with non-targeting shRNA and MEK5-specific shRNA. D. Western blot analyzes MEK5 expression in HuH7 cell lines transfected with non-targeting shRNA and MEK5-specific shRNA. E. MTT analyzes the cell proliferation rate in HepG2 cell lines transfected with non-targeting shRNA and MEK5-specific shRNA. F. MTT analyzes the cell proliferation rate in HepG2 cell lines transfected with non-targeting shRNA and MEK5-specific shRNA. (NS: Not significant, ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$).

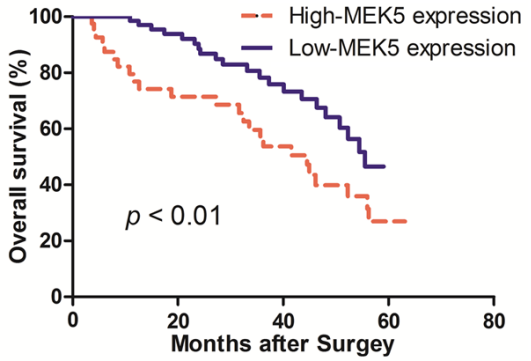

Figure 3. Survival analysis of HCC patients (n = 124). Kaplan-Meier estimates the overall survial according to MEK5 expression in 124 patients. The overall survival was significantly lower in patients with High-MEK5 expression when compared with patients who had Low-MEK5 expression.

have a primary understanding of the significance of MEK5 in HCC. The MEK5 expression status in HCC cell lines HepG2 and HuH7 and normal liver cell line L02 were measured by QRT-PCR and western blot at first. As shown in **Figure 2A** and **2B**, MEK5 was overexpressed in HCC cell lines when compared to the normal liver cell line L02. According to the expression level analysis results, the HCC cell lines HepG2 and HuH7 which have a relatively high level MEK5 expression were selected to estimate the effects of MEK5 inhibition.

Then, the MEK5-sepcific shRNAs and non-targeting shRNA were transfected into the HepG2 and HuH7 cell lines. QRT-PCR was performed to examine the efficiency of shRNA and also to confirm the successful down-regulate of MEK5 expression in HepG2 and HuH7. According to the results shown in **Figure 2C** and **2D**, we can deduct that the expression of MEK5 was down-regulated in HepG2 and HuH7 after MEK5-sepcific shRNA transfection. To study the effect of MEK5 expression on cell proliferation, MTT assay was conducted to measure the cell proliferation rate of the cell lines with or without shRNA transfection. The results showed that MEK5 depletion significantly suppressed the proliferation rate of HepG2 and HuH7 cell lines with MEK5-specific shRNA transfection compared to those with non-targeting shRNA transfection (**Figure 2E**, **2F**). Meanwhile, the proliferation rate is associated with the expression level of MEK5. These results reveal that MEK5 increases the cell proliferation rate of HCC cell lines.

Correlation between MEK5 expression and clinicopathological features

A total of 124 patients were enrolled in this study and 76 of them were proved to be MEK5 overexpressed. Meanwhile, the clinicopatholoigcal features were obtained from all the patients. The correlation between these features and the MEK5 expression was investigated as well and shown in **Table 1**. The results demonstrated that the expression of MEK5 was closely associated with tumor size (P = 0.016), TNM stage (P = 0.018), and differentiation grade (P = 0.049). However, there was no significance relationship between the expression of MEK5 and the other features, including gender, age, HBsAg and smoking status (all P > 0.05).

Correlation between MEK5 expression and overall survival

The association between MEK5 expression and overall survival of HCC patients was investigated by Kaplan-Meier analysis and long-rank test. The 5-year overall survival rate of the 124 patients with HCC was 58.06%, with 52 deaths observed during the follow-up period. Among the total 52 deaths, 38 were from the high-MEK5 expression group and the rest of them were from the low-MEK5 expression group. Meanwhile, the overall survival curve shown in **Figure 3** demonstrated that the patients in high-MEK5 expression group had shorter 5-year survival rates than patients in low-MEK5 expression group (P < 0.018).

According to the univariate analyses results, MEK5 expression (P = 0.015), tumor size (P = 0.021), TNM stage (P = 0.030), and differentiation grade (P = 0.027) were significantly associated with the patients' overall survival. However, no significant association was found for gender, age, HBsAg, smoking status, AFP and patient outcome (**Table 2**). Then, we conducted multivariate analysis using Cox proportional hazards model for all the significant variables in the univariate analysis. We found the MEK5 expression along with tumor size, TNM stage, and differentiation grade were independent prognostic predictors for overall survival (**Table 2**).

Table 2. Univariate analysis and multivariate analyses of overall survival

Variables	Univariate analysis		*P* value	Multivariate analysis		*P* value
	HR	95% CI		HR	95% CI	
MEK5	2.143	1.163-3.950	0.015	2.142	1.146-4.004	0.017
Age	1.702	0.910-3.183	0.096	-	-	-
Gender	1.664	0.884-3.135	0.115	-	-	-
Tumor size	2.039	1.114-3,734	0.021	2.160	1.171-3.983	0.014
Smoking status	1.742	0.918-3.305	0.089			
HBsAg	1.763	0.956-3.250	0.069	-	-	-
Differentiation grade	2.448	1.107-5.414	0.027	2.022	1.104-3.702	0.022
TNM stage	1.941	1.067-3.531	0.030	2.036	1.112-3.729	0.021

HR: hazard ratio; CI: Confidence Index; MEK-5: Mitogen/extracellular signal regulated kinase kinase-5; HBsAg, hepatitis B virus surface antigen.

Discussion

Hepatocellular carcinoma (HCC) is a common malignancy and accounts for as many as 1 million deaths annually worldwide. Though the survival of patients with HCC has improved due to the advances in surgical techniques, the long-term survival rate of HCC patients remained poor. Although numbers of clinicopathological features which are useful to evaluate the prognosis of HCC patients have been revealed, they still cannot meet the requirements for precise prediction of HCC course. Therefore, it is still an urgent demand to search for novel biomarker to predict the prognosis of HCC and therapeutic strategies.

The MEK5/ERK5 pathway was reported to regulate a variety of cellular processes during development [20]. Also, recent researches have demonstrated the MEK5/ERK5 signaling pathway is upregulated in many human cancers, including prostate cancer, breast cancer, malignant mesotheliomas, leukemia and colorectal cancer. In breast cancer cells, researchers found the activated oncogene STAT3 could bind to the promoter region of MEK5 and thus results in gene transcription, conferring a critical survival signal. Meanwhile, in metastatic prostate cancer, the overexpression of MEK5 is correlated with bony metastases, and poor prognosis is caused by upregulated BMK1-induced activator protein-1 (AP-1) activity [5]. However, no available research regarding the expression of MEK5 in HCC was found. Therefore, to explore the potential role of MEK5 in the tumorigenesis and progression of HCC, we examined the expression status of MEK5 in HCC tissues and also analyzed the relationship between MEK5 expression and the clinicopathological features.

In our study, we demonstrated that MEK5 mRNA and protein expression level in HCC tissues were significantly higher compared to that in the adjacent normal tissues which suggested that MEK5 might play an important role in the tumorigenesis and development of HCC. To further confirm the location of MEK5 in HCC tissues, IHC was performed in 124 archived paraffin-embedded HCC samples. The results illustrated that MEK5 was overexpressed in HCC tissues and was associated with tumor differentiation.

To investigate the potential role of MEK5 in the development of HCC, the upregulated mRNA and protein expression in HCC cell lines were confirmed using qRT-PCR and western Blot. Furthermore, the MEK5-specific shRNA was transfected into the HCC cell lines to knockdown the expression of MEK5 and evaluate the effect of MEK5 down-regulation on cell proliferation. Results indicated the MEK5 expression in HCC cell lines was effectively down-regulated, and the cell proliferation rate of the stable transfected cells was significantly decreased. Taken together, we proved the down-regulation of MEK5 could inhibit the cell proliferation *in vitro*, and negatively affected development of tumors *in vivo*, which were consistent with our data from the western blot and immunohistochemistry analyses in the HCC samples.

Moreover, the statistic analysis between MEK5 expression and clinicopathological features

demonstrated that the expression of MEK5 in HCC was closely correlated with tumor size, TNM stage, and differentiation grade. Kaplan-Meier analysis of the survival curves showed a significantly worse 5-year overall survival rate for patients with overexpressed MEK5, which suggests that MEK5 may be a biomarker for poor prognosis of HCC patients. In the univariate and multivariate analyse, we showed that MEK5 overexpression was an independent predictor of poor overall survival. Therefore, the patients with MEK5 overexpression require an intensive follow-up to improve the survival quality of HCC patients.

In conclusion, our current study demonstrated that MEK5 was upregulated in HCC tissues and the downregulation of MEK5 in HCC cell lines could inhibit cell proliferation. Moreover, this study provides the clinical evidence that MEK5 is an independent prognostic for outcome in HCC. To the best of our knowledge, this is the first report to investigate the MEK5 expression pattern and its clinical significance in HCC.

Disclosure of conflict of interest

None.

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