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Original Article

HGF induces oncoprotein HCCR-1 expression through the Wnt/β-catenin pathway in gastric cancer

Sung Ae Koh[1], Ji Yoon Jung[1], Jae-Ryong Kim[2,3], Kyung Hee Lee[1]

[1]*Department of Hematology-Oncology, College of Medicine, Yeungnam University, Daegu, Republic of Korea;* [2]*Department of Biochemistry and Molecular Biology, College of Medicine, Yeungnam University, Daegu, Republic of Korea;* [3]*Aging-Associated Vascular Disease Research Center, College of Medicine, Yeungnam University, Daegu, Republic of Korea*

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Abstract: Objective: Human cervical cancer oncogene (HCCR)-1, previously identified in cervical cancer and its cell lines, has been reported to play an important role in tumor progression in several cancers as a suppressor of apoptosis. However, the role of *HCCR-1* in the tumorigenesis of stomach cancer has not been identified. This study examined the role of *HCCR-1* as a suppressor of apoptosis during tumorigenesis in gastric cancer, along with its possible regulatory pathway. Methods: We employed several techniques including western blotting, semiquantitative reverse transcription polymerase chain reaction, diphenyltetrazolium bromide assay, chromatin immunoprecipitation assay, fluorescence-activated cell sorting, and *HCCR-1* knockdown with short hairpin RNA to elucidate the role of *HCCR-1*. Results: We observed that hepatocyte growth factor (HGF) upregulated *HCCR-1* expression. In addition, the expression levels of β-catenin, T cell factor-1 (TCF1), and B-cell lymphoma 2 (*bcl2)* were increased, whereas that of tumor protein 53 (*p53*) was decreased following HGF treatment. *HCCR-1* knockdown in NUGC-3 and MKN-28 cells decreased the expression of TCF1 and phosphorylated β-catenin and increased the binding activity on the binding site of the *HCCR-1* promoter. This identifies the possible involvement of the Wnt/β-catenin pathway in HGF-induced *HCCR-1* regulation. We also confirmed the role of *HCCR-1* in HGF-induced anti-apoptotic activity. p53 protein expression was increased, whereas that of bcl2 was decreased with HGF treatment in *HCCR-1* knockdown cells, while the apoptotic activity was increased. Conclusion: Our study suggests the anti-apoptotic activity of HGF-induced *HCCR-1* expression and that HGF may regulate HCCR-1 via TCF1/β-catenin in stomach cancer.

Keywords: Hepatocyte growth factor, *HCCR-1*, gastric cancer

Introduction

Tumorigenesis is a complex process involving germline mutations, suppressor gene mutations, amplification of oncogenes, and hormonal activity [1]. Several growth factors, including epidermal growth factor (EGF), hepatocyte growth factor (HGF), and vascular endothelial growth factor (VEGF), are associated with cancer progression and metastasis [2-4]. Cell surface receptors bind to growth factors and several ligands, subsequently activating signal transduction by tyrosine kinase, which promotes cancer proliferation by regulating cell survival, protein synthesis, and angiogenesis [5].

We have previously explored novel genes that were upregulated by HGF and its cognitive N-methyl-N'-nitroso-guanidine human osteosarcoma transforming gene (c-MET) receptor-associated cancer progression and metastasis, particularly in stomach cancer, using a complementary DNA (cDNA) microarray approach [6]. HGF is a known effector of the cell membrane expressing c-MET tyrosine kinase receptor and acts as a cytokine on the cells of epithelial origin to stimulate mitogenesis and angiogenesis [7, 8]. In tumor cells, HGF plays an important role in cell proliferation and angiogenesis and facilitates cancer cells to progress and infiltrate the surrounding tissues and to metastasize [9, 10].

In the present study, we identified a novel gene, human cervical cancer oncogene (HCCR)-1, which was upregulated by 3.1 fold following HGF treatment. *HCCR* is located on chromosome 12q and encodes HCCR-1 and HCCR-2 proteins, which were first identified in cervical

cancer and cervical cancer cell lines [11]. Some studies have reported that *HCCR* is overexpressed and acts as a suppressor of apoptosis, leading to tumor progression in colorectal, pancreatic, and breast cancers [12-14]. Moreover, one study reported that the *HCCR-1* oncogene is activated by the Wnt/β-catenin pathway [15]. We had previously demonstrated that HGF upregulates the Wnt/β-catenin pathway in stomach cancer cells [16]. We hypothesized that HGF-mediated *HCCR-1* may play a role in tumorigenesis, including anti-apoptotic activity, and it may also be activated by the Wnt/β-catenin pathway in stomach cancer. Thus, the present study identified the function and activation pathway of a novel gene, *HCCR-1*, which is upregulated by HGF in stomach cancer.

Materials and methods

Cell culture

Two human gastric cancer cell lines (NUGC-3, MKN-28) were obtained from the Korea Cell Line Bank (Seoul, Korea). They were from a poorly differentiated adenocarcinoma and a moderately differentiated adenocarcinoma. The cells were cultured in RPMI 1640 medium (Gibco, MA, USA) including 10% fetal bovine serum (FBS) at 37°C and maintained in a humidified atmosphere of 5% CO_2 and 95% air.

Reagents and antibodies

Horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies were purchased from Bio-Rad Laboratories (Hercules, CA, USA). Recombinant human HGF was procured from R&D Systems, Inc. (Minneapolis, MN, USA). Antibodies against β-catenin and phospho-β-catenin were purchased from Cell Signaling Technology (Beverly, MA, USA). HCCR-1 and TCF1 rabbit polyclonal antibodies were purchased from Abcam (Cambridge, UK).

Semiquantitative reverse transcription polymerase chain reaction (RT-PCR)

Total RNA from cells were extracted with TRIzol reagent. Complementary DNA (cDNA) was generated by reverse transcription using MMLV reverse transcriptase (Promega Corp., Madison, WI, USA) following the oligo (dT) priming method. PCR was amplified in a 10 μL reaction containing 10 mM Tris-HCl (pH 8.5), 50 mM KCl, 1 μL cDNA, 200 μM deoxyribonucleotide triphosphates (dNTPs), 1 mM $MgSO_4$, 1 U platinum Pfx Taq polymerase, and 2 μM primer. The reaction cycle was as follows: 95°C for 4 min; followed by 30 cycles at 94°C for 15 s, 60°C for 15 s, and 72°C for 30 s and 72°C for 10 min. The PCR products were separated by gel electrophoresis on a 1% agarose and visualized by ethidium bromide stain on an ultraviolet transilluminator [17].

Western blot analysis

To detect cellular proteins, cells were lysed with RIPA lysis buffer containing protease inhibitors. Proteins (50 μg) of an aliquot were separated by 10% SDS-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes. The membranes were preincubated with 5% skimmed milk in Tween-Tris-buffered saline (TTBS) for 30 min at room temperature and then incubated overnight with the primary antibodies at 4°C. After washing, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 90 min at 4°C. The blots were detected using enhanced ECL reagent. Protein bands were visualized using Fujifilm LAS-3000 image system (Stanford, CT, USA).

MTT assay

Cell proliferation ability was measured using the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. Control and transfected cells (1,500 cells/well) were seeded in 96-well plates. After serum starvation for 24 h, the cells were treated with or without HGF (10 ng/mL). After treatment for 72 h, 1 mg/mL MTT solution was added to the cells. After incubating for 4 h, the medium was removed, and the formazan crystals were dissolved in 100 μL dimethyl sulfoxide. For viability detection, the absorbance was measured at 570 nm using Bio-Rad multiscan plate reader (Hercules, CA, USA).

HCCR-1 knockdown with short hairpin RNA (shRNA)

The human HCCR-1-specific shRNA expression vector (HCCR-1-shRNA) containing HCCR-1-targeted shRNA sequence was purchased from Santa Cruz Biotechnology. (Santa Cruz, Inc, CA,

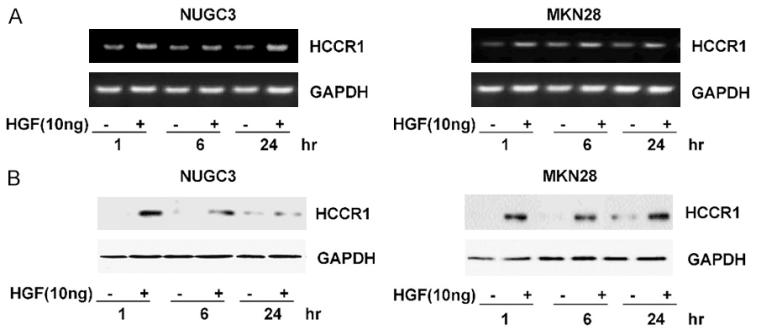

Figure 1. Effects of HGF on *HCCR-1* expression. Cells were serum-starved for 24 h, treated with/without HGF (10 ng/mL) for the indicated time and harvested. The expression levels of *HCCR-1* mRNA and protein were measured via reverse transcription polymerase chain reaction (A) and western blot analysis (B). The figure illustrates representative data from three independent experiments. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1.

USA). Following the instructions for Lipofectamine2000 (Invitrogen, Carlsbad, CA, USA), cells were transfected with HCCR1-shRNA for 24 hr. After transfection with HCCR1-shRNA, clonal selection was performed by culturing with puromycin (10 μg/mL) until the puromycin-resistant cells formed colonies. Stably transfected colonies were identified by HCCR1 antibody via western blot analysis.

Standard two-chamber invasion assay

A total of $1*10^4$ cells (Control and HCCR-1shRNA-expressing cells) were seeded into the upper chamber of Matrigel transwell chamber (0.8-μm pores, Thermo Fisher Scientific, Houston, TX, USA) with/without HGF 10 ng/ml adding serum-free medium. After incubation for 48 h, the cells that migrated on the lower membrane were fixed and stained using HEMA-3 stain kit (Curtis Matheson Scientific, Houston, TX, USA) following to the manufacturer's protocol. The stained filter membrane was cut and placed on a glass slide. The number of cells that migrated was calculated under a microscope (10 fields at 200× magnification).

Chromatin immunoprecipitation (ChIP) assay

Chromatin immunoprecipitation (ChIP) was done using a ChIP assay kit (Upstate Biotechnology, Waltham, MA, USA) according to the manufacturer's protocol. Cells were cross-linked by 1% formaldehyde at 37°C for 10 min and the reaction was stopped by adding 0.125 M glycine for 5 min. Cell lysates were achieved by sonication to shear chromatin. Lysates containing soluble chromatin were immunoprecipitated and Protein A/G plus agarose beads were used to collect the immunoprecipitated complexes. The resulting DNA fragments were visualized on an agarose gel. PCR primers were as follows *HCCR-1* promoter region 5'-ccagactgactggaaagtgg-3' (forward) and 5'-cttcacagcgagagaagcgg-3' (reverse).

Apoptosis

The cell cycle profile was measured by staining with propidium iodide. Trypsinized cells were incubated with 70% ethanol in -20°C for at least 1 h. Subsequently, the pellets were washed twice with ice-cold PBS. The pellets resuspended in 0.5 mL ice-cold PBS containing RNase (50 μg/mL) for 30 min and treated with propidium iodide (50 μg/mL) at 37°C for 30 min. The analysis of cells were performed by fluorescence-activated cell sorting (FACS; Becton-Dickinson, San Jose, CA, USA) at an excitation wavelength of 480 nm [18].

Results

HCCR-1 upregulation following HGF treatment

To validate *HCCR-1* upregulation by HGF, NUGC-3 and MKN-28 gastric cell lines were analyzed via western blotting and RT-PCR with HGF stimulation. An increased expression level of *HCCR-1* messenger RNA (mRNA) was confirmed after HGF treatment (**Figure 1A**). Western blot analysis revealed that HCCR-1 protein level was also increased by HGF treatment (**Figure 1B**). These test results suggested that *HCCR-1* was upregulated by HGF treatment in NUGC-3 and MKN-28 gastric cancer cell lines.

Dose-dependent effects of HGF on HCCR-1

Western blot analysis was conducted to identify the dose-dependent effects of HGF on *HCCR-1*. HCCR-1 protein expression increased with increasing concentrations of HGF (0, 10, and 40 ng/mL) (**Figure 2A**). In addition, we ana-

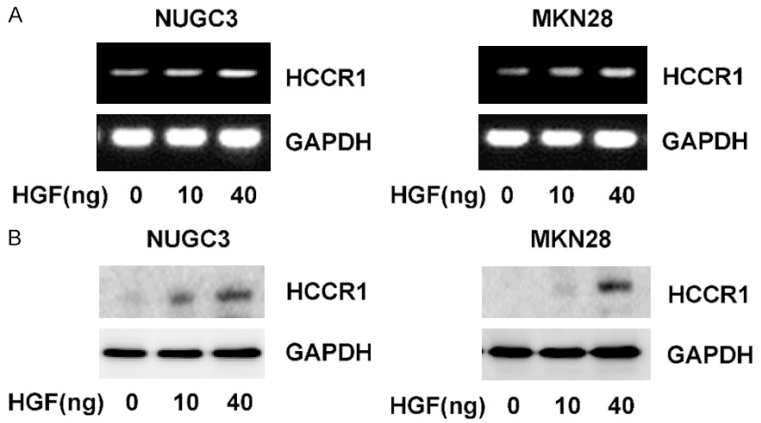

Figure 2. Effects of different doses of HGF on *HCCR-1* expression. Cells were serum-starved for 24 h and treated with HGF (0, 10, and 40 ng/mL) for 1 h and harvested. The expression levels of *HCCR-1* mRNA and protein were measured via reverse transcription polymerase chain reaction (A) and western blot analysis (B). The figure illustrates representative data from three independent experiments. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1.

lyzed the expression of *HCCR-1* mRNA through RT-PCR analysis and observed that *HCCR-1* mRNA levels increased with increasing concentrations of HGF in both gastric cancer cell lines (0, 10, and 40 ng/mL) (**Figure 2B**).

Dose-dependent effects of HGF on the Wnt/β-catenin pathway

To determine the association of *HCCR-1* with the Wnt/β-catenin pathway, we analyzed the protein levels of T cell factor-1 (TCF1), which is considered the best binding transcription factor of β-catenin and phosphorylated (p)-β-catenin, following HGF treatment via western blotting. The protein levels of TCF1 and p-β-catenin in the Triton-insoluble fraction were increased by HGF in a dose-dependent manner (0, 10, and 40 ng/mL) (**Figure 3A** and **3B**). These results suggest that HGF treatment upregulates the Wnt/β-catenin pathway.

Effects of HCCR-1 knockdown on apoptosis

To identify the association of *HCCR-1* with apoptosis, we examined the effect of *HCCR-1* knockdown on apoptosis markers, tumor protein 53 (p53), and B-cell lymphoma 2 (bcl2) via western blotting. It is known that *p53* is an apoptosis gene, whereas *bcl2* is an oncogene having an anti-apoptotic role. The expression level of p53 increased in both cell lines following *HCCR-1* knockdown. In contrast, the expression level of bcl2 was decreased in both *HCCR-1* knockdown cell lines (**Figure 4**). These results indicate that *HCCR-1* may be associated with HGF-mediated anti-apoptotic activity in gastric cancer cells.

Effects of HCCR-1 knockdown on the Wnt/β-catenin pathway

To identify the association between HGF-induced *HCCR-1* expression and Wnt/β-catenin signaling, we examined the effects of *HCCR-1* knockdown on TCF1 and β-catenin expression via western blotting. The knockdown of *HCCR-1* resulted in decreased TCF1 expression (**Figure 5A**). We also analyzed the effect of *HCCR-1* knockdown on β-catenin in NUGC-3 and MKN-28 cell lines. The level of p-β-catenin was increased in the Triton-soluble fraction, whereas it was decreased in the Triton-insoluble fraction in the *HCCR-1* knockdown cell lines (**Figure 5B**).

Effect of HCCR-1 knockdown on HGF-mediated proliferation

We then checked the effect of *HCCR-1* on HGF-mediated proliferation of gastric cancer cells by comparing *HCCR-1* knockdown and control cells. HCCR-1-shRNA-transfected and control cells were treated with HGF, and cell proliferation was measured after 72 h using the MTT assay. We analyzed the cell proliferation of control cells with HGF treatment in advance and observed that it was increased compared to that of cells without HGF. We observed that HGF-mediated proliferation was lower in *HCCR-1* knockdown cells than in control cells in both NUGC-3 and MKN-28 cell lines ($P<0.05$) (**Figure 6**).

Effect of HCCR-1 knockdown on HGF-mediated cell invasion

HGF/c-MET plays an important role in cell invasion, which can transport cancer cells and cause micrometastasis. We speculated that

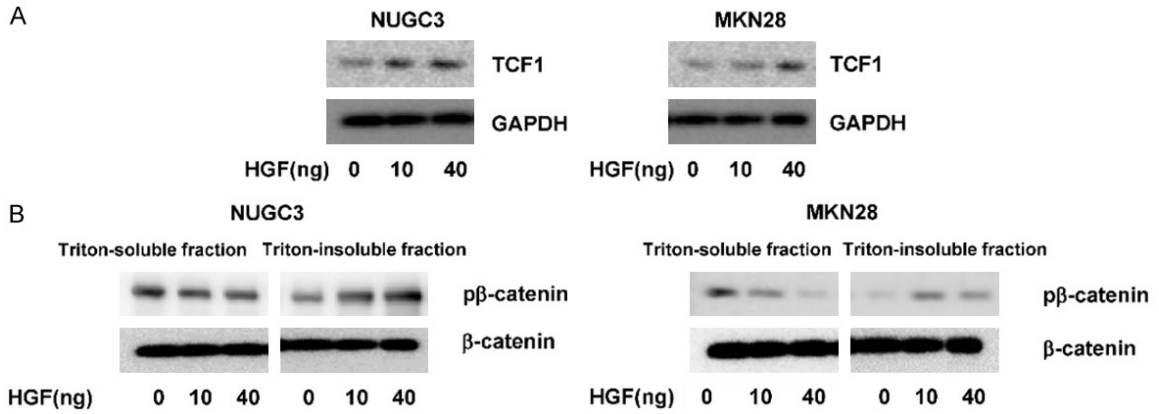

Figure 3. Effects of HGF on TCF1 and β-catenin expression. Cells were serum-starved for 24 h and treated with HGF (0, 10, and 40 ng/mL) for 1 h and harvested. The expression level of TCF1 was measured via western blotting (A). Proteins in Triton X-100-soluble or -insoluble fractions were immunoprecipitated with an antibody against β-catenin and separated on 10% SDS-polyacrylamide gel. The protein levels of β-catenin and phosphorylated β-catenin were analyzed via western blotting (B). The figure illustrates representative data from three independent experiments. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; TCF1: T cell factor 1.

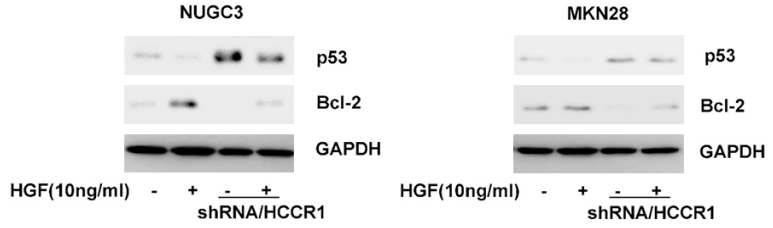

Figure 4. Effects of *HCCR-1* knockdown on HGF-mediated changes to expression levels of p53 and bcl2. Control cells and HCCR-1-shRNA-transfected cells were serum-starved and treated with/without HGF (10 ng/mL). The p53 and bcl2 expressions were measured via western blot analysis. The figure shows representative data from three independent experiments. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; p53: tumor protein p53; bcl2: B-cell lymphoma 2.

HCCR-1 plays a role in HGF-mediated cell invasion and performed an *in vitro* invasion assay using Matrigel-coated transwell chambers. HCCR-1-shRNA-transfected and control cells were treated with HGF. After 72 h of incubation, the cells were used for invasion assays. The data revealed that HGF-mediated cell invasion decreased in HCCR-1-shRNA-transfected cells compared to control cells in both gastric cell lines ($P<0.05$) (**Figure 7**).

Binding of TCF1 to the HCCR-1 promoter region

To verify whether TCF1 regulates the transcriptional activity of *HCCR-1* mRNA by binding to the *HCCR-1* promoter site, we identified the putative binding sequence of TCF1 in the *HCCR-1* promoter region using sequence analysis software (**Figure 8A**). To confirm TCF1 binding activity, we treated HCCR-1-shRNA-transfected and control cells with HGF and measured the binding activity of TCF1 to the putative binding site by ChIP assay. The TCF1 binding activity to the *HCCR-1* promoter region was enhanced by HGF in the control cells but did not enhance in HCCR-1-shRNA-transfected cells treated with or without HGF (**Figure 8B**). These results showed that HGF-mediated *HCCR-1* expression is controlled by the direct binding of TCF1 to the *HCCR-1* promoter region.

Effect of HCCR-1 knockdown on HGF-mediated cell apoptosis

Some studies have reported that *HCCR-1* acts as a suppressor of apoptosis in tumors. We investigated the role of *HCCR-1* in HGF-mediated apoptosis. We treated HCCR-1-shRNA transfected and control cells with HGF and measured cell apoptosis after 30 min using FACS. The results showed that HGF-mediated cell apoptotic activity was increased in HCCR-1 shRNA cells compared to control cells ($^{*}P<0.05$, $^{**}P<0.1$) (**Figure 9**).

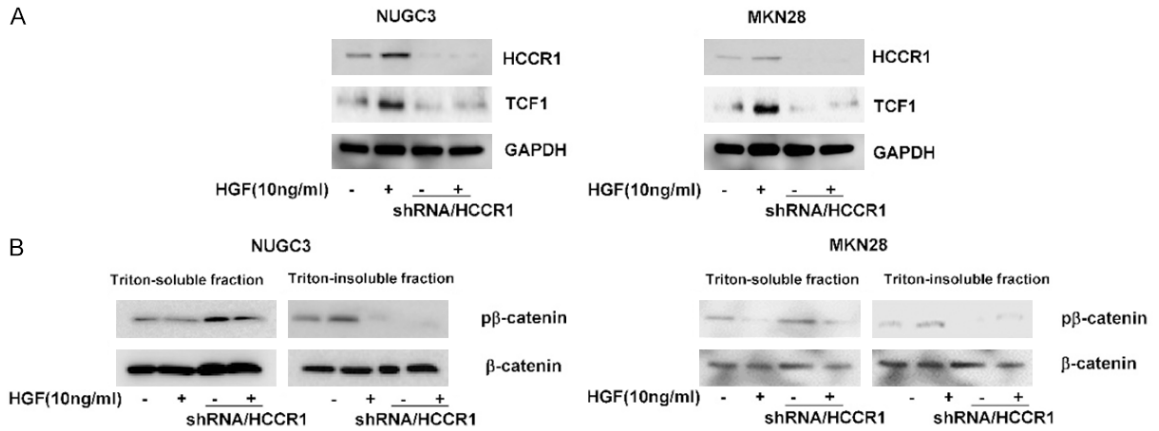

Figure 5. Effects of *HCCR-1* knockdown on HGF-mediated changes to expression levels of HCCR-1, TCF1, and β-catenin. Control cells and stable HCCR-1-shRNA-transfected cells (1×10^6 cells/well) were plated overnight in complete medium, starved for 24 h, treated with/without HGF (10 ng/mL) for 1 h, and harvested. The expression levels of HCCR-1 and TCF1 were analyzed via western blotting (A). Serum-starved cells were pretreated with 10 ng/mL HGF for the indicated time and harvested. Proteins in Triton X-100-soluble or -insoluble fractions were immunoprecipitated with an antibody against β-catenin and separated on 10% SDS-polyacrylamide gel. The protein level of β-catenin and phosphorylated β-catenin were analyzed via western blotting (B). The figure illustrates representative data from three independent experiments. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; TCF1: T cell factor 1; shRNA: short hairpin RNA.

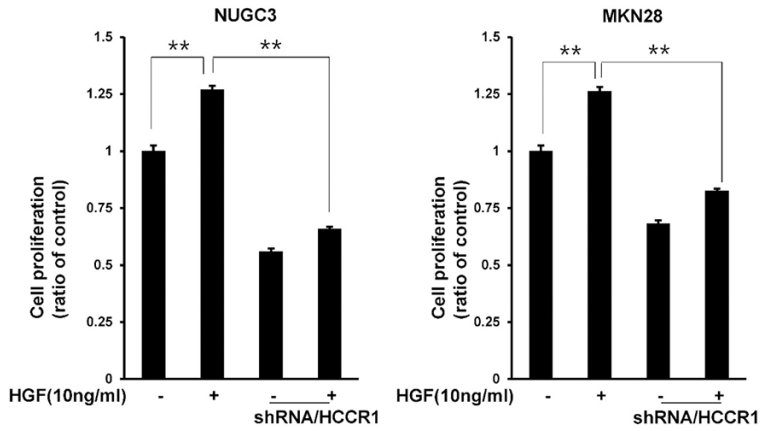

Figure 6. Effect of *HCCR-1* on cell proliferation. Control cells and stable HCCR-1-shRNA-transfected cells were seeded in 96-well plates in media supplemented with 5% FBS and incubated for 24 h. After serum starvation for 24 h, cells were treated/untreated with HGF (10 ng/mL) for 72 h. Cell proliferation was measured via the MTT assay and expressed as a percentage of HGF-untreated control cells. The results of three independent experiments were expressed as the means ± SD and were analyzed by Student's *t*-test. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; shRNA: short hairpin RNA; FBS: fetal bovine serum; SD: standard deviation.

Discussion

HGF is a paracrine growth factor that was originally discovered as a mitogen in primary hepatocytes [19]. Subsequent studies have reported several effects of HGF in normal cells, including cell motility, angiogenesis, cell differentiation, immune responses, and anti-apoptosis [20]. c-Met is a high-affinity receptor of HGF located on the surface of cells; HGF binding induces c-MET activation in a paracrine or autocrine manner [21]. HGF/c-MET axis activation promotes carcinogenesis in various types of cancer by regulating tumor cell proliferation, migration, invasion, metastasis, tumor angiogenesis, and anti-apoptotic activity [22-24].

We have previously investigated HGF/c-MET signaling and the functions of novel genes associated with the HGF/c-MET axis in gastric cancer cell lines. We have reported that novel genes upregulated by HGF/c-MET signaling might play a role in tumor cell proliferation and invasion, angiogenesis, and anti-apoptotic activity in gastric cancer. In the present study, among the genes upregulated by HGF, we selected *HCCR-1*, which is a known oncogene and is overexpressed in various cancers. One study reported the role of *HCCR-1* in carcinogenesis

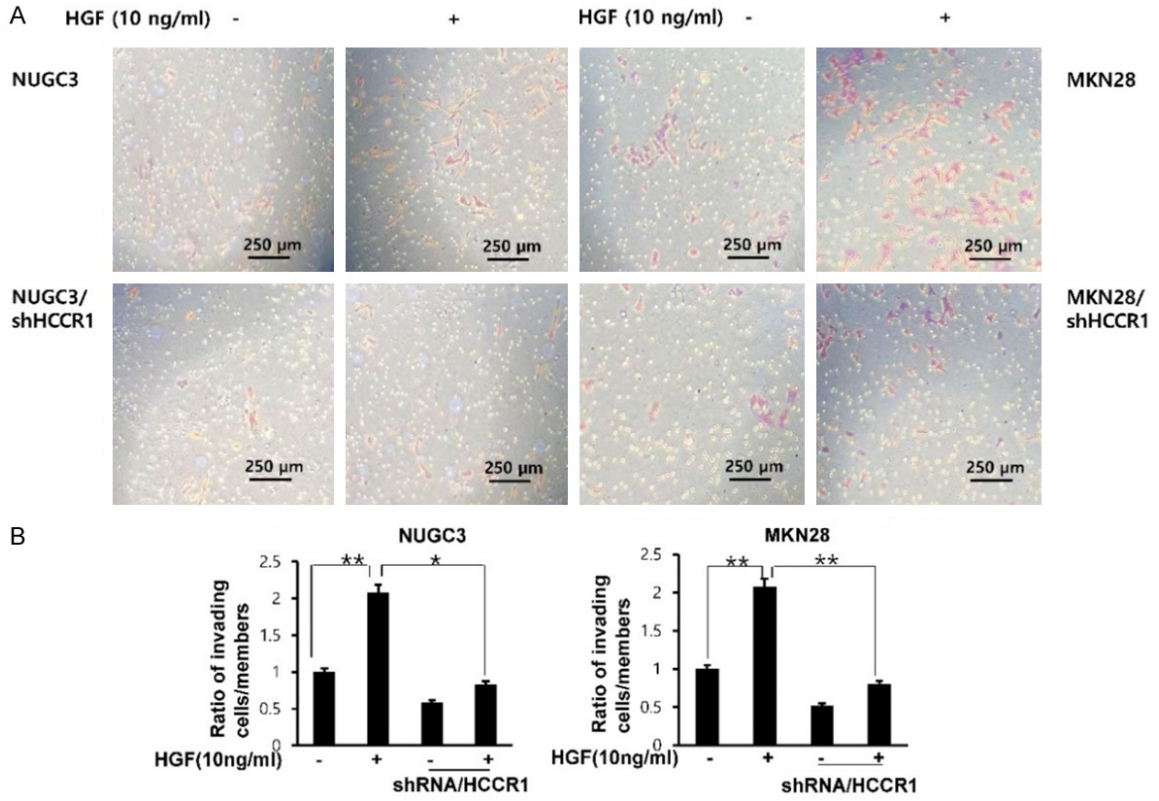

Figure 7. Effect of *HCCR-1* on HGF-mediated cell invasion. Control cells and stable HCCR-1-shRNA-transfected cells were treated with/without HGF (10 ng/mL) for 48 h. Cell invasion capacity was measured using Matrigel transwell chambers (A). Images were captured under a light microscope (scale bar =1 mm, all images are equal to scale bar) (B). The results of three independent experiments were expressed as the means ± SD and were analyzed via Student's *t*-test. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; shRNA: short hairpin RNA; SD: standard deviation.

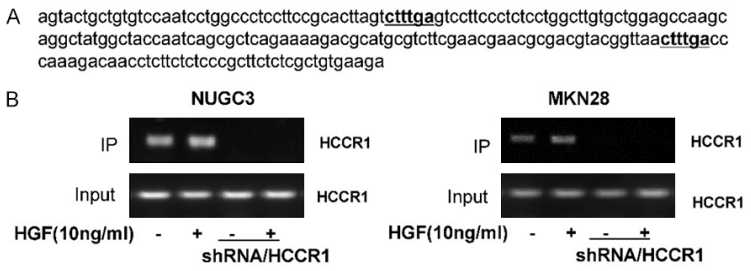

Figure 8. Effect of HGF treatment on TCF1 binding to *HCCR-1* promoter. Sequence of the proximal *HCCR-1* promoter. Underlined sequences mark the location of the putative TCF1 binding sites (A). ChIP assay results show amplification of a fragment of the proximal *HCCR-1* promoter containing TCF1 binding site. Immunoprecipitation was carried out using an anti-TCF1 antibody. The figure illustrates representative data from three independent experiments (B). HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; TCF1: T cell factor 1; ChIP: chromatin immunoprecipitation assay.

as a negative regulator of *p53* [11]. A subsequent study revealed that *HCCR-1* transgenic mice developed breast cancer and metastasis via the suppression of *p53* action [25]. In addition, another study reported that HCCR-1 expression is regulated by the Wnt/β-catenin pathway [15]. We speculated that HGF/c-MET-induced *HCCR-1* expression plays an important role as a suppressor of apoptosis during tumorigenesis in gastric cancer cells. In addition, we investigated the pathways regulated by *HCCR-1*.

We validated the upregulation of *HCCR-1* by HGF via western blotting and RT-PCR. HGF induced the expression of *HCCR-1* in a dose-dependent manner, as observed after 1 h of treatment. Moreover, we observed that TCF1, the best binding transcription factor in the Wnt/β-catenin

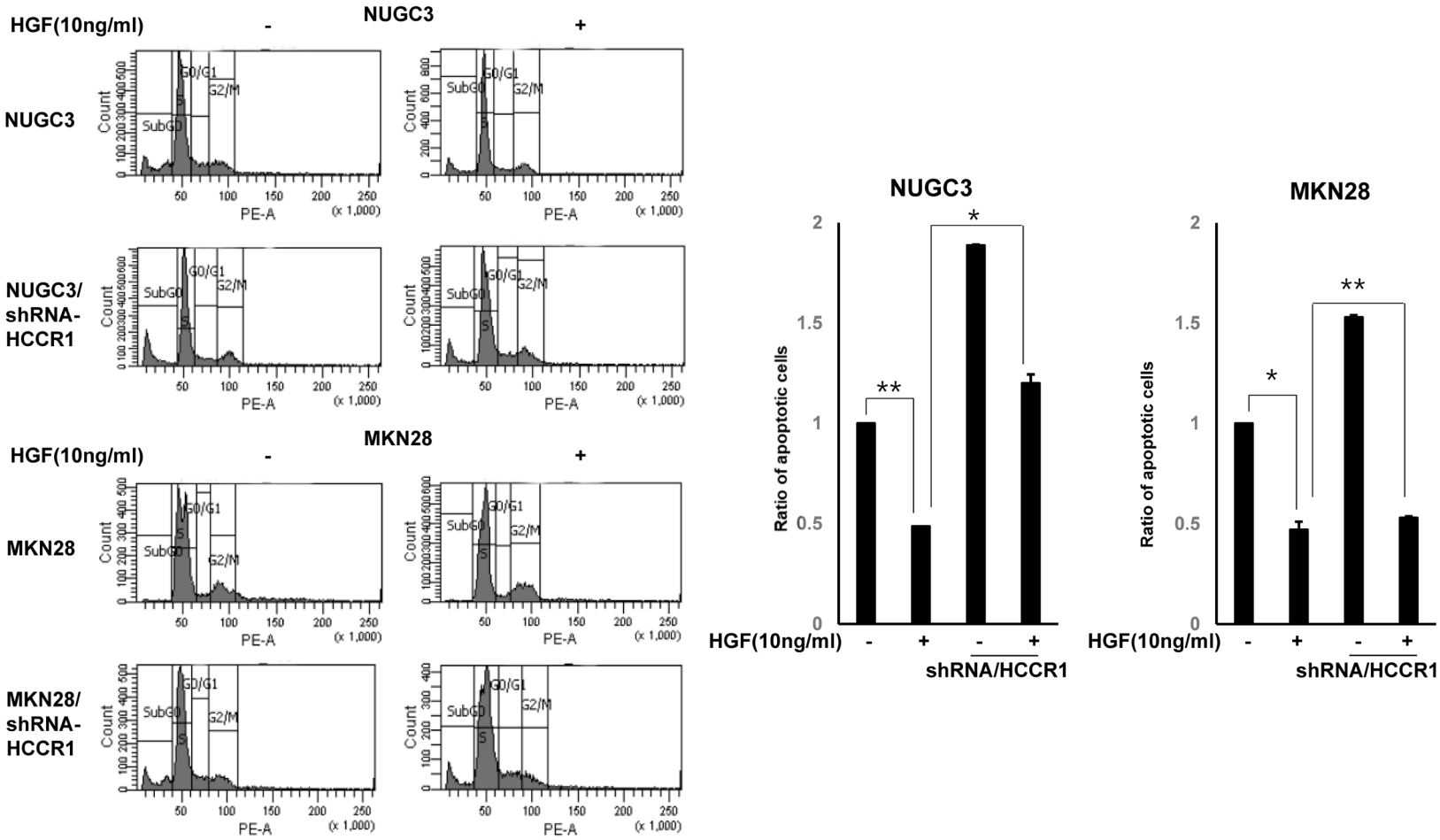

Figure 9. Effect of HGF on apoptosis in stable HCCR-1-shRNA-transfected cells. Stable HCCR-1-shRNA expressing cells were treated with/without HGF (10 ng/mL); then cell pellets were fixed and stained with PBS containing 10 μg/mL propidium iodide and analyzed by FACS. The figure shows representative data from three independent experiments. These results were expressed as the means ± SD and were analyzed via Student's *t*-test. HGF: hepatocyte growth factor; *HCCR-1*: human cervical cancer oncogene 1; shRNA: short hairpin RNA; TCF1: T cell factor 1; PBS: phosphate-buffered saline; FACS: fluorescence-activated cell sorting.

pathway, and p-β-catenin levels in the Triton-insoluble fraction were upregulated by HGF treatment. These results suggest that HGF induces the phosphorylation of β-catenin in the cell nucleus, which in turn interacts with TCF1 and plays a role in gene transcription [26].

We also observed decreased levels of p-β-catenin and TCF1 in *HCCR-1* knockdown cells compared to control cells in both gastric cancer cell lines. It is known that the upstream factors of the pathway could be upregulated or downregulated when the downstream factors are knocked down. The levels of p-β-catenin and TCF1 are considered to be decreased in *HCCR-1* knockdown cells because they are transcription factors that can bind directly to the *HCCR-1* promoter, resulting in its expression; one study have reported that TCF1 and its cofactor β-catenin could directly bind to the *HCCR-1* promoter [15]. We also tested whether TCF1, which is activated via the Wnt/β-catenin pathway, can directly bind to the *HCCR-1* promoter in gastric cancer cell lines. We identified the TCF1 putative binding site on the *HCCR-1* promoter proximal sequence. Subsequently, using ChIP assay, we demonstrated that the expression of TCF1 was increased in the *HCCR-1* promoter region following HGF treatment, and this expression was absent in the *HCCR-1* promoter region in *HCCR-1* knockdown gastric cancer cells. These results suggest that HGF-induced *HCCR-1* expression might be regulated by TCF/β-catenin.

Furthermore, we investigated the role of *HCCR-1* in the tumorigenesis of stomach cancer. Cell proliferation was decreased by HGF treatment in *HCCR-1* knockdown NUGC-3 and MKN-28 cells compared to that in control cells. Moreover, invasion was decreased in *HCCR-1* knockdown cells compared to that in control cells. Thus, HGF-induced *HCCR-1* expression may promote cell proliferation and metastasis in stomach cancer. However, in this study, we did not investigate the direct association of *HCCR-1* expression with HGF-induced cancer progression and invasion. Additional studies are required to identify the role of HGF-induced *HCCR-1* expression in stomach cancer tumorigenesis and metastasis.

With respect to its anti-apoptotic activity, *HCCR-1* was identified as a negative regulator of *p53,* which plays important roles in the regulation of cell cycle, apoptosis, and genomic stability and is a known tumor suppressor gene [27]. We observed increased levels of *p53* and decreased levels of *bcl2*, an anti-apoptotic marker, after HGF treatment in *HCCR-1* knockdown cells compared to control cells. In addition, using FACS, we found that the number of apoptotic cells in *HCCR-1* knockdown cells was higher than that in control cells. These results indicate that HGF-induced *HCCR-1* expression may inhibit apoptosis, thereby affecting cancer progression in gastric cancer. However, we did not identify the direct regulatory pathway of *HCCR-1* that involves *p53* and *bcl2*.

In this study, we identified the role of *HCCR-1* and its regulatory pathways via HGF treatment in stomach cancer cells. However, this study has limitations in terms of presenting only *in vitro* data on HGF-induced *HCCR-1* and its pathways. Additional studies, such as *in vivo* experiments using knockout mice, are required to identify and validate the role of *HCCR-1* in stomach cancer tumorigenesis.

In conclusion, we demonstrated that HGF/c-MET-induced *HCCR-1* expression promotes cancer proliferation and invasion in stomach cancer cells. In addition, we identified that HGF-induced *HCCR-1* expression might be regulated by the Wnt/β-catenin pathway and recognized the TCF1 binding site in the *HCCR-1* promoter and its binding activity. Furthermore, we demonstrated that HGF-induced *HCCR-1* expression decreased apoptotic activity, which could promote cancer progression. Additional studies are warranted to confirm the specific role of *HCCR-1* in stomach cancer and determine how *HCCR-1* can be used as a potential therapeutic target in stomach cancer treatment.

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Disclosure of conflict of interest

None.

Address correspondence to: Kyung Hee Lee, Department of Hematology-Oncology, College of Medicine, Yeungnam University, 170 Hyeonchung-ro, Nam-gu, Daegu 42415, Republic of Korea. Tel: +82-536203845; E-mail: lkhee@med.yu.ac.kr

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