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ARTICLE

Hepatitis B virus core protein promotes the expression of neuraminidase 1 to facilitate hepatocarcinogenesis

Fanyun Kong[1] · Nan Li[1,2] · Tao Tu[1,3] · Yukai Tao[1] · Yanwei Bi[1] · Dongchen Yuan[1] · Ning Zhang[1] · Xiaoying Yang[1] · Delong Kong[1] · Hongjuan You[1] · Kuiyang Zheng[1,4] · Renxian Tang[1,4]

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Abstract

Neuraminidase 1 (NEU1) has been reported to be associated with hepatocellular carcinoma (HCC). However, the function and associated molecular mechanisms of NEU1 in hepatitis B virus (HBV)-related HCC have not been well investigated. In the present study, the expression of NEU1 mediated by HBV and HBV core protein (HBc) was measured in hepatoma cells. The expression of NEU1 protein was detected via immunohistochemical analysis in HBV-associated HCC tissues. The role of NEU1 in the activation of signaling pathways and epithelial-mesenchymal transition (EMT) and the proliferation and migration of hepatoma cells mediated by HBc was assessed. We found that NEU1 was upregulated in HBV-positive hepatoma cells and HBV-related HCC tissues. HBV promoted NEU1 expression at the mRNA and protein level via HBc in hepatoma cells. Mechanistically, HBc was able to enhance the activity of the NEU1 promoter through NF-κB binding sites. In addition, through the increase in NEU1 expression, HBc contributed to activation of downstream signaling pathways and EMT in hepatoma cells. Moreover, NEU1 facilitated the proliferation and migration of hepatoma cells mediated by HBc. Taken together, our findings provide novel insight into the molecular mechanism underlying the oncogenesis mediated by HBc and demonstrate that NEU1 plays a vital role in HBc-mediated functional abnormality in HCC. Thus, NEU1 may serve as a potential therapeutic target in HBV-associated HCC.

Introduction

Hepatitis B virus, an infectious pathogen, is one of the leading causes of hepatocellular carcinoma (HCC) development [1–3]. Its viral genome contains partially double-stranded DNA and harbors four open reading frames (ORFs), namely, C, S, P, and X. The C ORF encodes the hepatitis B core (HBc) and HBe proteins. The S ORF contains HBs, preS1 and preS2 domains and encodes three viral envelope proteins. The P and X ORFs are responsible for expression of the viral polymerase (HBp) and HBx proteins [2, 3]. Among the encoded viral proteins, HBc is a structural component of the viral nucleocapsid and is involved in the viral life cycle and the pathogenesis induced by the virus [4]. Recent reports have shown that HBc plays a vital role in HCC development. For example, HBc suppresses the expression of death receptor 5 and Fas to inhibit apoptosis of hepatoma cells [5, 6]. The viral protein contributes to hepatoma cell proliferation via upregulation of telomerase reverse transcriptase [7] and activation of the Src/PI3K/Akt pathway [8], and promotes the expression of multiple metabolic enzymes and the secretion of

These authors contributed equally: Fanyun Kong, Nan Li, Tao Tu

✉ Hongjuan You
youhongjuan_00@163.com

✉ Renxian Tang
tangrenxian-t@163.com

[1] Jiangsu Key Laboratory of Immunity and Metabolism, Department of Pathogenic Biology and Immunology, Xuzhou Medical University, Xuzhou, Jiangsu, P.R. China

[2] Jiangsu Provincial Xuzhou Pharmaceutical Vocational College, Xuzhou, Jiangsu, P.R. China

[3] Shuyang Traditional Chinese Medicine Hospital, Shuyang, Jiangsu, P.R. China

[4] National Demonstration Center for Experimental Basic Medical Sciences Education, Xuzhou Medical University, Xuzhou, Jiangsu, P.R. China

metabolites from hepatoma cells to mediate pathogenesis during HBV infection [9]. Apart from these functions, HBc has also been shown to accelerate liver cancer metastasis via a miR-382-5p/DLC-1 axis [10]. Further understanding of the molecular mechanisms associated with HCC development mediated by HBc may help us find potential targets for treatment of HCC with HBV infection.

Neuraminidase 1 (NEU1) is a human sialidase that regulates the sialylation of multiple proteins and thus participates in many physiological processes, including cellular proliferation, adhesion, migration, and immune surveillance [11, 12]. Currently, alteration of NEU1 expression is reported in various tumors, and the protein is considered a novel therapeutic target in tumorigenesis [11]. In particular, Hou et al. found that NEU1 expression was significantly elevated in HCC tissues [13] and identified NEU1 as a new biomarker for HCC diagnosis and prognosis. Importantly, studies from different groups have shown that NEU1 is primarily associated with HCC induced by HBV infection [14, 15]. Whether HBV is capable of inducing the expression of NEU1 to regulate HCC pathogenesis is still unclear.

In the present study, the expression, molecular function, and associated mechanisms of NEU1 mediated by HBV were explored in hepatoma cells. Our results demonstrated that HBc contributes to NEU1 upregulation in HBV-associated hepatoma cells and that increased NEU1 expression is associated with activation of intracellular signaling pathways and epithelial-mesenchymal transition (EMT) in hepatoma cells. These results could help us gain a better understanding of the molecular mechanisms underlying hepatocarcinogenesis associated with NEU1 and mediated by HBV.

Materials and methods

Reagents and plasmids

Antibodies against HBsAg (HBs), vimentin, E-cadherin, β-catenin, and NEU1 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The antibody against HBc was from Abcam (Cambridge, MA, USA). Antibodies against ERK, P65, and IkBα were obtained from Proteintech (Wuhan, Hubei, China). Phosphorylated ERK (p-ERK, Thr202/Tyr204) and phosphorylated P65 (p-P65, Ser276) were purchased from Bioss Biotechnology Co., Ltd. (Beijing, China). GAPDH, β-actin, rabbit anti-goat IgG-HRP, goat anti-mouse IgG-HRP, and goat anti-rabbit IgG-HRP antibodies were obtained as mentioned previously [16, 17]. BAY11-7082, an NF-κB inhibitor, and 2′-(4-methylumbelliferyl)-α-D-N-acetylneuraminic acid sodium salt hydrate (4-MU-NANA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trizol reagent, G418, and TIANScript RT Kit were collected as previously described [17]. Matrigel solution was obtained from BD Biosciences (San Diego, CA, USA). Clarity™ ECL western blot substrate was purchased from BIO-RAD (Richmond, CA, USA). LipoMax DNA transfection reagent was bought from Sudgen Biotechnology (Nanjing, Jiangsu, China). Oseltamivir phosphate (OP) (Tamiflu®) was purchased from MCE (Medchem Express, Shanghai, China). The dual-luciferase reporter assay system was obtained from Promega (Madison, WI, USA).

HBV plasmids (pUC18-HBV1.2) and plasmids containing different HBV genes, including HBc, HBx, HBe, HBp, HBs, preS1, and preS2 plasmids, were obtained as mentioned previously [18]. PGL3-Basic plasmid and pRL-TK plasmid were obtained from Promega. A plasmid containing short hairpin RNA (shRNA) against NEU1 (GGGCTC TGGTATTCAGAAACA), pGPU6/GFP/Neo-NEU1, an shRNA vector against P65 (GGAGATGAAGACTTCTC CTCC), pGPU6/GFP/Neo-P65, and a control plasmid were purchased from GenePharma (Suzhou, Jiangsu, China).

Cell culture and transfection

The culture protocol for the human HepG2 and Huh7 hepatoma cell lines and L02 hepatocyte line has been described previously [16–19]. The cells were transfected with different plasmids using LipoMax DNA transfection reagent according to the manufacturer's instructions. In addition, HepG2 and Huh7 cells stably transfected with HBc plasmids or control plasmids were selected with G418 and named HepG2-HBc cells, Huh7-HBc, HepG2-Mock, and Huh7-Mock cells.

Clinical samples

HBV-negative adjacent tissues from 10 patients, HBV-negative HCC tissues from 30 patients, HBV-positive adjacent tissues from 30 patients, and HBV-related HCC tissues from 50 patients were collected from the Department of Pathology in the affiliated hospital of Xuzhou Medical University and purchased from Shanghai Outdo Biotech Co., Ltd. The study associated with these tissues followed the principles of the Declaration of Helsinki. Approval for the study was obtained from the ethics committee of Xuzhou Medical University, and written informed consent was obtained from the patients.

Reverse transcription-polymerase chain reaction (RT-PCR) and real-time PCR

RT-PCR and real-time PCR were conducted as mentioned previously [16]. Briefly, using Trizol reagent, total cellular RNA was collected from the target cells. Reverse

transcription was performed using a TIANScript RT Kit. The primer sequences and PCR amplification conditions used for GAPDH were as previously described [16]. The primers for NEU1, NEU2, NEU3, and NEU4 were TC TACCATGTTGGTATGGAGCAAG and GCTCATAGG GCTGGCATTC; AGCGGGCAAGCAAGAAGGATG and CCCAGGGATGGCAATGAAGAA; GCCAAGGGAGTG TGGTAAGTT and CAAGGGGGTCTGGTTGAGATA; and GTCCAGGGTCCTGACGTAGCC and AGCACTG TCCGTGAAGGGGTA. The conditions for amplification were as follows: 2 min at 94 °C followed by 30 s at 94 °C, 30 s at 58 °C, and 30 s at 72 °C for 45 cycles. The relative expression of target genes was normalized against that of GAPDH.

Construction of NEU1 plasmid and plasmids encoding different regions of the NEU1 promoter

The NEU1 gene was amplified by PCR and cloned into a pcDNA3.1 vector using Nhel and EcoRI sites to construct the NEU1 plasmid. The primer sequences for the NEU1 gene were as follows: CCCAAGCTTATGACTGGGGA GCGACCCAG and CCGGAATTCTTAGAGTGTCCCAT AGACACTGATTTTGG. Five different regions of the NEU1 promoter were cloned into PGL-3 plasmids. Briefly, using specific primers, five different 5′-flanking regions (−1849/+100, −1398/+100, −724/+100, −469/+100, and −168/+100) of NEU1 were amplified and inserted into the KpnI/BglII site of the PGL3-Basic vector and named PGL3-P (−1849/+100), PGL3-P (−1398/+100), PGL3-P (−724/+100), PGL3-P (−469/+100), and PGL3-P (−168/ +100). The forward primers for the NEU1 promoter with different regions were as follows: CATGGGTACCACGG GTCATGTCTTCCAGA, CATGGGTACCTCAGGAGTT CGAGACCAG, CATGGGTACCGTGACAGCTCCGGC CCTGAT, CATGGGTACCCATGCACCTGTCATCCCA GC and CATGGGTACCTAAGGATGGTGCCAGTCCC, respectively; the reverse primer was CATGCTCGAGCT AGACTCCACAGAGTCG. The PGL3-P (−1398/+100) mutants, which carried two substitutions (CCACTTTGG GGTCT and CCCCTAAAAGA) in the potential NF-κB binding sites (GGTGAAACCCCTCT and GGGGATTTT CT) in the NEU1 promoter region, were constructed by overlapping extension PCR as previously reported [17].

Luciferase reporter gene assays

The luciferase reporter gene assays were conducted as already described [17]. Briefly, 1×10^5 HBc-positive cells or control cells were placed into 24-well plates. After culture for 24 h, the cells were cotransfected with plasmids encoding different regions of the NEU1 promoter or with PGL3-Basic plasmid, along with pRL-TK plasmid. After transfection for 24 h, the cells were collected and then lysed in 1× passive lysis buffer. Next, the results were detected using the dual-luciferase reporter assay system as described [17].

Western blot analysis

Western blotting was performed as previously described [19]. Briefly, total protein was collected and subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Then, the proteins were transferred onto polyvinylidene difluoride (PVDF) membranes. After blocking with 5% milk in Tris-buffered saline for 2 h at room temperature (RT), different primary antibodies were incubated with the PVDF membranes at 4 °C overnight. The next day, the PVDF membranes were incubated with HRP-conjugated secondary antibodies for 2 h at RT. The results were measured using Clarity™ ECL western blot substrate.

Bioinformatics analysis of NEU1 expression in microarray data

Bioinformatics analysis of NEU1 expression in GSE14520 microarray data from the GEO database [20, 21] was performed with Affymetrix Expression Console and Affymetrix Transcriptome Analysis Console. The samples in GSE14520 were mostly HBV-associated HCC samples detected using an Affymetrix HT Human Genome U133A Array. During analysis, the samples without HBV infection were removed, and 212 HBV-HCC and 220 non-HCC cases were ultimately used in the study [22]. Details of the analysis of NEU1 expression in GSE14520 have been described previously [23].

Immunohistochemistry (IHC) analysis

IHC was performed as previously described [17]. Briefly, target tissues were fixed with 4% formaldehyde, embedded in paraffin, and sectioned. Sequentially, the tissue sections were deparaffinized and rehydrated. Next, the tissue sections were incubated with 0.01 M sodium citrate to retrieve antigen. After treatment with 3% H_2O_2 and subsequent blocking with 10% goat serum for 1 h, the tissue sections were incubated with anti-NEU1 or anti-HBc antibodies overnight, followed by incubation with HRP-conjugated antibodies for 2 h. The tissue sections were stained with 3,3′-diaminobenzidine, and the reaction was terminated using double-distilled water. The tissue sections were next counterstained with hematoxylin, and the results were observed using an Olympus microscope. The expression of NEU1 and HBc in target tissues was calculated as described by Wang et al. [24].

Immunofluorescence analysis

The location of NEU1 and P65 protein in hepatoma cells was detected with immunofluorescence assays as previously described [19]. Briefly, hepatoma cells were seeded on coverslips for 24 h in 12-well plates. Then, the coverslips were fixed for 5 min with ice-cold acetone and blocked for half an hour with 5% bovine serum albumin (BSA) in phosphate-buffered saline (PBS). Next, the coverslips were incubated with anti-NEU1 or anti-P65 antibodies for 2 h at room temperature, washed with PBS three times and then incubated with Alexa Fluor 488- and Alexa Fluor 594-conjugated antibodies for 2 h at room temperature. Cell nuclei were stained for 10 min with DAPI. The results were observed and acquired using an Olympus microscope.

Sialidase activity assay

Sialidase activity was measured using 4-MU-NANA as the substrate as referred to in the method described by Chen et al. [25], with some modifications. Briefly, first, HBc-positive cells and control cells were harvested and suspended for 15 min in 100 µl of lysis buffer (pH 7.6) containing 20 mM Tris-HCl, 1% Triton X-100, 150 mM NaCl, and protease inhibitor mixtures. The cell-containing mixtures were sonicated and centrifuged at 12,000 rpm for 10 min. Then, 10 µl of the supernatants was incubated with 15 µM 4-MU-NANA for 30 min at 37 °C in 50 µl reaction buffer (50 mM sodium phosphate, pH 5.0). The reaction was terminated with 600 µl stop buffer (0.25 M glycine-NaOH, pH 10.4), and the fluorescence intensity was detected with a BioTeK Synergy2 Multi-Detection Microplate Reader (Biotek Instruments, Winooski, VT, USA) (excitation at 360 nm and emission at 460 nm).

Cell viability assay

Cell viability assays were performed as previously described [19]. Briefly, 100 µL cell suspensions from each group were seeded into 96-well plates with six parallel wells. After the cells were measured with a CCK-8 kit at 24, 48, 72, and 96 h, the relative proliferation rate of cells was detected by determining optical density (OD) values at 450 nm with a ClinBio-128 plate reader (SLT, Austria).

Plate clone formation assay

Cells in each group were added into a 6-well plate with three parallel wells. After incubation for 2 weeks, the cells were washed with PBS twice and stained with crystal violet staining solution. The efficiency of clone formation was calculated as previously reported [19].

Animal transplantation

The animal experiments in the study were performed following the ethical principles for scientific experiments on animals of the Swiss Academy of Medical Sciences and were approved by the Animal Care and Use Committee of Xuzhou Medical University. Female BALB/c nude mice (4 weeks old) were purchased from Shanghai Laboratory Animal Co., Ltd. (Shanghai, China) and fed under specific-pathogen-free and temperature-controlled conditions. After transfection of HepG2-HBc cells with NEU1 shRNA or control plasmids for 48 h, the cells were harvested and resuspended in sterile PBS at a concentration of 1×10^7/mL. One hundred microliters of the cell suspension with 0.1 ml Matrigel solution was injected into the null mice in each group ($n = 6$) at the shoulder. A caliper was used to assess the length and width of each tumor, and the volume of tumors was calculated using the following formula: $(\text{length} \times \text{width}^2) \times 0.5$. After being fed for 28 days, the mice were killed, and tumors were excised.

Transwell assay

Transwell assays were conducted as previously described [19]. Briefly, cells resuspended in serum-free medium were placed in the upper chamber of the transwell plate and incubated at 37 °C for 24 h. One milliliter of medium containing 10% fetal bovine serum as a chemoattractant was placed in the bottom chamber. The cells that did not pass through the polycarbonate membrane in the upper chamber were removed with a cotton swab. The cells that passed through the polycarbonate membrane were considered migrating cells. The migrating cells were fixed with 4% paraformaldehyde, stained with crystal violet, and observed with an Olympus microscope (×200).

Wound healing assay

Wound healing assays were conducted as previously described [19]. After the cells reached 90% confluence in 6-well plates, a wound was created with a micropipette tip. The results were detected using an Olympus microscope (×400), and migration distances were calculated.

Statistical analysis

The data are presented as the means ± standard deviation (SD) and analyzed using a *t*-test, one-way ANOVA, or a Mann–Whitney test where appropriate. In addition, a chi-square test was used to analyze the relative expression of NEU1 and HBc proteins detected by IHC analysis and to determine the significance of correlations between NEU1 and expression of HBc proteins using SPSS 19.0 software

(IBM, Armonk, NY, USA). Based on density measurements, the results of semiquantitative western blot analysis were determined with ImageJ software (NIH, Bethesda, MD, USA). A p-value < 0.05 was considered statistically significant.

Results

HBc contributes to NEU1 expression in HBV-related hepatoma cells

First, we investigated whether HBV could promote the expression of sialidases in hepatoma cells. HepG2 and Huh7 cells were transfected with HBV plasmids or control plasmids. The mRNA expression levels of four types of human sialidases, namely, NEU1, NEU2, NEU3, and NEU4 [12], were then detected in HBV-related hepatoma cells and control cells. The results showed that only the expression of NEU1 mRNA was elevated by HBV in hepatoma cells (Fig. 1a). The increased expression of NEU1 protein mediated by HBV was also confirmed by western blot analysis in two types of hepatoma cells (Fig. 1b). Using GSE14520 microarray data, the expression of NEU1 mRNA was investigated in HBV-related HCC and non-tumor tissues. The results showed a change in NEU1 mRNA greater than 2.0-fold in HBV-related HCC tissues compared with non-HCC tissues (Fig. 1c). NEU1 protein expression in HBV-negative adjacent tissues, HCC tissues, HBV-positive adjacent tissues, and HBV-positive tumor tissues was identified through IHC analysis. As shown in Fig. 1d, compared with the expression in HBV-negative adjacent tissues, HCC tissues, and HBV-positive adjacent tissues, the expression of NEU1 protein was increased in HBV-related tumor tissues ($p < 0.05$).

We further explored the role of different HBV genes in NEU1 expression. HBc and HBx were found to significantly induce NEU1 expression at the mRNA and protein level, while the HBp gene downregulated NEU1 expression in HepG2 and Huh7 cells (Fig. 1e, f). These results indicate that the comprehensive effect of HBV genes was an increase in NEU1 expression and that the role of HBc in promoting NEU1 expression was greater than that of HBx in hepatoma cells. Thus, we next mainly focused on the role of HBc in NEU1 expression in this study. We also measured whether HBV and HBc could induce NEU1 expression in hepatocytes by transfecting the hepatocyte line L02 with HBV, HBc, and control plasmids. The results showed that HBV and HBc could promote NEU1 expression at the mRNA and protein level in L02 cells (Fig. 1g, h).

Next, we mainly focused on the role of HBc in NEU1 expression in hepatoma cells. The expression levels of NEU1 in hepatoma cells stably transfected with HBc or control plasmids were investigated. The results showed that NEU1 expression was increased in stable HBc-expressing cells compared with control cells (Fig. 1i, j). IHC analysis was also performed to detect the relationship between HBc and NEU1 expression in HBV-related HCC tissues. We found that the expression of NEU1 was positively correlated with HBc expression in HBV-related HCC tissues (Fig. 1k).

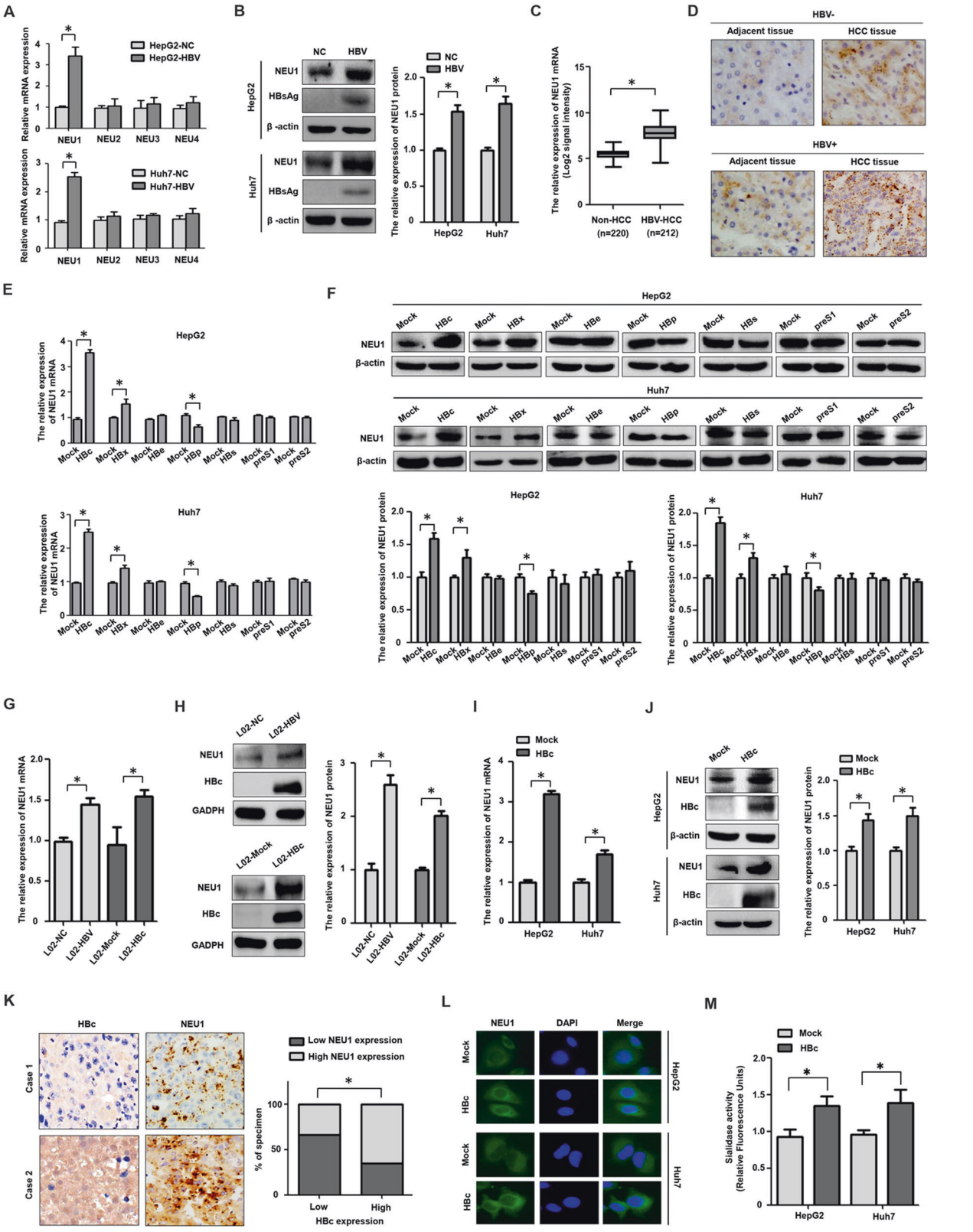
We also measured the location of NEU1 in HBc-positive cells and control cells via immunofluorescence assays. As shown in Fig. 1l, the increased NEU1 protein induced by HBc was found to be mainly located in the cytoplasm in both HepG2 and Huh7 cells.

Because NEU1 is a type of sialidase and the expression of NEU1 is elevated in HBc-positive hepatoma cells, we were interested in exploring whether sialidase activity is increased in HBc-positive hepatoma cells. Expectedly, compared with control cells, elevated sialidase activity was found in HBc-positive HepG2 and Huh7 cells (Fig. 1m).

HBc promotes activity of the NEU1 promoter via the NF-κB pathway in hepatoma cells

To explore the mechanisms associated with HBc-mediated expression of NEU1, five NEU1 promoters with different lengths, specifically, −1849/+100 [PGL3-P(−1849/+100)], −1398/+100 [PGL3-P(−1398/+100)], −724/+100 [PGL3-P(−724/+100)], −469/+100 [PGL3-P(−469/+100)], and −168/+100 [PGL3-P(−168/+100)], were cloned into PGL-3 vectors. Hepatoma cells were cotransfected with these vectors along with HBc or control plasmids for 24 h to investigate the role of HBc in activation of different fragments of the NEU1 promoter. The results (Fig. 2a) showed that HBc could activate PGL3-P(−1849/+100) and PGL3-P(−1398/+100) but not PGL3-P(−724/+100), PGL3-P(−469/+100) or PGL3-P(−168/+100). These results suggest that the regulatory site mediated by HBc is located in the (−1398/−724) region of the NEU1 promoter.

Next, the transcription factor binding sites in the (−1398/−724) promoter region of NEU1 were predicted with AliBaba2.0 and TFSEARCH [17]. We found that two potential binding NF-κB sites were located in the (−1398/−724) promoter region (Fig. 2b). HBc has been shown to transactivate the NF-κB pathway to mediate the expression of IL-6 [26], and we speculated that HBc may activate the NEU1 promoter via the NF-κB pathway. Activation of the NF-κB pathway is associated with increased phosphorylation of P65, degradation of IkBα, and transfer of P65 into the nucleus of target cells [27]. We detected P65 phosphorylation, IkBα expression, and the location of P65 in HBc-positive cells and control cells. As shown in Fig. 2c, d, e, P65 phosphorylation was elevated, IkBα expression was reduced, and the level of nuclear P65 was increased in



HBc-positive HepG2 and Huh7 cells compared with control cells. In summary, these results indicate that HBc can promote activation of the NF-κB pathway in hepatoma cells.

Furthermore, we observed that the promoter activity of PGL3-P (−1398/+100) mediated by HBc was decreased when any one of the two potential NF-κB binding site

◀ **Fig. 1 The role of HBc in NEU1 expression in HBV-related hepatoma cells. a** The role of HBV in the expression of human sialidases in hepatoma cells at the mRNA level detected with real-time PCR. **b** The expression of NEU1 protein mediated by HBV measured via western blot analysis. **c** Bioinformatics analysis of NEU1 expression in HBV-related HCC tissues and non-HCC tissues using GSE14520 microarray data. **d** The expression of NEU1 protein in HBV-negative adjacent tissues ($n = 10$), HBV-negative HCC tissues ($n = 30$), HBV-positive adjacent tissues ($n = 30$), and HBV-related tumor tissues ($n = 50$) was assessed via IHC analysis (×200). **e** The role of encoding genes in the HBV genome in NEU1 mRNA expression in hepatoma cells. **f** The role of encoding genes in the HBV genome in NEU1 protein expression in HepG2 and Huh7 cells. **g** The role of HBV and HBc in NEU1 mRNA expression in L02 cells. **h** The role of HBV and HBc in NEU1 protein expression in L02 cells. **i** The expression of NEU1 mRNA in HBc stably transfected hepatoma cells. **j** The expression of NEU1 protein in HBc-positive hepatoma cells. **k** The expression of NEU1 and HBc proteins in HBV-related tumor tissues assessed via IHC analysis (×200). **l** The expression of NEU1 in HBc-positive cells and control cells assessed with immunofluorescence assays. **m** Sialidase activity in HBc-positive cells and control cells. HepG2-NC, Huh7-NC: HepG2 and Huh7 cells transfected with control plasmids. HepG2-HBV, Huh7-HBV: HepG2 and Huh7 cells transfected with HBV plasmids. HepG2-Mock, Huh7-Mock: HepG2 and Huh7 cells transfected with control plasmids. HepG2-HBc, Huh7-HBc: HepG2 and Huh7 cells transfected with HBc plasmids. $^*P < 0.05$.

sequences (−1368/−1354 and −1116/−1105) were mutated in hepatoma cells (Fig. 2b, f). To further explore whether HBc activates the NEU1 promoter via the NF-κB pathway, the NF-κB pathway inhibitor BAY11-7082 was administered to HBc-positive hepatoma cells. The results showed that BAY11-7082 significantly suppressed the activity of the NEU1 promoter (Fig. 2g). Next, HBc-positive hepatoma cells were transfected with specific shRNA plasmids targeting P65 (Fig. 2h), and the inhibition of P65 was found to significantly decrease the activity of the NEU1 promoter in HBc-positive cells (Fig. 2i). Furthermore, after HBc-positive hepatoma cells were treated with P65 shRNA or BAY11-7082, the expression of NEU1 mediated by HBc was downregulated (Fig. 2j, k). Taken together, these results suggest that the expression of NEU1 regulated by HBc was dependent on the NF-κB pathway in hepatoma cells.

NEU1 expression mediated by HBc facilitates activation of signaling pathways and EMT in hepatoma cells

Current studies indicate that NEU1 contributes to activation of the ERK pathway, the NF-κB pathway, and EMT [28–30]. We detected whether NEU1 could activate ERK, the NF-κB pathway and EMT in hepatoma cells by transfecting HepG2 and Huh7 cells with NEU1 plasmids or control plasmids. As shown in Fig. 3a, compared with control cells, transfection of hepatoma cells with NEU1 increased the sialidase activity in hepatoma cells. Furthermore, increased expression of phosphorylated ERK, enhanced phosphorylation of P65, decreased expression of IkBα, and elevated expression of nuclear P65 were found in NEU1 overexpressing HepG2 and Huh7 cells compared with control cells (Fig. 3b, c). In addition, compared with the results in control cells, NEU1 induced morphological changes in HepG2 cells, leading to development of a spindle-like morphology. However, the morphological changes in NEU1-positive Huh7 cells compared with control cells were not significant (Fig. 3d). Increased β-catenin and vimentin expression and decreased E-cadherin expression (EMT markers) were observed in NEU1 overexpressing HepG2 and Huh7 cells compared with control cells (Fig. 3e).

We next analyzed, based on NEU1, whether HBc could activate the ERK and NF-κB pathways. To better explore the role of NEU1 in HBc-positive cells, a NEU1 shRNA plasmid was constructed, and we found that NEU1 shRNA significantly inhibited the expression of NEU1 protein in HBc-positive cells (Fig. 3f). In addition, we found that sialidase activity was decreased after NEU1 was knocked down in HBc-expressing hepatoma cells (Fig. 3g). In HBc-expressing hepatoma cells, the phosphorylation level of ERK was found to be increased (Fig. 3h). Increased activation of P65, decreased expression of IkBα, and elevated expression of P65 in the nucleus were also found in HBc-positive cells (Fig. 3i–k). These results indicate that HBc can promote activation of the NF-κB pathway in hepatoma cells. After treatment of HBc-positive cells with NEU1 shRNA, the phosphorylation levels of ERK and P65 were significantly decreased, the expression of IkBα was elevated, and the expression of P65 in the nucleus was reduced in HepG2 and Huh7 cells (Fig. 3h–k). These results suggest that the activation of the ERK and NF-κB pathways mediated by HBc was primarily dependent on NEU1 in hepatoma cells.

We also used the NEU1 inhibitor OP to investigate the role of NEU1 in the activation of the ERK and NF-κB pathways in HBc-positive cells. First, we examined the inhibitory function of OP on sialidase activity in HBc-positive hepatoma cells. As shown in Fig. 4a, the sialidase activity was decreased after HBc-expressing hepatoma cells were treated with OP. Furthermore, after the HBc-positive cells were treated with OP, the phosphorylation of ERK and P65 significantly declined, the expression of IkBα increased, and the expression of nuclear P65 decreased (Fig. 4b–e).

We next measured whether HBc could induce the expression of NEU1 to promote EMT in hepatoma cells. We first detected whether HBc could induce a morphology change in HepG2 and Huh7 cells. As shown in Figs. 3l and 4f, compared with the results in control cells,

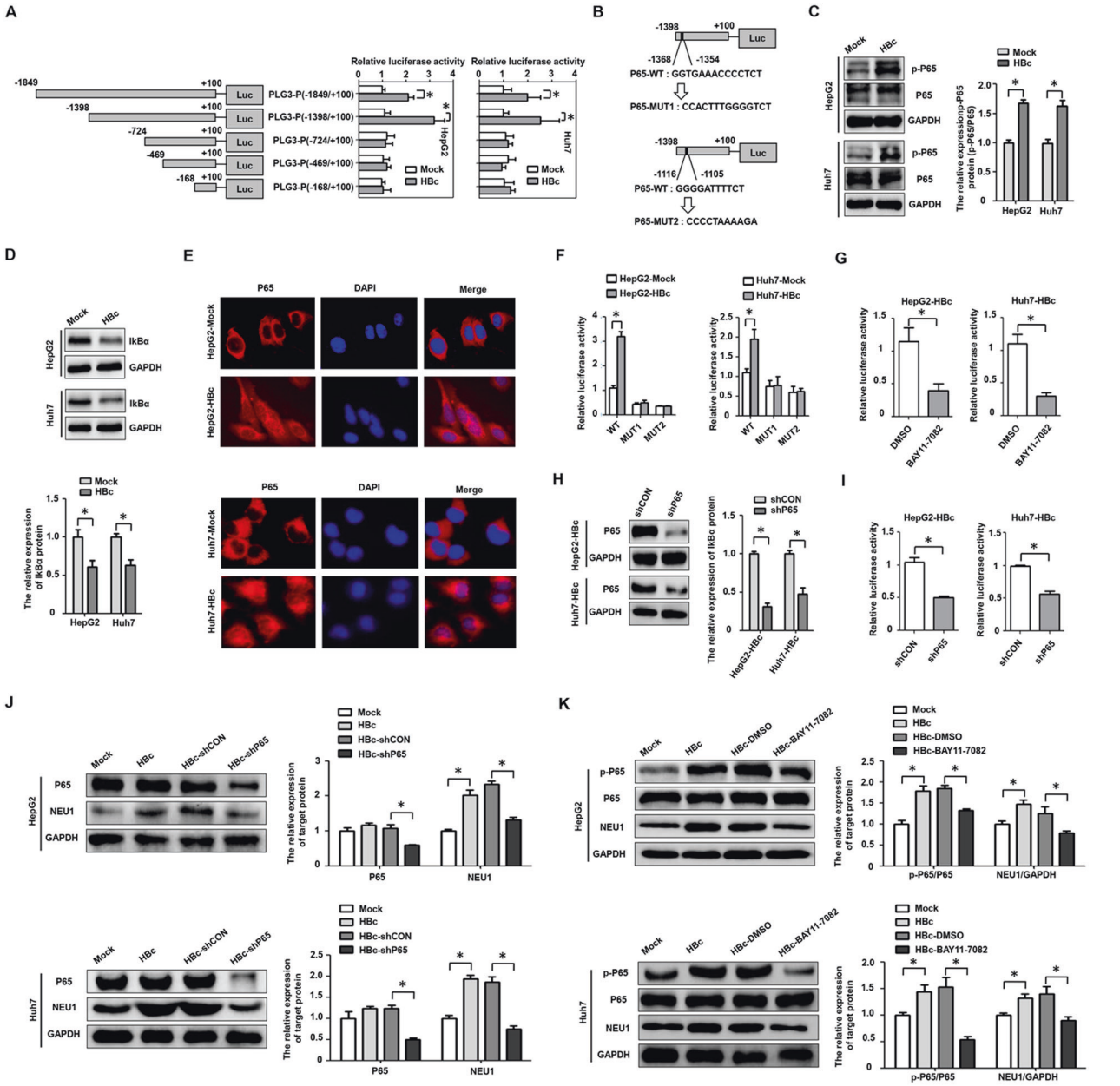


Fig. 2 The effect of the NF-κB pathway in activation of the NEU1 promoter mediated by HBc in hepatoma cells. a Identification of the regulatory region of the NEU1 promoter influenced by HBc via a luciferase reporter gene assay. **b** Information regarding the NF-κB binding site in the core region of the NEU1 promoter and associated mutations in the PGL3-P (−1398/+100) plasmid. **c** The activation of P65 in HBc-positive cells and control cells. **d** The expression of IkBα in HBc-positive cells and control cells. **e** The location of P65 in HBc-positive cells and control cells. **f** The influence of mutation in the NF-κB binding site on activation of the core region of the NEU1 promoter in the PGL3-P (−1398/+100) plasmid. **g** The effect of inhibition of NF-κB via BAY11-7082 on the activity of the core region of the NEU1 promoter in the PGL3-P (−1398/+100) plasmid in HBc-positive hepatoma cells. **h** The effect of P65 inhibition using shRNA plasmids on the expression of P65 protein in HBc-positive hepatoma cells. **i** The effect of P65 inhibition via shRNA plasmids on the activity of the core region of the NEU1 promoter in PGL3-P (−1398/+100) plasmid in HBc-positive hepatoma cells. **j** The effect of P65 inhibition using shRNA plasmids on NEU1 protein expression in hepatoma cells. **k** The effect of P65 inhibition via BAY11-7082 on NEU1 protein expression in hepatoma cells. $*P < 0.05$.

morphological changes in HepG2 cells carrying HBc were found. The cells were elongated and had a spindle-like morphology, as induced by NEU1. However, morphological changes in the HBc-positive Huh7 cells compared with control cells were not obvious. We also examined changes in the expression of the epithelial and mesenchymal markers E-cadherin, β-catenin, and vimentin in HepG2 and Huh7 cells. As shown in Figs. 3m and 4g, HBc induced EMT,

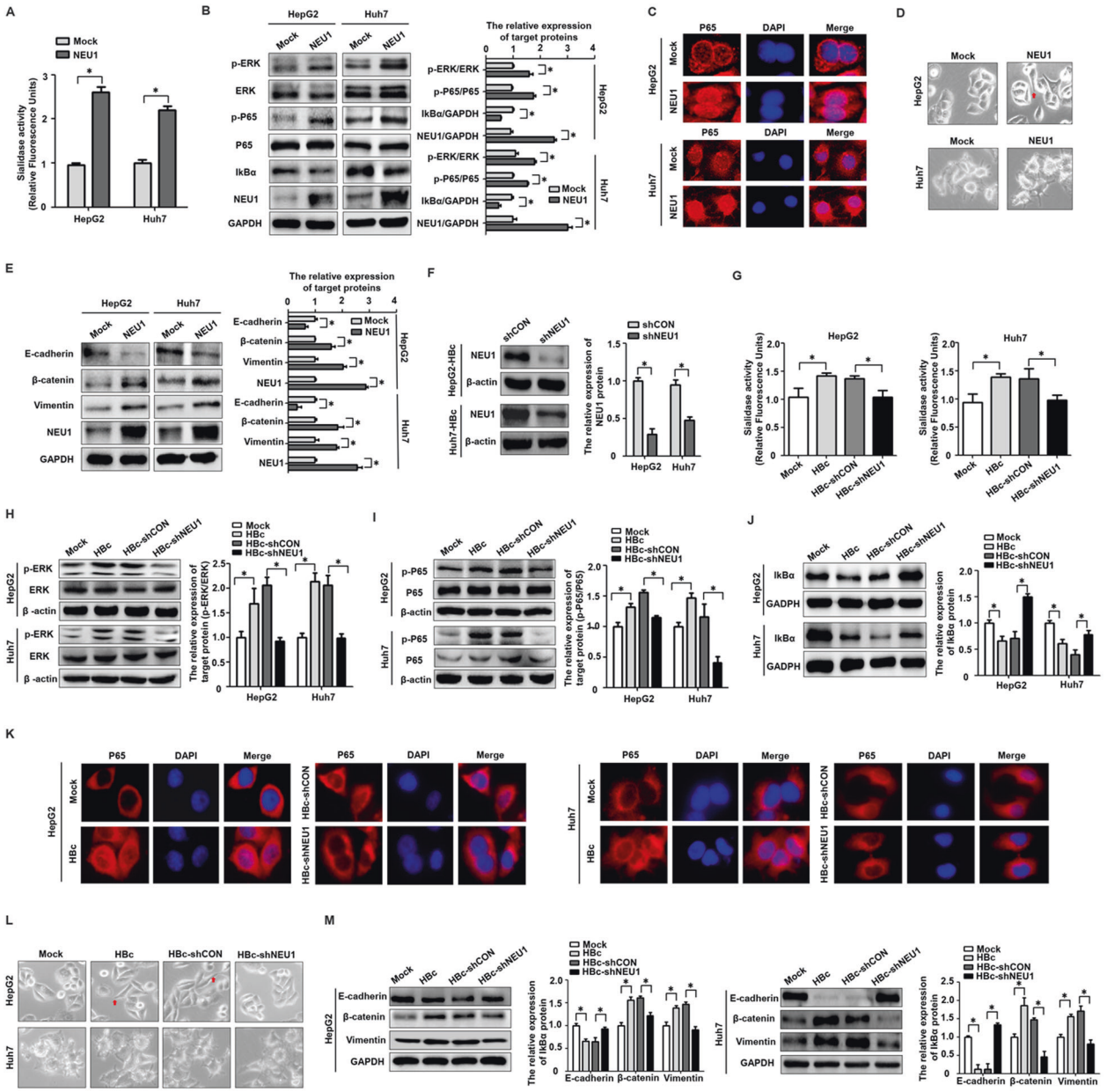
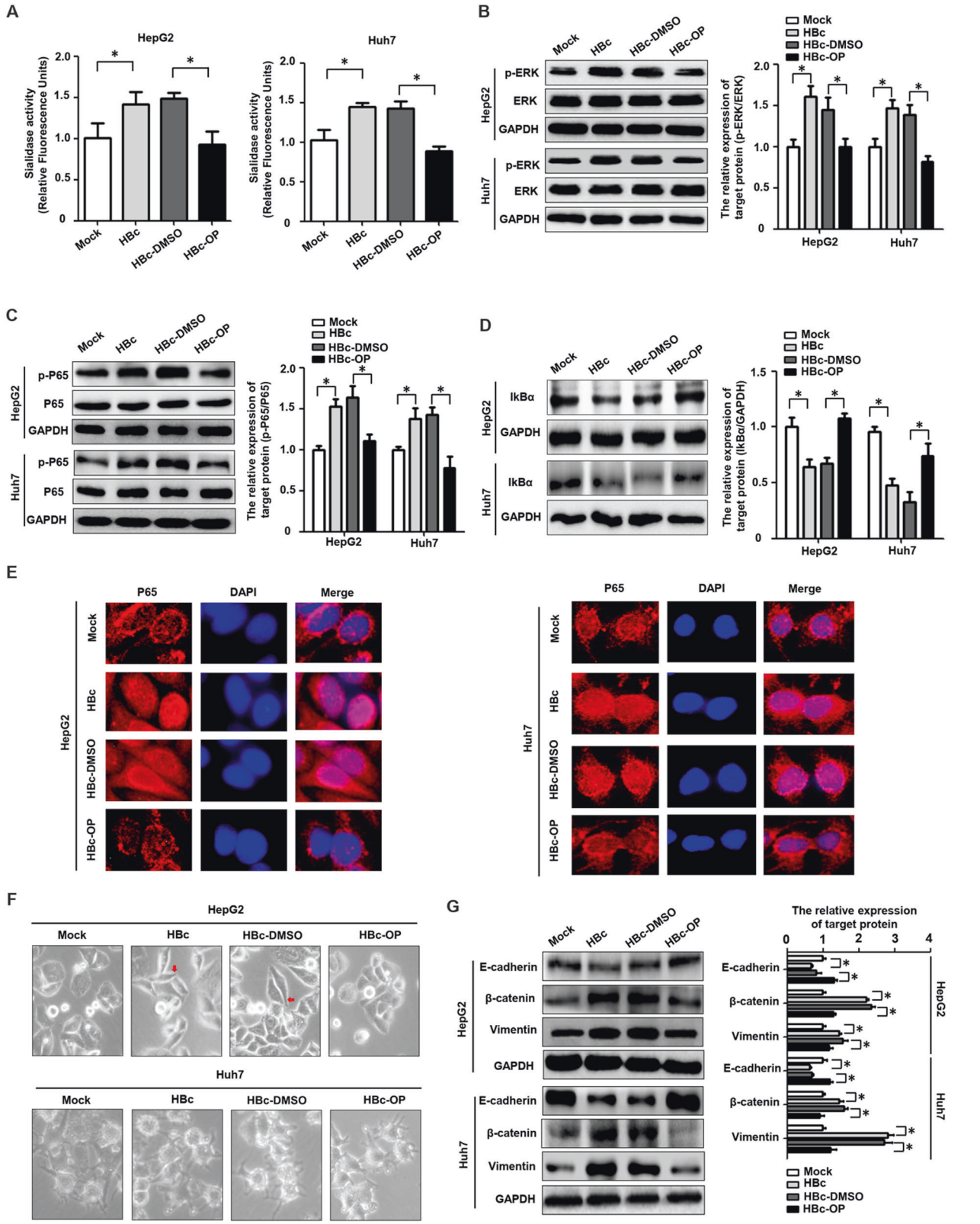


Fig. 3 The role of NEU1 inhibition via shRNA in intracellular signaling pathways and EMT in HBc-positive hepatoma cells. a The role of NEU1 in sialidase activity in hepatoma cells. **b** The role of NEU1 in activation of ERK and P65 and in the expression of IkBα in hepatoma cells. **c** The role of NEU1 in the expression of nuclear P65 in hepatoma cells. **d** The effect of NEU1 on morphological change in hepatoma cells (×400). **e** The effect of NEU1 on the expression of EMT markers in hepatoma cells. **f** The effect of NEU1 inhibition mediated by shRNA on the expression of NEU1 protein in HBc-positive hepatoma cells. **g** The effect of inhibition of NEU1 expression induced by shRNA on sialidase activity mediated by HBc in hepatoma cells. **h** The effect of inhibition of NEU1 expression mediated by shRNA on activation of the ERK pathway in hepatoma cells stably transfected with HBc. **i** The effect of inhibition of NEU1 expression mediated by shRNA on P65 activation in HBc-positive hepatoma cells. **j** The effect of inhibition of NEU1 expression mediated by shRNA on IkBα expression in HBc-positive hepatoma cells. **k** The effect of inhibition of NEU1 expression mediated by shRNA on P65 expression in the nucleus in HBc-positive hepatoma cells. **l** The effect of NEU1 inhibition mediated by shRNA on morphological changes in HBc-positive hepatoma cells (×400). **m** The effect of NEU1 inhibition mediated by shRNA on EMT markers in hepatoma cells mediated by HBc. Mock: cells transfected with control plasmid. HBc: cells transfected with HBc plasmid. HBc-shCON: HBc-positive cells treated with shRNA control plasmids. HBc-shNEU1: HBc-positive cells treated with shRNA plasmids targeting NEU1. $*P < 0.05$. The red arrow shows the changes in hepatoma cells that developed a spindle-like morphology.

with increased β-catenin and vimentin and decreased E-cadherin, in both HepG2 and Huh7 cells. When the cells were treated with NEU1 shRNA or OP, the morphological changes induced by HBc in HepG2 cells were restored (Figs. 3l and 4f). Furthermore, in both HepG2 and Huh7 cells, after HBc-positive cells were treated with



NEU1 shRNA or OP, the expression of β-catenin and vimentin decreased, while the level of E-cadherin significantly increased (Figs. 3m and 4g). In summary, these results suggest that NEU1 plays a positive role in EMT in hepatoma cells and that HBc-mediated EMT in hepatoma cells was mainly dependent on NEU1.

◀ **Fig. 4 The effect of NEU1 inhibition by OP on intracellular signaling pathways and EMT in HBc-positive hepatoma cells. a** The effect of inhibition of NEU1 expression by OP on sialidase activity mediated by HBc in hepatoma cells. **b** The effect of inhibition of NEU1 function by OP on activation of the ERK pathway in hepatoma cells stably transfected with HBc. **c** The effect of inhibition of NEU1 function mediated by OP on activation of P65 in HBc-positive hepatoma cells. **d** The effect of NEU1 inhibition mediated by OP on the expression of IkBα in HBc-positive hepatoma cells. **e** The effect of inhibition of NEU1 expression mediated by OP on P65 expression in the nucleus in HBc-positive hepatoma cells. **f** The effect of inhibition of NEU1 mediated by OP on morphological changes in HBc-positive hepatoma cells (×400). **g** The effect of NEU1 inhibition mediated by OP on EMT markers in hepatoma cells mediated by HBc. Mock: cells transfected with control plasmid. HBc: cells transfected with HBc plasmid. HBc-DMSO: HBc-positive cells treated with DMSO. HBc-OP: HBc-positive cells treated with OP. $*P < 0.05$. The red arrow shows the changes in hepatoma cells that developed a spindle-like morphology.

NEU1 promotes the proliferation and migration of hepatoma cells induced by HBc

HBc has been reported to be involved in the growth and metastasis of hepatoma cells [8, 10]. In addition, recent studies have indicated that NEU1 plays a vital role in the proliferation and migration of tumor cells of different types [13, 30]. We examined whether NEU1 was associated with the proliferation and migration of hepatoma cells mediated by HBc. The results of cell viability and plate clonal formation assays suggested that HBc-positive cells exhibited higher proliferation efficiency than control cells (Fig. 5). After HBc-positive cells were treated with NEU1 shRNA, the cellular proliferation mediated by HBc in hepatoma cells was reduced (Fig. 5a, b). In addition, we added OP into the medium of HBc-positive cells to inhibit the function of NEU1. After blocking of the function of NEU1, the proliferation of HBc-expressing hepatoma cells was suppressed (Fig. 5c, d).

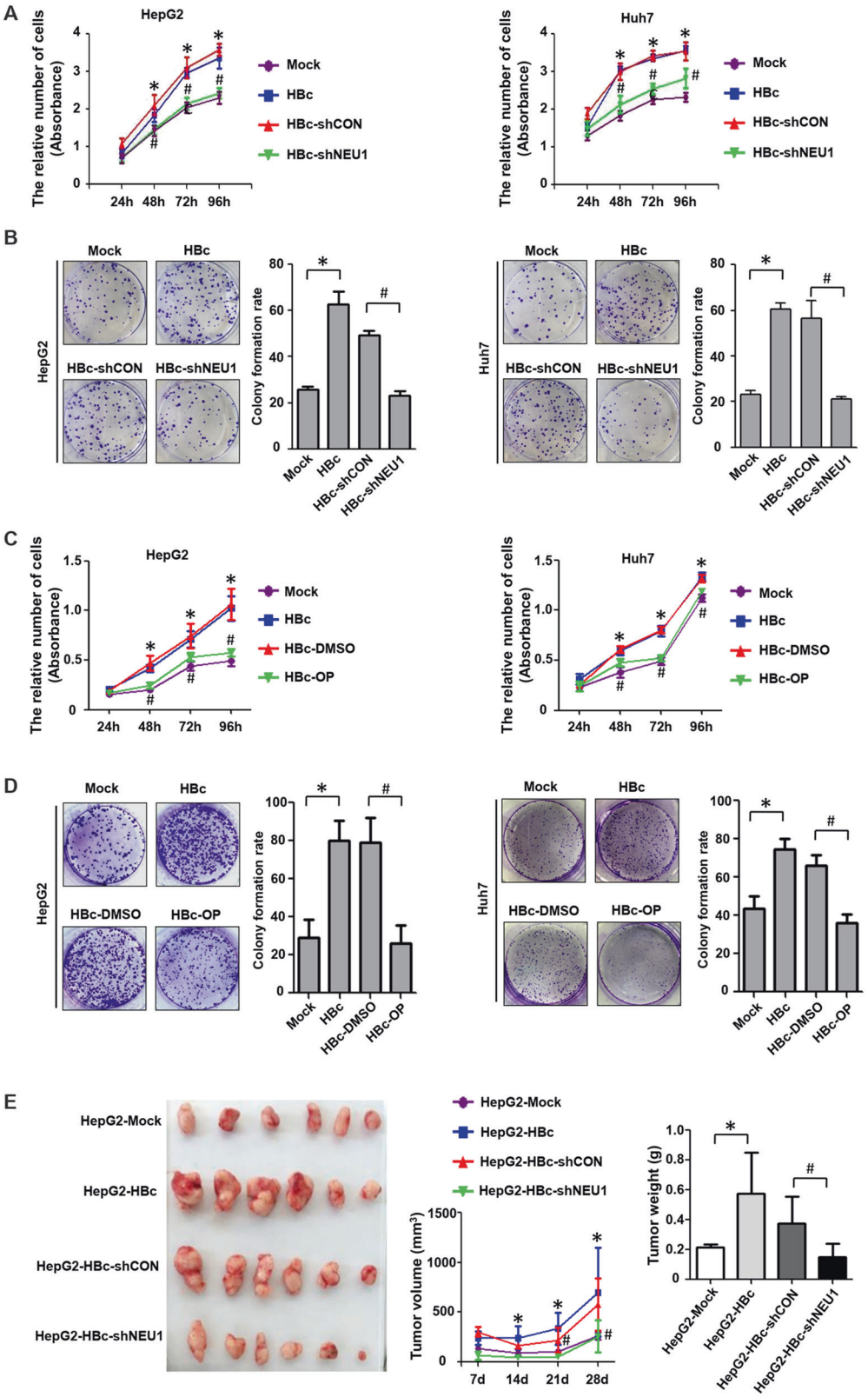
We also explored the effect of NEU1 on the development of hepatoma cells in vivo. After HepG2-HBc cells were treated with NEU1 shRNA or control plasmids for 48 h, the cells were subcutaneously injected into BALB/c nude mice. As shown in Fig. 5e, the volume and weight of the HepG2-HBc tumors were greater than those of control tumors. After HepG2-HBc cells were transfected with shRNA targeting NEU1, the ability of HepG2-HBc cells to form tumors in nude mice was significantly lower than that of cells transfected with shRNA control plasmids (Fig. 5e). Taken together, these findings suggest that NEU1 facilitates the development of hepatoma cells mediated by HBc in vivo.

Subsequently, we used transwell and wound healing assays to investigate the role of NEU1 in cell migration mediated by HBc. Our results showed that HBc promoted the migration of hepatoma cells (Fig. 6). When HBc-positive cells were treated with NEU1 shRNA or OP, the migration of hepatoma cells mediated by HBc was suppressed (Fig. 6). Taken together, these results indicate that NEU1 upregulation is involved in the proliferation and migration of hepatoma cells mediated by HBc.

Discussion

NEU1 is reported to be related to HCC with HBV infection. However, the biological function and associated mechanisms of NEU1 mediated by HBV in HCC are not well elucidated. In this study, we found that HBV facilitated the expression of NEU1 via HBc in hepatoma cells. The HBc-mediated upregulation of NEU1 was mainly dependent on the NF-κB pathway. Furthermore, NEU1 was found to promote the activation of intracellular signaling pathways and EMT and to contribute to the proliferation and migration of hepatoma cells mediated by HBc.

Although epidemiological evidence demonstrates that HBV contributes to the development of HCC, the molecular mechanism of oncogenesis mediated by HBV is still not well elucidated. Among the proteins encoded in the HBV genome, HBc is viewed as a viral structural protein that can self-assemble to form the viral nucleocapsid and participates in multiple steps in the HBV life cycle [4, 31]. Apart from these functions, accumulated evidence demonstrates that HBc interacts with various cellular factors to regulate different biological processes during HBV infection [4]. Importantly, HBc is also involved in the development of HCC mediated by the virus [8, 10]. In the present study, we found that HBV promoted the expression of NEU1 and that the role of HBV in NEU1 expression was dependent on HBc. As a member of the sialidase superfamily, NEU1 can cleave sialic acids from glycoconjugates and induce structural changes and alterations in the molecular function of substrate proteins [12]. Consistent with the increased expression of NEU1, we found that sialidase activity was also elevated in hepatoma cells carrying HBc. A previous study showed that NEU1 molecules are located on the cell surface and inside cells [32]. Based on immunofluorescence analysis, we found that the increased NEU1 protein mediated by HBc was mainly located in the cytoplasm in hepatoma cells. In addition, NEU1 has been found to be expressed in a variety of tissues and controls various biological processes, including cell proliferation, differentiation, and apoptosis [11, 12]. Moreover, aberrant expression of NEU1 has been reported in different tumors, and NEU1 was found to exert important biological functions during the generation and progression of tumors [11]. The results of our study indicate that HBc may upregulate NEU1 to facilitate HCC development.



Our study results showed that HBc induced the expression of NEU1 at the mRNA and protein level. In addition, previous studies have demonstrated that HBc disrupts the expression of target genes by regulating activation of the respective promoters via specific transcription factors, including c-Ets2 and CREB [7, 33].

◀ **Fig. 5 The role of NEU1 in the proliferation of hepatoma cells induced by HBc. a** The effect of inhibition of NEU1 expression using shRNA on the proliferation of HBc-positive hepatoma cells detected with CCK-8 assays. **b** The effect of inhibition of NEU1 expression via shRNA on the proliferation of HBc-positive hepatoma cells assessed with plate clone formation assays. **c** The effect of inhibition of NEU1 function using OP on the proliferation of HBc-positive hepatoma cells detected with CCK-8 assays. **d** The effect of inhibition of NEU1 function with OP on the proliferation of HBc-positive hepatoma cells as examined with plate clone formation assays. **e** The role of NEU1 in the proliferation of hepatoma cells mediated by HBc in nude mice. Mock: cells transfected with control plasmid. HBc: cells transfected with HBc plasmid. HBc-shCON: HBc-positive cells transfected with shRNA control plasmid. HBc-shNEU1: HBc-positive cells transfected with shRNA plasmid targeting NEU1. $^{*}P < 0.05$, the Mock group compared with the HBc group; $^{\#}P < 0.05$, the HBc-shCON group compared with the HBc-shNEU1 group.

Therefore, we speculated that HBc might modulate the expression of NEU1 via activation of the NEU1 promoter in a manner dependent on specific transcription factors. To better understand the molecular mechanisms associated with the upregulation of NEU1 mediated by HBc, the NEU1 promoter was cloned, and the results showed that HBc was capable of activating the NEU1 promoter. In addition, bioinformatics analysis of transcription factor binding sites revealed that two potential NF-κB binding sites were present in the core region of the NEU1 promoter. In hepatoma cells, we found that HBc could activate the NF-κB pathway. Interestingly, based on site mutation in potential NF-κB binding sites, we found that both two potential NF-κB binding sites could control the activation of NEU1 promoters. The results suggest that the transcription factor associated with the NF-κB pathway can interact with no less than one binding site to control expression of the NEU1 gene. Moreover, via the use of an inhibitor against the NF-κB pathway and shRNA targeting P65, we further demonstrated that HBc was capable of promoting NEU1 expression via the NF-κB pathway. In addition, Chen Z et al. showed that HBc was capable of inducing IL-6 expression through the NF-κB pathway [26]. These results indicate that by inducing the expression of multiple genes, including NEU1 and IL-6, the NF-κB pathway might play important roles in modulating various biological functions to promote the development of HCC mediated by HBc. In addition, although multiple transcription factor binding sites were found in the NEU1 promoter and some overlap with NF-kB binding sites was observed based on bioinformatics analysis, we only focused on two potential NF-κB binding sites in the NEU1 promoter in the present study. To achieve a more comprehensive understanding of the factors associated with the mechanisms that participate in the activation of the NEU1 promoter mediated by HBc, further work is needed in the future to explore the role of other predicted transcription factor binding sites.

NEU1 activation increases the phosphorylation levels of downstream signaling pathways, including ERK and NF-κB, to regulate various cellular functions in different cells [28, 29]. In the present study, we found that NEU1 could also promote activation of the ERK and NF-κB pathways in hepatoma cells. In addition, previous studies have indicated that HBc can activate the ERK and NF-κB pathways [26], but how HBc activates these two signaling pathways is still unknown. Our results indicate that HBc activates the ERK and NF-κB pathways through NEU1. In addition, O'Shea et al. reported that NEU1 contributed to EMT in pancreatic cancer cells [30]. We speculated that HBc might activate EMT via NEU1. Our results indicate that HBc and NEU1 can induce changes in cellular morphology and the expression of EMT markers in HepG2 cells. However, in Huh7 cells, HBc and NEU1 only induced a significant change in the expression of EMT markers but not in cellular morphology. These results indicate that the role of HBc and NEU1 in inducing EMT is heterogeneous among different cells. Current research indicates that certain proteins, such as N-cadherin and GCNT2, are essential for behavioral but not morphological changes during EMT in target cells [34, 35], and our results suggest that HBc and NEU1 cannot mediate morphological changes but can induce a change in cellular behavior by inducing EMT in Huh7 cells. Furthermore, consistent with our speculation, we found that EMT mediated by HBc was dependent on NEU1 expression in hepatoma cells.

Several studies have demonstrated that NEU1 participates in the proliferation and migration of different tumor cells [13, 36]. In addition, HBc has been reported to enhance the proliferation and migration of hepatoma cells [8, 10]. Consistent with previous reports, our results demonstrated that HBc increased the proliferation and migration of HepG2 and Huh7 cells. Furthermore, after the treatment of HBc-positive cells with NEU1 shRNA or inhibitor OP, we observed that the proliferation and migration of hepatoma cells were significantly reduced. These data indicate that NEU1 plays an important role in the proliferation and migration of hepatoma cells induced by HBc.

In conclusion, we found that HBc increased the expression of NEU1 via the NF-κB pathway to increase the activation of downstream signaling pathways and EMT and to contribute to the proliferation and migration of HBV-associated hepatoma cells. Our data broaden the understanding of the function and associated molecular mechanisms of NEU1 in HCC with HBV infection and may lay a foundation for providing a new therapeutic target for HBV-related HCC.

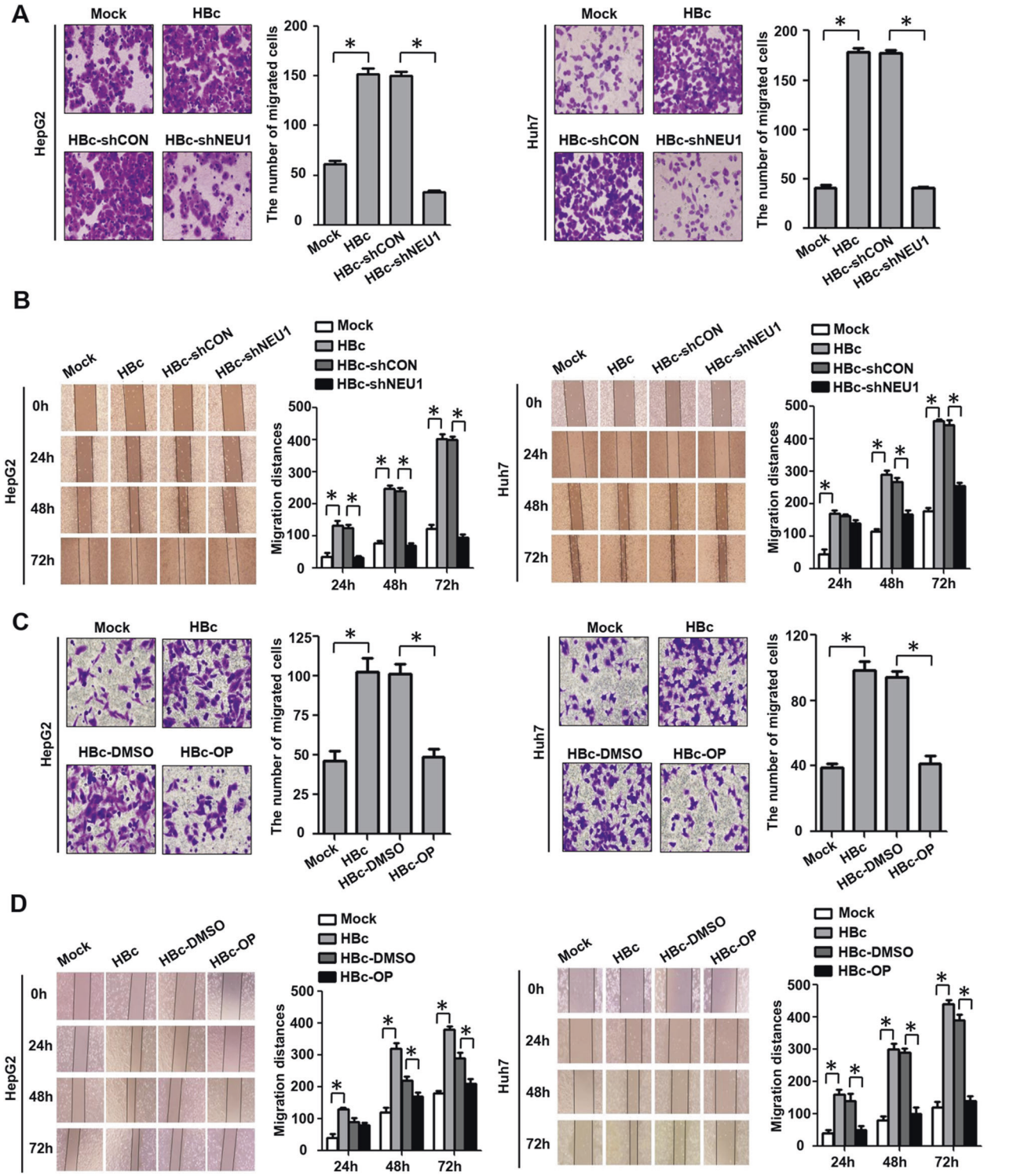


Fig. 6 The effect of NEU1 on the migration of hepatoma cells mediated by HBc. a The effect of inhibition of NEU1 expression via shRNA on the migration of HBc-positive hepatoma cells detected with transwell assays. **b** The effect of inhibition of NEU1 expression using shRNA on the migration of HBc-positive hepatoma cells examined with wound healing assays. **c** The effect of inhibition of NEU1 function using OP on the migration of HBc-positive hepatoma cells as detected with transwell assays. **d** The effect of inhibition of NEU1 function using OP on the migration of HBc-positive hepatoma cells as examined with wound healing assays. $^{*}P < 0.05$.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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References

1. Xu W, Yu J, Wong VW. Mechanism and prediction of HCC development in HBV infection. Best Pract Res Clin Gastroenterol. 2017;31:291–8.
2. Wang M, Xi D, Ning Q. Virus-induced hepatocellular carcinoma with special emphasis on HBV. Hepatol Int. 2017;11:171–80.
3. Levrero M, Zucman-Rossi J. Mechanisms of HBV-induced hepatocellular carcinoma. J Hepatol. 2016;64:S84–101.
4. Diab A, Foca A, Zoulim F, Durantel D, Andrisani O. The diverse functions of the hepatitis B core/capsid protein (HBc) in the viral life cycle: implications for the development of HBc-targeting antivirals. Antiviral Res. 2018;149:211–20.
5. Du J, Liang X, Liu Y, Qu Z, Gao L, Han L, et al. Hepatitis B virus core protein inhibits TRAIL-induced apoptosis of hepatocytes by blocking DR5 expression. Cell Death Differ. 2009;16:219–29.
6. Liu W, Lin YT, Yan XL, Ding YL, Wu YL, Chen WN, et al. Hepatitis B virus core protein inhibits Fas-mediated apoptosis of hepatoma cells via regulation of mFas/FasL and sFas expression. FASEB J. 2015;29:1113–23.
7. Gai X, Zhao P, Pan Y, Shan H, Yue X, Du J, et al. Hepatitis B virus core protein enhances human telomerase reverse transcriptase expression and hepatocellular carcinoma cell proliferation in a c-Ets2-dependent manner. Int J Biochem Cell Biol. 2013;45:1174–85.
8. Liu W, Guo TF, Jing ZT, Yang Z, Liu L, Yang YP, et al. Hepatitis B virus core protein promotes hepatocarcinogenesis by enhancing Src expression and activating the Src/PI3K/Akt pathway. FASEB J. 2018;32:3033–46.
9. Xie Q, Fan F, Wei W, Liu Y, Xu Z, Zhai L, et al. Multi-omics analyses reveal metabolic alterations regulated by hepatitis B virus core protein in hepatocellular carcinoma cells. Sci Rep. 2017;7:41089.
10. Du J, Bai F, Zhao P, Li X, Gao L, Ma C, et al. Hepatitis B core protein promotes liver cancer metastasis through miR-382-5p/DLC-1 axis. Biochim Biophys Acta Mol Cell Res. 2018;1865:1–11.
11. Haxho F, Neufeld RJ, Szewczuk MR. Neuraminidase-1: a novel therapeutic target in multistage tumorigenesis. Oncotarget. 2016;7:40860–81.
12. Glanz VY, Myasoedova VA, Grechko AV, Orekhov AN. Sialidase activity in human pathologies. Eur J Pharmacol. 2019;842:345–50.
13. Hou G, Liu G, Yang Y, Li Y, Yuan S, Zhao L, et al. Neuraminidase 1 (NEU1) promotes proliferation and migration as a diagnostic and prognostic biomarker of hepatocellular carcinoma. Oncotarget. 2016;7:64957–66.
14. Wang W, Peng JX, Yang JQ, Yang LY. Identification of gene expression profiling in hepatocellular carcinoma using cDNA microarrays. Dig Dis Sci. 2009;54:2729–35.
15. Amaddeo G, Cao Q, Ladeiro Y, Imbeaud S, Nault JC, Jaoui D, et al. Integration of tumour and viral genomic characterizations in HBV-related hepatocellular carcinomas. Gut. 2015;64:820–9.
16. Kong F, You H, Zhao J, Liu W, Hu L, Luo W, et al. The enhanced expression of death receptor 5 (DR5) mediated by HBV X protein through NF-kappaB pathway is associated with cell apoptosis induced by (TNF-alpha related apoptosis inducing ligand) TRAIL in hepatoma cells. Virol J. 2015;12:192.
17. You H, Kong F, Zhou K, Wei X, Hu L, Hu W, et al. HBX protein promotes LASP-1 expression through activation of c-Jun in human hepatoma cells. J Cell Physiol. 2018;233:7279–91.
18. Kong F, Hu W, Zhou K, Wei X, Kou Y, You H, et al. Hepatitis B virus X protein promotes interleukin-7 receptor expression via NF-kappaB and Notch1 pathway to facilitate proliferation and migration of hepatitis B virus-related hepatoma cells. J Exp Clin Cancer Res. 2016;35:172.
19. Tang R, Kong F, Hu L, You H, Zhang P, Du W, et al. Role of hepatitis B virus X protein in regulating LIM and SH3 protein 1 (LASP-1) expression to mediate proliferation and migration of hepatoma cells. Virol J. 2012;9:163.
20. Roessler S, Jia HL, Budhu A, Forgues M, Ye QH, Lee JS, et al. A unique metastasis gene signature enables prediction of tumor relapse in early-stage hepatocellular carcinoma patients. Cancer Res. 2010;70:10202–12.
21. Roessler S, Long EL, Budhu A, Chen Y, Zhao X, Ji J, et al. Integrative genomic identification of genes on 8p associated with hepatocellular carcinoma progression and patient survival. Gastroenterology. 2012;142:957–66.
22. Kong FY, Zhu T, Li N, Cai YF, Zhou K, Wei X, et al. Bioinformatics analysis of the proteins interacting with LASP-1 and their association with HBV-related hepatocellular carcinoma. Sci Rep. 2017;7:44017.
23. Kong FY, Wei X, Zhou K, Hu W, Kou YB, You HJ, et al. Bioinformatics analysis reveals distinct molecular characteristics of hepatitis B-related hepatocellular carcinomas from very early to advanced barcelona clinic liver cancer stages. PLoS ONE. 2016;11:e0158286.
24. Wang H, Shi J, Luo Y, Liao Q, Niu Y, Zhang F, et al. LIM and SH3 protein 1 induces TGFbeta-mediated epithelial-mesenchymal transition in human colorectal cancer by regulating S100A4 expression. Clin Cancer Res. 2014;20:5835–47.
25. Chen GY, Brown NK, Wu W, Khedri Z, Yu H, Chen X, et al. Broad and direct interaction between TLR and Siglec families of pattern recognition receptors and its regulation by Neu1. Elife. 2014;3:e04066.
26. Chen Z, Li YX, Fu HJ, Ren YL, Zou L, Shen SZ, et al. Hepatitis B virus core antigen stimulates IL-6 expression via p38, ERK and NF-kappaB pathways in hepatocytes. Cell Physiol Biochem. 2017;41:91–100.
27. Mulero MC, Huxford T, Ghosh G. NF-kappaB, IkappaB, and IKK: integral components of immune system signaling. Adv Exp Med Biol. 2019;1172:207–26.
28. Duca L, Blanchevoye C, Cantarelli B, Ghoneim C, Dedieu S, Delacoux F, et al. The elastin receptor complex transduces signals through the catalytic activity of its Neu-1 subunit. J Biol Chem. 2007;282:12484–91.
29. Natori Y, Nasui M, Edo K, Sato S, Sakurai T, Kizaki T, et al. NEU1 sialidase controls gene expression and secretion of IL-6 and

MCP-1 through NF-kappaB pathway in 3T3-L1 adipocytes. J Biochem. 2017;162:137–43.
30. O'Shea LK, Abdulkhalek S, Allison S, Neufeld RJ, Szewczuk MR. Therapeutic targeting of Neu1 sialidase with oseltamivir phosphate (Tamiflu(R)) disables cancer cell survival in human pancreatic cancer with acquired chemoresistance. Onco Targets Ther. 2014;7:117–34.
31. Mak LY, Wong DK, Seto WK, Lai CL, Yuen MF. Hepatitis B core protein as a therapeutic target. Expert Opin Ther Targets. 2017;21:1153–59.
32. Uemura T, Shiozaki K, Yamaguchi K, Miyazaki S, Satomi S, Kato K, et al. Contribution of sialidase NEU1 to suppression of metastasis of human colon cancer cells through desialylation of integrin beta4. Oncogene. 2009;28:1218–29.
33. Xiang A, Ren F, Lei X, Zhang J, Guo R, Lu Z, et al. The hepatitis B virus (HBV) core protein enhances the transcription activation of CRE via the CRE/CREB/CBP pathway. Antiviral Res. 2015;120:7–15.
34. Maeda M, Johnson KR, Wheelock MJ. Cadherin switching: essential for behavioral but not morphological changes during an epithelium-to-mesenchyme transition. J Cell Sci. 2005;118:873–87.
35. Zhang H, Meng F, Wu S, Kreike B, Sethi S, Chen W, et al. Engagement of I-branching {beta}-1, 6-N-acetylglucosaminyl-transferase 2 in breast cancer metastasis and TGF-{beta} signaling. Cancer Res. 2011;71:4846–56.
36. Ren LR, Zhang LP, Huang SY, Zhu YF, Li WJ, Fang SY, et al. Effects of sialidase NEU1 siRNA on proliferation, apoptosis, and invasion in human ovarian cancer. Mol Cell Biochem. 2016;411:213–9.