Sigma-1 receptor (σ1R) is downregulated in hepatic malignant tumors and regulates HepG2 cell proliferation, migration and apoptosis

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Abstract. Sigma-1 receptor (σ1R), an important transmembrane structural protein, has been demonstrated to be overexpressed in various types of human cancer, and has been confirmed to be involved in many biological behaviors during tumorigenesis and tumor progression. The aim of the present study was to explore the essential role of σ1R in hepatic malignant tumors (HMTs), which, to the best of our knowledge, has not been reported to date. We assessed σ1R expression in hepatocellular carcinoma (HCC) tissues and found that σ1R was significantly decreased in HCC when compared with that in benign liver tissues (P<0.01). Additionally, the expression of σ1R was shown to be inversely correlated with HCC grade (r=-0.424, P=0.021, Kendall's τ-b-test). We further used a FLAG-SV40-neomycin-plasmid strategy to increase σ1R expression in the HepG2 hepatoblastoma cell line. Overexpression of σ1R impaired cell proliferation, inhibited cell migration, induced cell cycle arrest at G1 phase, and increased cell apoptosis *in vitro*. Furthermore, overexpression of σ1R decreased the expression levels of STAT-3 and NF-κB, which provided insight into the underlying mechanisms of σ1R-associated HMT development and progression. These findings suggest that the decreased expression of σ1R plays an essential role in hepatic tumorigenesis, and that it may serve as a potential predictive factor and therapeutic target for the treatment of HMTs.

Introduction

Hepatocellular carcinoma (HCC) and hepatoblastoma (HB) are two common hepatic malignant tumors (HMTs) that typically occur in adults and children, respectively. HCC originates from hepatocytes, while the origin of HB is more complex, as it arises from primary hepatoblasts (1). HCC is the most common type of HMT, and results in 10,000 deaths worldwide every year, most of which occur in Asian countries (2). Additionally, the mortality rate of HCC ranks second among all primary cancer-related mortalities, and as many as 90% of these deaths are related to metastasis (3,4). Viral infection and chronic inflammatory liver diseases are common risk factors for HCC (5). Accurate diagnosis and medical advice in cases of HCC are frequently delayed due to the lack of obvious symptoms in the early stages (6). At present, the methods of diagnosis and treatment for HCC are numerous, but the short survival times of HCC patients indicate the poor prognosis associated with this disease.

Sigma (σ) receptors were initially described by Martin *et al* (7) in 1976 as subtypes of opioid receptors, and have since been reported to have high affinity for many antipsychotic drugs. Pharmacological studies have indicated that there are at least two σ receptor subtypes, of which only the σ1 receptor (σ1R) has been cloned. The cloned σ1R has 223 amino acids and shares 30% identity and 67% similarity with a yeast sterol C8-C7 isomerase (8,9). The σ1R gene encodes a 25.3-kDa protein with two putative transmembrane segments, although it remains unclear whether the N and C termini are cytoplasmic or extracytoplasmic (10,11).

Pharmacological studies have indicated that σ1R binds to a wide range of compounds, including opiates, antipsychotics and neurosteroids. Pentazocine and SKF10047 are two selective agonists for σ1R (12). However, endogenous ligands for σ receptors have not yet been defined. The function of σ1R has been explored by studying its interaction with its ligands. Brent and Pang revealed that certain σ1R ligands were potent inhibitors of cell proliferation in human mammary adenocarcinoma, colon carcinoma and melanoma (13). Meanwhile, the σ1R ligand SKF10047 was found to be effective at modulating cell proliferation in the metastatic breast cancer cell line MDA-MB-231 (14).

Previous studies have found that σ1R is highly expressed in various cancer tissues, including those of neural and non-neural origins (15). Additionally, the upregulation of σ1R was reported to correlate with the biological behavior of tumors,

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such as proliferation, adhesion and cell death (14,16). Our previous study showed that σ1R was overexpressed in human esophageal squamous cell carcinoma and that the overexpression of σ1R was significantly associated with the pathological TNM classification and lymph node metastasis (18). However, the expression of σ1R and its biological relevance in HCC have not yet been identified.

In the present study, we first evaluated the expression status of σ1R in HCC, showing that σ1R expression was decreased with the progression of HCC and was significantly correlated with tumor grade. We then increased σ1R expression in HepG2 cells using a FLAG-SV40-neomycin-plasmid strategy, which indicated that σ1R upregulation impaired cell proliferation and induced cell cycle arrest and cell apoptosis in HepG2 cells. Furthermore, immunoblotting analysis revealed that multiple migration-associated pathways were regulated by σ1R upregulation, providing valuable evidence of σ1R-associated mechanisms in HMT development and progression. Taken together, the present findings strongly suggest a causal relationship between σ1R expression and HMT development, indicating that σ1R may serve as a potential target in research on the pathogenesis and prognosis of HMT.

Materials and methods

Materials and cell lines. HepG2 and SMCC-7721 cell lines, which originate from primary hepatoblasts (19) and mature hepatocytes, respectively, were used in the present study. The human HB cell line HepG2 and the HCC cell line SMCC-7721 were obtained from GeneChem Co. Ltd. (Shanghai, China) and cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), in an atmosphere of 5% CO_2 at 37˚C. Polyclonal anti-σ1R (AP2747A; Abgent, San Diego, CA, USA), anti-NF-κB (8242S; Cell Signaling Technology, Inc., Danvers, MA, USA), anti-STAT-3 (Ab68153; Abcam, Cambridge, UK), and anti-β-actin (SAB5500001; Sigma-Aldrich® Co. LLC, St. Louis, MO, USA) antibodies were used for immunohistochemistry or western blotting. The peroxidase-conjugated anti-rabbit secondary antibody was purchased from Santa Cruz Biotechnology, Inc. (sc-2004; Santa Cruz, CA, USA).

Specimen collection. For the retrospective analysis, archived formalin-fixed, paraffin-embedded human hepatic tissues from 40 patients were obtained from the Department of Pathology of Jining First People's Hospital between 2012 and 2014. The 40 samples included 30 specimens of HCC and 10 specimens of hepatic cavernous hemangioma (HCH). The HCC patients comprised 26 men and 4 women (median age, 59 years). Information regarding sex, age, stage of disease, and histopathological parameters was retrieved from the medical records, and the patient data are summarized in Table I. All the tumors were confirmed as HCC by the pathologists at the Clinical Pathology Department of Jining First People's Hospital. In addition, 26 samples of cirrhotic liver tissue, from ≥2 cm away from the tumor edge, were obtained from the 30 HCC patients to represent precancerous lesions. The evaluation of tumor differentiation was based on histological criteria of the WHO Pathological Classification of Tumors guidelines. The study was approved by the Ethics Committee

Table I. Clinical parameters of the HCC patients.

Clinical parameters	No.
Age (years)	
≤59	15
>59	14
Sex	
Male	25
Female	4
Tumor size (cm)	
≤3	10
3-5	8
>5	11
Differentiation	
G1	10
G2	10
G3	9
AFP (ng/ml)	
≤20	13
>20	16
Hepatitis B surface antigen	
Negative	7
Positive	22
Microvascular invasion	
Absent	17
Present	12
Tumor number	
Single	27
Multiple	2

AFP, α-fetoprotein; HCC, hepatocellular carcinoma.

of the Central Hospital of Jining, the local ethics committee, and only patients who had provided written informed consent were included.

Immunohistochemical staining. The sections were dewaxed in xylene and rehydrated in a series of graded alcohols. Subsequently, the slides were submerged for 10 min in a peroxidase quenching solution containing one part 30% hydrogen peroxide to 9 parts absolute methanol. After rinsing in PBS, antigen retrieval was carried out by autoclaving the tissue in 0.01 M sodium citrate buffer (pH 6.0) at 120˚C for 3 min. Following autoclaving, the sections were blocked in 10% normal goat serum for 10 min at room temperature, and then incubated overnight at 4˚C with an anti-σ1R polyclonal antibody (1:400; Abgent). Subsequently, the sections were subjected to staining with a 2-Step Plus Poly-HRP Anti-Mouse/Rabbit IgG Detection System (PV-9000; ZSGB-BIO, Beijing, China) and a Liquid DAB substrate kit (Invitrogen, Shanghai, China). Samples were rinsed well with distilled water, then counterstained with Mayer's hematoxylin, dehydrated and mounted.

The immunohistochemical staining results were assigned a mean score considering both the intensity of staining and

the proportion of tumor cells showing unequivocal positive reactivity. Each section was independently assessed by two histopathologists without prior knowledge of the patient data. Positive reactions were defined as the presence of brown staining in the cell cytoplasm, nucleus and membrane. For σ1R, a staining index (values 0-12) was determined by multiplying the score for staining intensity (0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining) by the score for the positive stained area (1, positive staining in 0-25% of tumor cells; 2, positive staining in >25-50% of tumor cells; 3, positive staining in 51-75% of tumor cells; 4, positive staining in >75-100% of tumor cells). For the purpose of statistical analyses, scores of 0-4 were considered low expression (-), and scores of 5-12 were considered high expression (+).

Reverse transcription-quantitative PCR (RT-qPCR). Total RNA was extracted and purified from the HepG2 and SMCC-7721 cells using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RT was performed using a RevertAid First Strand cDNA Synthesis kit (Thermo Fisher Scientific, Inc., Waltham, MA, USA) to obtain cDNA. The expression level of σ1R mRNA was measured by RT-qPCR. Primers used were as follows: σ1R, 5'-ACCATCATCTCTGGC ACCTTCC-3' (forward), and 5'-GCCAAAGAGGTAGGT GGTGAGC-3' (reverse); β-actin, 5'-CACCCAGCACAATGA AGATCAAGAT-3' (forward), and 5'-CCAGTTTTTAAATCC TGAGTCAAGC-3' (reverse). PCR amplification consisted of 40 cycles (95°C for 15 sec and 60°C for 60 sec), after an initial denaturation step (95°C for 10 min). The relative expression of σ1R was normalized to β-actin mRNA level using the $2^{-\Delta\Delta Cq}$ method. All samples were examined in triplicate.

Construction of expression vectors and cell transfection. To achieve σ1R overexpression, Lentivector Expression Systems (GeneChem, Shanghai, China) were obtained to construct lentiviruses encoding σ1R, which were then transfected into HepG2 cells. The primers for the σ1R gene were as follows: forward, 5'-ACGGGCCCTCTAGACTCGAGCGCCACCATG CAGTGGGCCGTGGGCCG-3'; and reverse, 5'-AGTCAC TTAAGCTTGGTACCGAAGGGTCCTGGCCAAAGAGGT AGG-3'. The designed DNA sequence was inserted into the lentivirus-based GV141 vector (GeneChem) with *XhoI/KpnI* sites. An empty vector was used as a negative control (NC). For transfection, HepG2 cells were plated into 6-well plates at $2x10^5$ cells per well. After 24 h, cells were transfected with the lentivirus expressing σ1R or the NC lentivirus, and then cultured in a 5% CO_2 incubator at 37°C for another 3 days. Cells were then harvested and total proteins were extracted to determine the overexpression efficiency by western blotting.

Protein extraction and immunoblotting analysis. The total cellular protein was extracted from cells using 2X RIPA buffer containing protease inhibitors. The protein concentration was estimated using the Pierce 660nm protein assay (Thermo Fisher Scientific, Inc.). Equal amounts of tissue lysates (50 µg) were electrophoresed on 12% polyacrylamide gels at 40 V for 30 min, followed by 60 V for 3 h. The lysates were then transferred to polyvinylidene fluoride (PVDF) membranes (EMD Millipore, Bedford, MA, USA). Subsequently, the membranes were blocked in 5% skim milk in PBS-Tween (0.01 M PBS, 0.05% Tween-20) for 1 h at room temperature, followed by the addition of the primary antibody (anti-STAT-3 dilution, 1:1,000; anti-NF-κB dilution, 1:1,000 and anti-β-actin dilution, 1:2,000, respectively) for 2 h at room temperature. After that, the membranes were washed and incubated with peroxidase-conjugated anti-rabbit secondary antibodies (dilution, 1:2,000) and analyzed using a Pierce™ ECL Western Blotting Substrate kit (Thermo Fisher Scientific, Inc.). β-actin was examined as an internal control in this experiment (dilution, 1:2,000).

MTT assay. Cell viability in the σ1R-overexpressing and NC groups was assessed with 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide (MTT), which is converted to a colored, soluble formazan product in metabolically active cells. HepG2 cells ($5x10^3$) were seeded into 96-well plates and incubated for 48 h, after which 10 µl of sterile MTT (5 mg/ml in PBS, pH=7.4) was added to each well and incubated for 4 h. The medium was then removed from the wells and replaced with 150 µl dimethyl sulfoxide (DMSO) (Amresco Inc., Solon, OH, USA)/well, and the absorbance at 490 nm was measured within 10 min of DMSO addition. Each experiment was conducted in triplicate.

Cell migration assay. Cell migration was determined by a Transwell migration assay. Cells ($5x10^4$) were seeded into the top chambers of 24-well Transwell chambers with 8-µm micropore membrane filters (Corning Inc., Corning, NY, USA), and the bottom chambers were filled with 0.5 ml DMEM/F-12 medium with 20% FBS as a chemoattractant. After 24 h, the non-invaded cells on the upper surface were carefully removed with a cotton swab, and the membranes were fixed and stained with crystal violet reagent. Migration was quantified by counting 3 random fields under a light microscope (magnification, x200). Data obtained from 3 separate chambers were presented as mean values.

Cell cycle analysis. Flow cytometry was used to determine the cell cycle distribution and to detect apoptosis, and was performed as previously described (19). Initially, HepG2 cells ($2x10^5$) were transfected with GV141-σ1R or NC plasmids and incubated at 37°C for 4 days. Harvested cells were fixed with 75% cold ethanol at 4°C overnight, washed twice with ice-cold PBS, incubated with 1 mg/ml RNase at 37°C for 40 min, and stained with propidium iodide (PI; 100 µg/ml; GeneChem). The fluorescence of DNA-bound PI in cells was measured with a flow cytometer (FACSCalibur; BD Biosciences, San Jose, CA, USA) and the cell populations in different phases of the cell cycle were analyzed with ModFit 3.0 software (Verity Software House, Inc., Topsham, ME, USA). Each experiment was performed in triplicate.

Annexin V-FITC apoptosis assay. An Annexin V-FITC apoptosis detection kit (KeyGen Biotech, Nanjing, China) was used for the labeling of apoptotic cells, according to the manufacturer's protocol. HepG2 cells ($2x10^5$) were transfected with σ1R-expressing or NC lentiviruses. After incubation for 4 days, the cells were harvested, washed with PBS buffer, and resuspended in 200 µl binding buffer. Then, 5 µl Annexin V-FITC was added into the cell suspension and

incubated at room temperature for 15 min. The cell cycle was monitored using PI (50 μg/ml; Sigma-Aldrich Co. LLC) staining of nuclei. Signals were detected with a FACSCalibur flow cytometer (Beckman Coulter, Inc., Brea, CA, USA). All experiments were performed in triplicate.

Statistical analysis. Data are expressed as the mean ± standard deviation of 3 independent experiments. Differences were compared using a Student's t-test. Associations between σ1R expression and clinicopathological characteristics were assessed with the Kendall's τ-b test. All statistical analyses were performed with SPSS 20.0 software (IBM Corp., Armonk, NY, USA). Each P-value is two-tailed, and P<0.05 was considered to indicate a statistically significant difference.

Results

σ1R is decreased in HCC tissues. Immunohistochemical staining was used to examine the expression patterns of σ1R in tissue samples from 30 HCC patients, as well as benign liver tissues from 10 HCH patients. From the 30 HCC patients, 29 samples of malignant tumor tissue (after 1 sample was excluded due to extensive necrosis preventing scoring) as well as 26 samples of cirrhotic tissue, representing precancerous lesions, were evaluated. As shown in Fig. 1A, in benign hepatic tissues, the positive signals were intense and well distributed in the cytoplasm, whereas weak to moderately positive signals were observed in precancerous lesions, and weak or negative staining was observed in the tumor tissues. The rates of σ1R+ expression in benign hepatic tissue, hepatic cirrhosis and HCC specimens were 90 (9/10), 65.38 (17/26) and 27.59% (8/29), respectively. The difference was statistically significant between benign hepatic tissues and HCC (P<0.01) (Fig. 1B). Therefore, we concluded that σ1R is downregulated in HCC tissues.

σ1R expression is positively correlated with HCC grade. Immunohistochemical staining was used to explore the expression of σ1R in 30 cases of HCC. σ1R immunoreactivity, which mainly showed a cytoplasmic staining pattern, was analyzed with respect to various clinicopathological parameters in these cases (Table II). A significant correlation was found between σ1R level and the degree of histological differentiation of the tumors (r=-0.424, P=0.021). Notably, intense staining was more frequent in well-differentiated cases of HCC, and σ1R+ expression was observed in 50% of grade I cases and 15.8% of grade II/III cases (Fig. 2). There were no significant correlations between σ1R expression and other clinical parameters.

σ1R mRNA expression in HepG2 and SMCC-7721 cell lines. The mRNA levels of σ1R were assessed in HepG2 and SMCC-7721 cell lines by RT-qPCR. It was observed that σ1R mRNA was expressed in the two cell lines, and notably, that the expression level of σ1R in SMCC-7721 was ~55% of the level in HepG2 cells (Fig. 3A). As the expression level in HepG2 was higher than that in SMCC-7721, we selected the HepG2 cell line for subsequent experiments.

σ1R expression is increased efficiently by lentiviral expression vector transfection in HepG2 cells. To investigate the

Table II. Association between σ1R expression and clinical pathological parameters in HCC.

Clinical parameters	σ1R status[a]		r[b]	P-value[c]
	-	+		
Age (years)				
≤59	12	3	0.176	0.427
>59	9	5		
Sex				
Male	19	6	0.201	0.552
Female	2	2		
Tumor size (cm)				
≤3	7	3	-0.199	0.256
3-5	4	4		
>5	10	1		
Histological differentiation				
G1	5	5	-0.424	0.021
G2	7	3		
G3	9	0		
AFP (ng/ml)				
≤20	8	5	-0.219	0.406
>20	13	3		
Hepatitis B surface antigen				
Negative	5	2	0.012	1.000
Positive	16	6		
Microvascular invasion				
Absent	10	7	0.362	0.093
Present	11	1		
Tumor number				
Single	19	8	-0.168	0.586
Mutiple	2	0		

[a]-, negative, scores of 0-4; +, positive, scores of 5-12. [b]The Kendall's tall-b test; r, Kendall tau coefficient value; [c]Each P-value is two-tailed and significance level is 0.05. AFP, α-fetoprotein; σ1R, sigma-1 receptor; HCC, hepatocellular carcinoma.

mechanism by which σ1R contributes to the malignancy of HMTs, we performed lentiviral-mediated overexpression of σ1R in HepG2 cells. The upregulation efficiency of σ1R was evaluated by western blot analysis. As shown in Fig. 3B and C, the protein expression level of σ1R was significantly upregulated compared with that in the NC group.

σ1R overexpression inhibits HepG2 cell growth and migration. The effect of σ1R overexpression on the proliferative ability of HepG2 cells was determined by MTT assay. As illustrated in Fig. 4A, the cellular proliferative rate of the σ1R-GV141 group was markedly reduced from the first day compared with
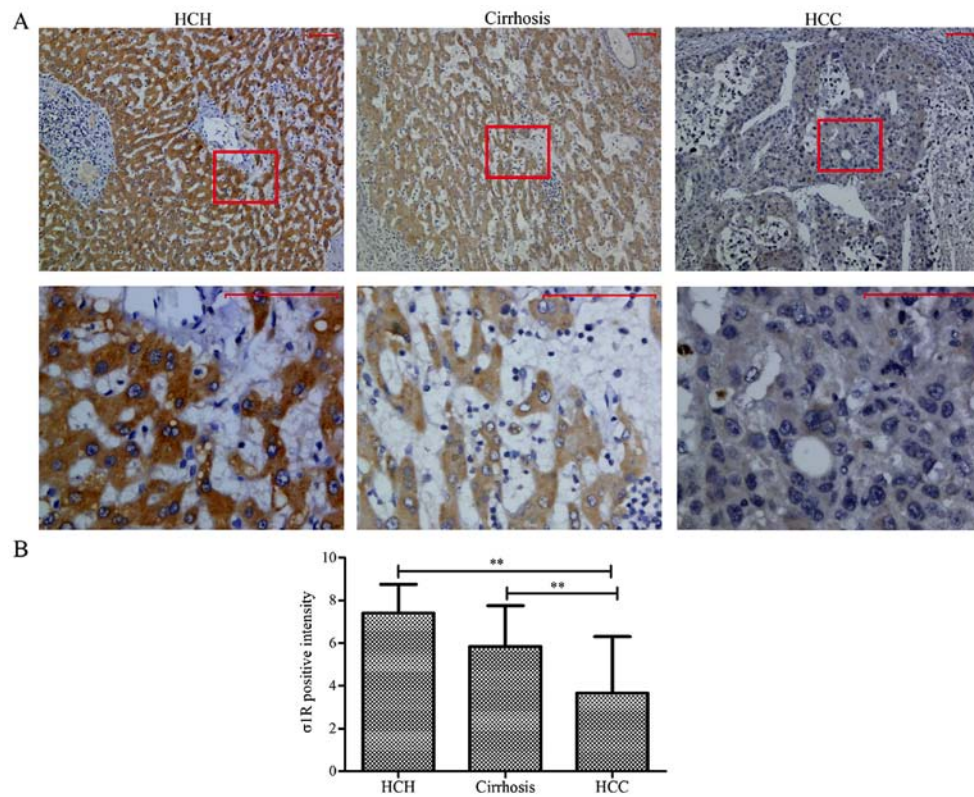
Figure 1. Immunohistochemical analysis of σ1R in benign hepatic tissue of HCH, cirrhotic cancer-adjacent liver tissues, and malignant HCC tissues. (A) σ1R expression in the progression from benign hepatic tissue to HCC. The staining was mainly located in the cytoplasm. In HCH tissues, the immunostaining of σ1R was intense and well-distributed compared with that in hepatic cirrhosis. Weak or negative staining was observed in HCC. Scale bars, 50 μm. (B) Rates of positive σ1R expression in HCH, hepatic cirrhosis and HCC. Statistically significant differences were observed between HCH and HCC (**P<0.01), and between hepatic cirrhosis and HCC (**P<0.01), respectively. σ1R, sigma-1 receptor; HCC, hepatocellular carcinoma; NCH, hepatic cavernous hemangioma.
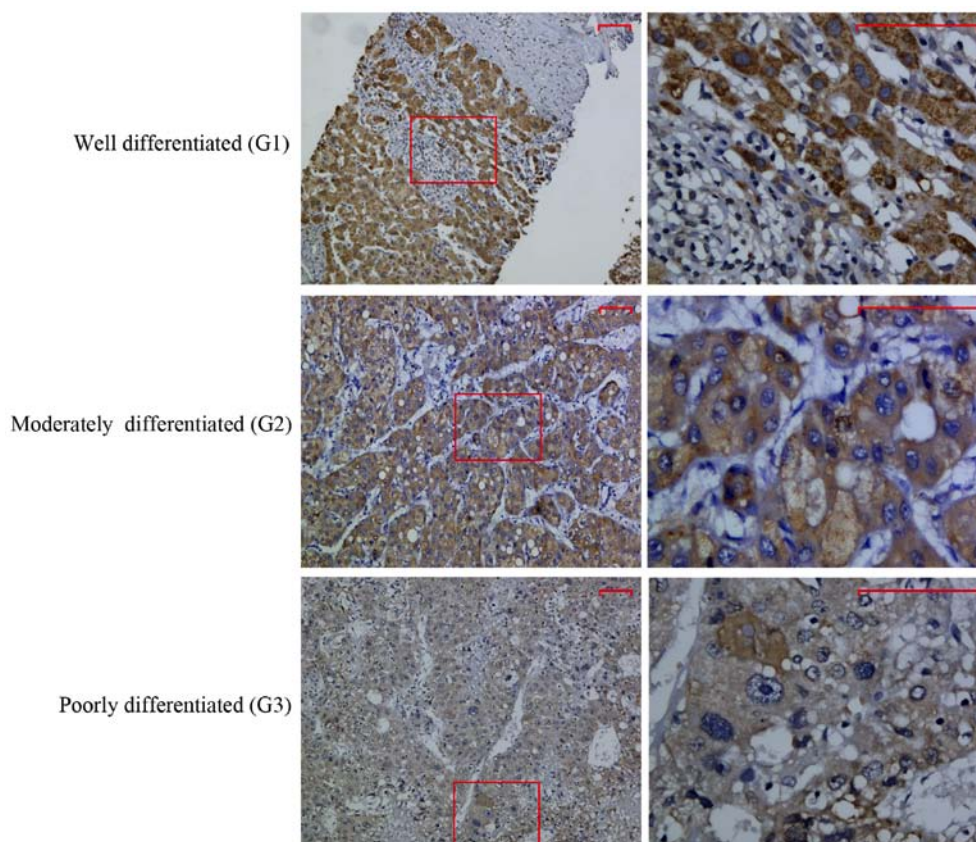

Figure 2. Immunohistochemical staining of σ1R in HCC according to the degree of differentiation (grades 1, 2 and 3). The staining of σ1R was found to gradually weaken with increased differentiation grade. Scale bars, 50 μm. σ1R, sigma-1 receptor; HCC, hepatocellular carcinoma.
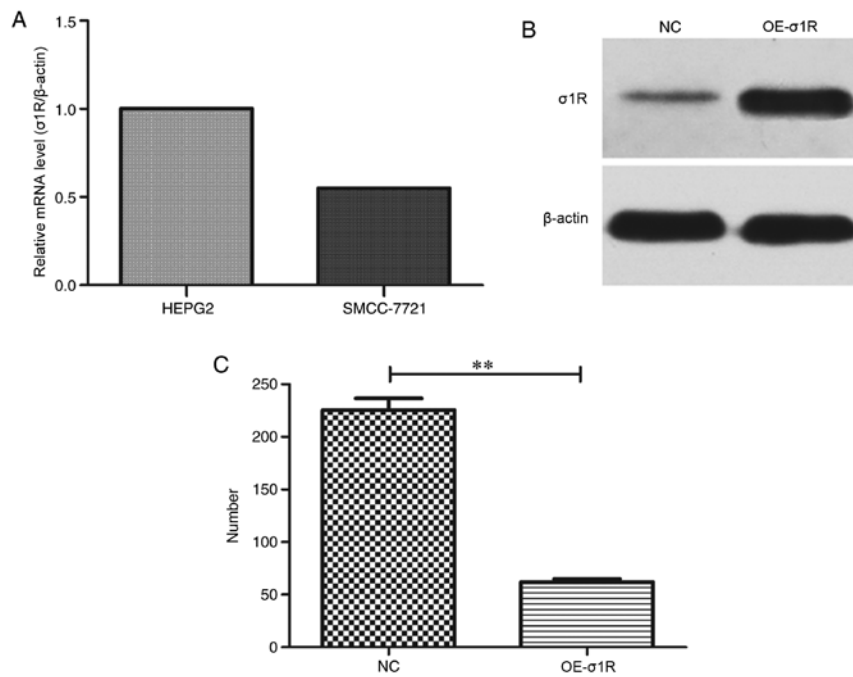
Figure 3. mRNA and protein expression levels of σ1R in hepatic malignant tumor cell lines. (A) σ1R mRNA level in HepG2 and SMCC-7721 cell lines. Expression of σ1R mRNA was measured by RT-qPCR in the indicated cell lines. The constitutively expressed β-actin gene was used as an internal control. (B) Overexpression of σ1R (OE-σ1R) protein in HepG2 cells. σ1R protein expression was analyzed by western blotting in control-transfected (negative control; NC) and σ1R-GV141-transfected HepG2 cells after 4 days of cell culture. (C) Confirmation of σ1R overexpression in HepG2 cells. HepG2 cells were transfected with σ1R-GV141 or NC lentivirus and σ1R protein levels were analyzed by western blotting; **$P<0.01$. β-actin was used as an internal control. σ1R, sigma-1 receptor; HCC, hepatocellular carcinoma. σ1R, sigma-1 receptor.
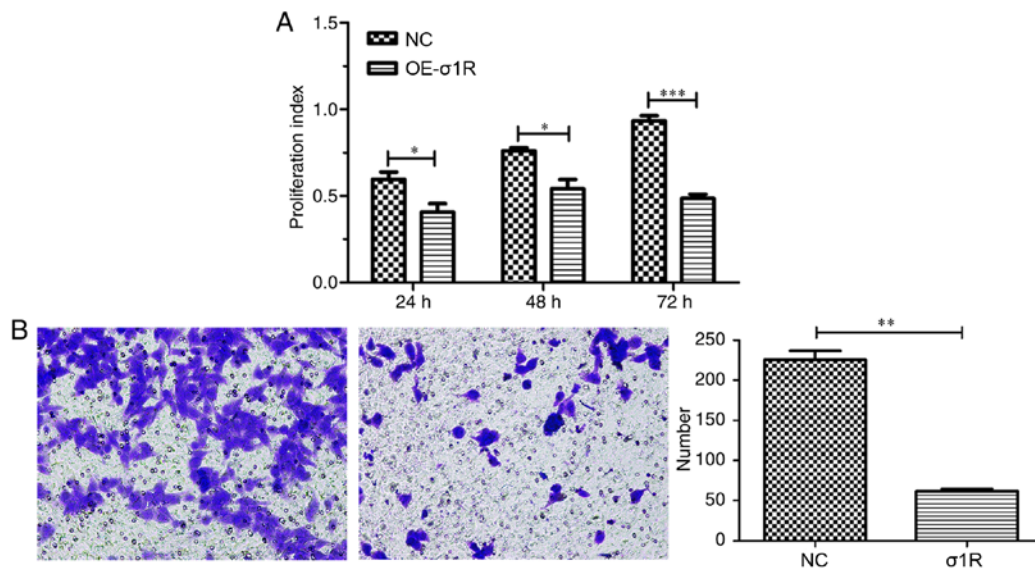

Figure 4. Overexpression σ1R suppresses cell proliferation and migration in HepG2 cells. (A) The proliferation index of σ1R-GV141-transfected HepG2 cells compared with the control group (NC) was determined by MTT assay; *$P<0.05$, ***$P<0.001$. (B) A similar trend was observed in the cell migration assay. Bright field microscopic images are shown (magnification, x200); **$P<0.01$. Data are shown as the mean ± SD. σ1R, sigma-1 receptor.

the NC-transfected cells ($P<0.05$), and the inhibitory effect on the third day was more obvious compared with that on the first day of cell incubation ($P<0.001$). Furthermore, a Transwell migration assay was performed to determine the effect of σ1R overexpression on the invasive ability of HepG2 cells *in vitro*. The results showed that σ1R overexpression in HepG2 cells caused a significant reduction in migration compared with the NC cells (Fig. 4B; $P<0.01$). Thus, it was demonstrated that the overexpression of σ1R suppressed the proliferation and migration of HepG2 cells.

σ1R overexpression causes cell cycle arrest in the G1 phase and induces apoptosis in HepG2 cells. As changes in cell numbers may result from arrest of cell cycle progression or induction of apoptosis, both were analyzed by flow cytometry. As shown in Fig. 5A, for HepG2 cells, the σ1R-overexpressing
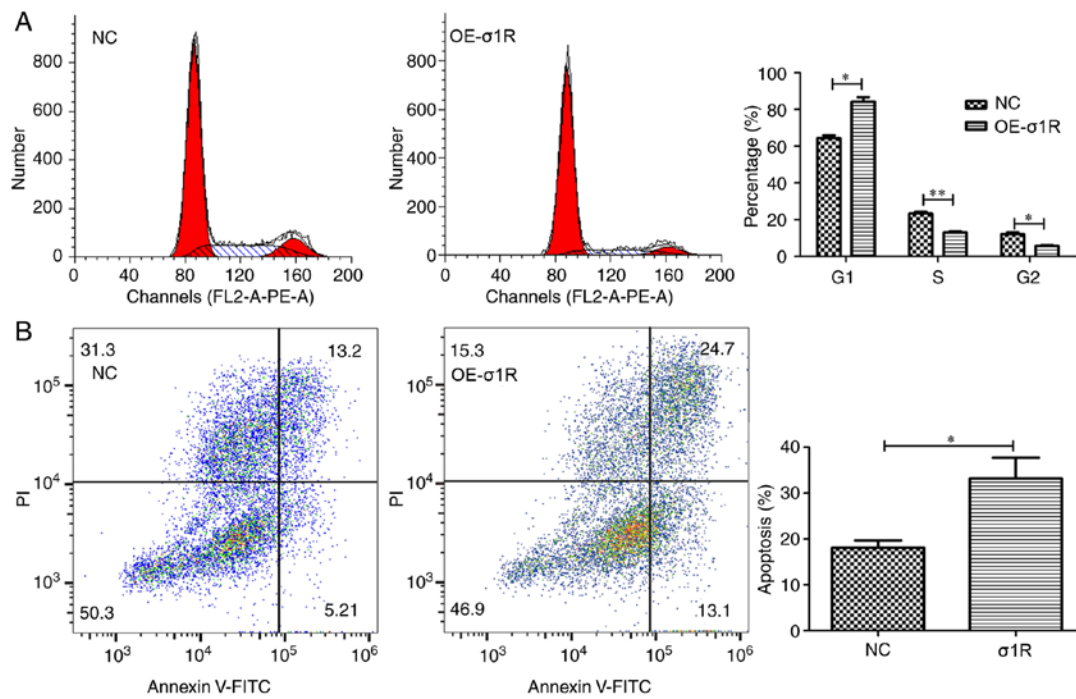
Figure 5. Overexpression σ1R leads to cell cycle arrest and induces cell apoptosis. (A) Flow cytometric analysis of cell cycle distribution revealed that overexpression of σ1R induced cell cycle arrest at the G1 phase; *P<0.05, **P<0.01. (B) Cell apoptosis was determined by Annexin V staining and flow cytometry. The apoptosis of HepG2 cells was induced by σ1R-GV141 transfection; *P<0.05. Data are shown as the mean ± SD. σ1R, sigma-1 receptor.
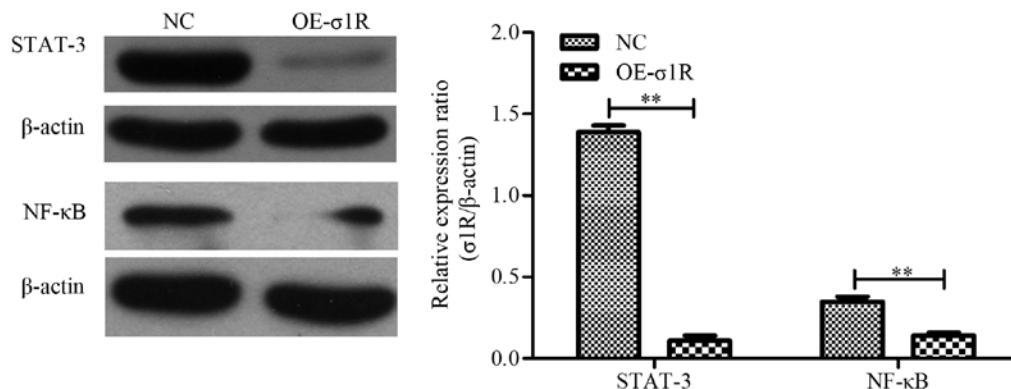

Figure 6. Overexpression of σ1R suppresses the expression of STAT-3 and NF-κB. HepG2 cells were transfected with σ1R-GV141 lentivirus for 3 days and subjected to western blotting with the indicated antibodies. β-actin was used as a loading control; **P<0.01. Data are shown as the mean ± SD. σ1R, sigma-1 receptor.

group and NC group exhibited the following cell cycle distributions: G1 phase, 84.17±4.19 vs. 64.42±2.66%, respectively; S phase, 13.00±1.30 vs. 23.40±1.37%, respectively; and G2/M phase, 5.59±0.81 vs. 12.17±1.51%, respectively. The results showed a significant decrease in the proportion of cells in the S phase (P<0.01) or G2/M phase (P<0.05), and an increase in the proportion of cells in G1 phase (P<0.05) in the σ1R-overexpressing group compared with the NC group, suggesting that σ1R overexpression led to cell cycle arrest in G1 phase. The ability to resist apoptosis is an important feature of tumor cells (21). In the present study, the apoptosis analysis following Annexin V staining showed that the apoptotic rate of σ1R-overexpressing cells was significantly increased with respect to the control group (Fig. 5B; 33.20±4.52 vs. 18.13±1.57%; P<0.05). These data indicated that σ1R may be associated with apoptosis in HMT.

σ1R overexpression inhibits STAT-3 and NF-κB expression. To explore the molecular mechanisms underlying the involvement of σ1R in HMT development, we analyzed the effect of σ1R on oncogenic signaling pathways. Western blotting showed that STAT-3 and NF-κB were decreased in HepG2 cells overexpressing σ1R (Fig. 6; P<0.01). These results suggested that the upregulation of σ1R may be responsible for STAT-3 and NF-κB downregulation in the context of HepG2 cell proliferation, apoptosis and migration.

Discussion

HCC is a type of malignant tumor associated with rapid progression and a poor survival rate (3). Although there have been extensive studies on HMT, poor prognosis and the limited value of established prognostic markers have prompted

researchers to search for new biomarkers that are able to predict the prognosis and act as possible treatment targets in patients with HMT. Sigma-1 receptor (σ1R) was first discovered in the nervous system by Martin *et al* (7), although its expression has since been identified in other organs, including the liver, kidneys, lungs and gonads (14,17). More recently, studies have found that σ1R is highly expressed in various cancer tissues of neural and non-neural origins (12,15,16,22,23), and the upregulation of σ1R has been reported to be associated with biological behavior, such as proliferation, adhesion and cell death, in tumors (13,24). However, the expression and biological significance of σ1R in HMT remain unknown.

A novel finding in the present study was that σ1R was expressed at low levels in HCC, and that there was a statistically significant difference between its expression levels in benign hepatic tissue and HCC (P<0.01). The decreased expression of σ1R in HCC was an interesting phenomenon, which was inconsistent with former studies in other tumor types (16,18,25). We considered that this contradiction may indicate a different molecular biological function in HCC compared with other tumor types. It has been reported that certain narcotic drugs and oxygen and glucose deprivation are important factors in inducing σ1R expression (26,27). The liver is an organ that is central to metabolism and produces a wide range of chemicals essential to bodily functioning. External and internal factors may interact with HCC tumor cells to regulate the expression of σ1R.

Histological differentiation is a significant factor in estimating the prognosis of cancer patients. Patients with high-grade HCC often have a poor prognosis. In the present study, a significant inverse correlation was observed between σ1R expression and the grade of differentiation of HCC (r=-0.424, P=0.021), and HCC cases with a high level of σ1R expression were more likely to have low grade disease. Therefore, we speculated that σ1R may play a role in the initiation or progression of HCC, and particularly in histological differentiation.

In the present study, lentivirus-GV141-mediated σ1R overexpression markedly inhibited HMT cell proliferation and migration *in vitro*; the cell proliferation rate and migratory ability in the σ1R-GV141 group were significantly decreased or decelerated compared with the NC lentivirus-transfected group. Furthermore, σ1R overexpression induced cell cycle arrest in the G1 phase and promoted cell apoptosis. These findings demonstrate that the downregulation of σ1R has an important effect on HMT cell proliferation and migration, and that it may serve as a potential predictive factor and therapeutic target in the treatment of HMT.

The scientific basis underlying the inhibitory effect of σ1R overexpression on tumor proliferation is an important aspect for further research. Ion channels are considered to play a crucial role in many tumor types (16). Aydar *et al* found that Kv1.4 and Kv1.5 ion channels were highly sensitive to σ1R ligands in the presence of σ1R, while the modulation was weak in the absence of σ1R in *Xenopus* oocytes, which suggested that σ1R may form a functional complex with the expressed ion channels (10). In addition, Spruce *et al* (28) considered that σ1R ligands may be involved in the calcium-dependent activation of phospholipase C and concomitant calcium-independent inhibition of phosphatidylinositol 3'-kinase pathway signaling in some cancer cells.

Signal transducer and activator of transcription-3 (STAT-3), a member of the STAT family, is commonly activated in human epithelial cancers (29,30). Constitutive activation of STAT3 contributes to oncogenesis and progression, including increased cell proliferation and survival (31). Wu *et al* (32) demonstrated that increased STAT-3 expression was correlated with higher tumor stage and decreased patient survival in cases of HMT.

Nuclear factor-κB (NF-κB) is a well-known nuclear transcription factor that regulates the expression of a variety of genes critical for the regulation of apoptosis (33). The activation of NF-κB induces antiapoptotic gene expression to promote cell survival. In the present study, we found that the expression levels of STAT-3 and NF-κB were decreased in σ1R-overexpressing HepG2 cells relative to the NC group. Our findings preliminarily indicate that σ1R overexpression may suppress HepG2 cell proliferation and migration through inactivation of STAT-3 and NF-κB.

In summary, the present findings indicate that σ1R is decreased in HCC and is closely correlated with histological differentiation. The overexpression of σ1R suppressed cell proliferation, inhibited cell migration and induced cell cycle arrest and cell apoptosis in HepG2 hepatoblastoma cells. Therefore, we hypothesized that σ1R has an important role in promoting HCC cell differentiation. Elucidation of the functions and detailed mechanisms of σ1R in regulating HMT tumorigenesis and progression are the subjects of our ongoing research.

Acknowledgements

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