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Research paper

Role of gelsolin in cell proliferation and invasion of human hepatocellular carcinoma cells

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ABSTRACT

Objective: Gelsolin (GSN), one of the most important actin structure regulating proteins, has been implicated in the oncogenesis of some cancers. In this study, we investigated the expression of GSN in hepatocellular carcinoma (HCC) and revealed its potential mechanisms. The mRNA and protein levels of GSN were overexpressed in HCC cells and HCC tissues compared to adjacent noncancerous tissues. GSN expression was correlated with venous invasion ($P = 0.0199$) and Edmonson grading ($P = 0.0344$) expression in HCC. Overexpression of GSN in Huh7 and SMMC-7721 cells significantly promoted cell proliferation and the number of Matrigel™-invading cells compared with control cells, with increased expression of matrix metalloproteinase MCL-1, MMP-2 and MMP-9, a key regulator of growth and invasion. In contrast, knockdown of GSN expression with small interfering RNA (siRNA) in MHCC-97L and MHCC-97H cell lines resulted in decreased cell viability and cell invasion. Our findings indicated that GSN expression promoted tumor-associated phenotypes by facilitating proliferative and invasive capacities of HCC cells, which might serve as a potential therapeutic target for HCC treatment.

1. Introduction

HCC is the third leading cause of death from cancer and the fifth most common malignancy worldwide (Thorgeirsson and Grisham, 2002; Roberts, 2008). HCC develops commonly in a setting of liver cell injury, which leads to inflammation, hepatocyte regeneration, liver matrix remodeling, fibrosis, and ultimately, cirrhosis. The vast majority of HCC worldwide is attributed to hepatitis B virus (HBV) and HCV, but other risk factors include alcohol abuse, hemochromatosis, fatty liver disease, androgenic steroid use, and other metabolic disorders (El-Serag and Rudolph, 2007; Colombo, 2009; Pan et al., 2014). The mechanisms by which these varied etiologies lead to cirrhosis and HCC are not well understood.

Current research includes seeking for genes that are disregulated in HCC, protein-markers, non-coding RNAs and other predictive biomarkers (Zhu et al., 2012; Qin et al., 2014). It is hoped that identifying the aberrant genes and the key molecular targets and pathways involved in hepatocarcinogenesis could lead to the identification of pharmacological interventions for HCC (Thomas and Zhu, 2005; El-Serag and Rudolph, 2007). On the other hand, it is well-known that metastasis, the spread of malignant tumor cells from a primary site to distant sites, is the most life-threatening complication of cancer and a major problem of cancer treatment (Dimitroff et al., 1998; Fidler, 1999). Enhanced motility of cancer cells by remodeling of the actin cytoskeleton is crucial in the process of cancer cell invasion and metastasis.

GSN is a widely distributed actin-regulatory protein that can nucleate and sever actin that may play an important role in cell motility (Yin and Stossel, 1979; Sun et al., 1999). GSN is also involved in controlling cell morphology, growth and apoptosis (Kwiatkowski, 1999a). GSN has been reported to be frequently downregulated in various cancers, such as prostate cancer, breast cancer and lung cancer (Asch et al., 1999; Lee et al., 1999; Sagawa et al., 2003). In addition, GSN suppressed the epithelial–mesenchymal transition in mammary epithelial cells (Tanaka et al., 2006) and acted as a metastasis suppressor by inhibited cell migration and metastasis in vitro and in vivo in melanoma cells (Fujita et al., 2001). On the contrary, it has been reported that GSN overexpression promoted tumor cell motility and invasion through modulation of several pathways, including PI3K and Ras–PI3K–Rac In vitro (Chen et al., 1996; Lader et al., 2005). Among these, GSN has been the one studied most extensively, its role in tumor development remains controversial. However, the expression pattern and the potential mechanism of GSN in GC have not been elucidated.

Abbreviations: HCC, hepatocellular carcinoma; RNAi, RNA interference; GSN, Gelsolin; IHC, immunohistochemistry; qRT-PCR, quantitative real-time reverse-transcription.

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In our present study, we evaluated the expression pattern of GSN in HCC tissues for the first time. We further found that GSN could promote growth and motility of the human HCC cell lines. Additionally, the tumor malignant roles and potential mechanisms of GSN on HCC were determined.

2. Materials and methods

2.1. Tissue samples

Tumor specimens including HCC tissues and paired non-tumor tissues from 48 patients with HCC were obtained between May 2006 and Oct 2008 following surgical resection at the Shanghai First People's Hospital of Shanghai Jiao Tong University. Written consent was obtained from all enrolled patients in this study. Histological diagnoses and tumor differentiation were assayed by hematoxylin and eosin (H&E) stained tumor tissue sections, according to the World Health Organization (WHO) classification guidelines (2004). This study was approved by the Medical Ethical Committee of Shanghai First People's Hospital of Shanghai Jiao Tong University. Fresh tissue was harvested from patients, snap-frozen, and preserved at −80 °C until use.

2.2. Cell cultures

Two human HCC cell lines (Huh7 and SMMC-7721) and an immortalized normal human liver cell line (LO2) were purchased from the Shanghai Cell Bank of the Chinese Academy of Sciences (Shanghai, China) and cultured as previously described (Qu et al., 2014). The HCC cell lines, MHCC-97L and MHCC-97H (low and high metastatic potential, respectively), used in this study were obtained from the Liver Cancer Institute, Fudan University (Shanghai, China), and maintained in Dulbecco's Modified Eagle Medium (Life Technologies,. Inc., Gaithersburg, MD, USA) containing 10% fetal bovine serum (Life Technologies), 100 U/ml of penicillin G and 100 μg/ml of streptomycin at 37 °C in a water-saturated atmosphere of 5% CO_2.

2.3. Quantitative real-time reverse-transcription (qRT-PCR)

A TRIzol reagent (Life Technologies) was used to extract total RNA from HCC tissue specimens and cultured cells. The total RNA (500 ng) was used in reverse transcription reactions in 20-μL reaction mixtures with iScript™ cDNA Synthesis Kit (Bio-Rad, Hercules, CA, USA) according to the manufacturer's instructions. Real-time PCR was carried out in a total volume of 20 μl with the reverse-transcribed cDNA using the SYBR Green master mix (Life Technologies) in an ABI 7900HT real-time PCR system (Life Technologies) as directed by the manufacturer's protocol. The reactions were preheated in a 96-well optical plate at 95 °C for 10 min, followed by 40 cycles of 95 °C for 30 s and 60 °C for 1 min. The primer sequences used in the experiments were: 5′-AGATGGACTACCCCAAGCAGA-3′(sense) and 5′-GGTCCCGCCAGTTCTTGAA-3′(antisense) for GSN; 5′-TGATGACATCAAGAAGGTGGTGAAG-3′(sense) and 5′-TCCTTGGAGGCCATGTGGGCCAT-3′(antisense) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH). GAPDH was used as an internal control, and the relative amount of GSN normalized to GAPDH. All of the reactions were run in triplicate.

2.4. Immunohistochemistry

Immunohistochemical analysis was described in detail previously (Qu et al., 2014). Briefly, paraffin-embedded specimens were cut into 4-μm thick sections and deparaffinized with xylenes. After dehydration in a graded ethanol series, an antigen retrieval procedure was performed by heating the slides in 10 mM sodium citrate buffer at 95 °C for 20 min. The endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide, followed by incubation with normal goat serum to block non-specific binding. Primary Rabbit polyclonal antibodies against GSN (Proteintech Group, Inc., Chicago, IL, USA) were applied at 1:40 dilution and incubated at 4 °C overnight. The color was developed with Dako's horseradish peroxidase Envision kit (Dako Cytomation, Glostrup, Denmark). All sections were counterstained with hematoxylin, dehydrated, cleared and mounted.

2.5. Plasmid construction and synthesis of siRNA

The code sequence of GSN was obtained from cDNA of HEK293T by PCR amplification and cloned separately into multiple cloning sites of the eukaryotic expression plasmid pcDNA3.1(+) (Invitrogen, Carlsbad, CA, USA). All procedures were performed as previously described (Cao et al., 2014). The primer sequences used were: 5′-CCCAAGCTTATGGTGGTGGAACACCCCG-3′(sense) and 5′-AAAGGATCCTCAGGCAGCCAGCTCAGC-3′(antisense). The sequence of GSN was confirmed by DNA sequencing (Sangon, Shanghai, China).

For knockdown of GSN, small interfering RNA (siRNA) targeted to GSN and negative control (NC) siRNA were synthesized and purchased from GenePharma (Shanghai, China). The sequence of the GSN-specific antisense oligonucleotides was 5′-UUUCAGAACAAAGGCAUCGdTdT-3′, and that of control oligonucleotides was 5′-UUCUCCGAACGUGUCACGUdTdT-3′.

Transfections were performed using X-tremeGENE (Roche, Basel, Switzerland) according to the manufacturer's instructions. After 48 h of transfection, the cells were harvested for Western blot analysis or used for cell proliferation and invasion assay.

2.6. MTT assays and invasion assays

Transfected cells were seeded at 3×10^3 cells per well in 96-well flat-bottomed plates with a final volume of 100 μl culture medium. The MTT assay (Sigma) was used to assess the relative cell growth every 24 h. MTT (20 μl of 5 mg/ml) was added into a corresponding test well and incubated for 2 h at 37 °C. The plates were shaken for 10 min to allow the formazan crystals to dissolve after DMSO (200 μl) (Sigma) was added. Absorbance was measured at 490 nm using a Coulter Counter (Beckman Coulter, Fullerton, CA, USA).

The invasive abilities of the cells were determined by culturing the cells on an 8-μm pore-sized membrane (Millipore, Billerica, MA, USA) coated with Matrigel (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA) as previously described (Cao et al., 2014). Briefly, the Matrigel (20 μl) was first incubated in pre-chilled basal medium (80 μl) at 37 °C for 1 h, 5×10^5 cells in 100 μL DMEM medium without FBS were seeded, followed by incubation at 37 °C for 48 h. The cells on the lower side of the filter were stained with crystal violet and were counted by an IX71 inverted microscope (Olympus, Tokyo, Japan) at 100× magnification.

2.7. Western blot analysis

As described before (Qu et al., 2014), the whole-cell lysate was extracted using RIPA buffer (CST, Danvers, MA, USA) with a protease inhibitor cocktail (Roche Applied Science, Nutley, NJ, USA). After cell lysates were determined for protein content using a BCA protein assay kit (Pierce, Rockford, IL, USA), aliquots of protein was separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto a 0.22-μm polyvinylidene difluoride (PVDF) membrane (Millipore, Bedford, MA, USA). The membranes were blocked for 1 h in 5% nonfat dry milk in TBS + 0.05% Tween™ 20 and incubated with appropriate primary antibodies at 4 °C overnight. The following antibodies were used for Western blot: Rabbit Anti-GSN antibody (Proteintech), Rabbit Anti-MMP-2 antibody, Rabbit Anti-MMP-9 antibody and Rabbit Anti-Mcl-1 Antibody (Cell signaling Technology, Beverly, MA, USA), or Anti-GAPDH (Bioworld, Nanjing, China).