RESEARCH ARTICLE

Inhibition of SMP30 Gene Expression Influences the Biological Characteristics of Human Hep G2 Cells

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Abstract

Senescence marker protein 30 (SMP30), a hepatocellular carcinoma (HCC) associated antigen had been identified by our research group. To study its mechanisms of regulation and associations with the occurrence and development of HCC, we inhibited expression by RNAi technique, and observed effects on the biological characteristics of Hep G2 cells. In cell viability assays, cell growth in the experimental group (with siRNA transfection) was elevated. In Transwell invasion assays, compared with blank and control groups, numbers of invading cells in the experimental group were significantly increased, whereas in apoptosis assays, the percentage apoptosis demonstrated no differences, but after UV irradiation, that in the experimental group was higher than the other two groups. In a word, SMP30 can inhibit the proliferation and invasion of human hepatoma cells and thus can be regarded as a cancer suppressive factor.

Keywords: SMP30 - Hep G2 - proliferation - invasiveness

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Introduction

It has been confirmed that SMP30 is a tumor-related molecular of Hepatocellular Carcinoma (Luo et al., 1999; Stenner et al., 2000; Mo et al., 2007), and its high expression is the early event of HCC.then, anti-SMP30 antibody was found in serum of HCC patients using recombinant human SMP30 protein (Zhou et al., 2004; Huang et al., 2012), suggesting that it may be used as one of serum diagnostic indexes for HCC. Although diagnostic value was confirmed, its regulation mechanism on the occurrence and development of HCC has not been studied deeply.in this study, we inhibited the expression of SMP30 by RNAi technique, to observe its effect on the cell proliferation, apoptosis, cell cycle and invasiveness of the Hep G2 cells.and investigate the possible mechanism, to find out a new target in the treatment of HCC for investigating a high efficient and feasible method.The cell number in the lower chamber can reflect the invasion capacity of Hep G2.comparing with blank group and control group, the invaded cell number in experimental group significantly increased, difference between them was significant, it suggested that the inhibition of SMP30 can promote the invasion ability of Hep G2 cells. In means SMP30 maybe inhibit the secretion of MMPs.

Materials and Methods

Materials

Human Hep G2 cells were provided by Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences.Xfect Transfection Reagent Kit and Matrigel were from Clontech, American; SiRNA was from GenePharma, Shanghai ; Cell proliferation kit (MTT)was purchased from Roche Ltd. Annexin V-FITC apoptosis detection kit and Cell Cycle detection kit were purchased from Nanjing KeyGEN Biotech. CO., LTD.

Culture and transfection

SMP30 siRNA (Sense:5'-GAAGUUGCCUGUUGAU AAATT-3'; Anti-sense: 5'-UUUAUCAACAGGCAACU UCTT-3') and the control siRNA were synthesized by Genepharma Co., Ltd (Shanghai). Hep G2 (1×10^5 cells/well) were added to a 24-well plate. When cell confluence reached 40–60%, cells were transfected with siRNA by using Xfect GENE siRNA transfection reagent and cultured for 48 h. Interference of SMP30 expression was tested by RT (reverse transcription)-PCR and Western blot.

Cell viability assay

The 3- (4, 5-dimethylthiazolyl-2)-2, 5-diphenyl

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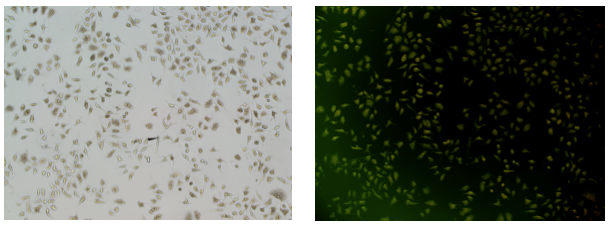

Figure 1. The Transfection Efficiency of FAM-siRNA(×100). A: under common path ; B: under fluorescence path

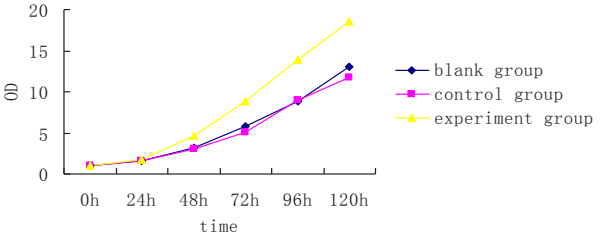

Figure 2. Hep G2 Cell Proliferation and Growth Curve. The OD value of 0 h was taken for standard "1"

tetrazoliumbromide (MTT) assay was used as a qualitative index of cell proliferation. We cultured Hep G2 cells in 96-well plates (5×10^3 cells/well). After 24 h, 48 h, 72 h, 96 h or120h incubation with above indicated compounds, 20 μl MTT (5 mg/ml) was added and the cells were cultured for additional 4h. Subsequently, the cells were lysed using dimethylsulfoxide (150μl/well, Sigma, USA). When the formanzan crystals were completely dissolved, the optical density (OD) was measured at 570 nm using a Microplate Reader (Biotek synergy, USA) the OD value of 0 h was taken for standard "1", draw cell proliferation and growth curve.

Invasion assay

The membrane invasion culture system chamber was used to measure invasiveness of the Hep G2 cells. Briefly, transwell inserts with 8 μm pores were uniformly coated with 50 μg of Matrigel and air-dried before being rehydrated. prepared after trypsinisation, Single-tumor-cell suspensions were seeded into the upper wells at a concentration of 1×10^5 per well. After a 24 h incubation in a humidified incubator with 5% CO_2, the inserts were fixed in methanol and stained for 20 min with crystal violet. The cells on the upper surface of the inserts were wiped away with a cotton swab. The cells that had migrated through the matrix and adhered to the lower surface of the inserts were counted as nine separate fields at 40×magnification. Each cell line was tested at least twice and within a single experiment, each assay was performed in quadruplicate.

Apoptosis assay

Before and after UV irradiation, Hep G2 cells were gently treated with trypsin, washed once with serum-containing medium, and collected by centrifugation. The cells were suspended in 500 μL of 1×binding buffer, and 5 μL of annexin V-FITC and propidium iodide (PI) were added according to the manufacturer's instructions . After incubation at room temperature for 5 min in the dark, the cells were evaluated for annexin V-FITC and PI binding by flow cytometry using a FACS Calibur (BD Biosciences, Inc., USA).

Statistical Analysis

Data are presented as means±standard deviation (SD) of three separate experiments. One-way ANOVA with the Student-Newman-Keuls test was used to compare values and to assess statistical significance ($p\leq0.05$).

Results

Transfection efficiency

12 hours after transfection with FAM-siRNA, counted the percent of fluorescent cells under fluorescence microscope (Olympus, Japan), the transfection efficiency was about 86.8% (Figure 1).

Proliferation and Apoptosis

Hep G2 cell proliferation and growth curve (Figure 2)was made, there was no difference in blank group and control group ($P>0.05$), The level of cell growth in experiment group was higher than the other two ($P<0.05$);The percent of apoptosis in three groups had no difference ($P<0.05$), after UV irradiation, the percent of apoptosis in blank group and control group were (25.54±4.33) % and (26.45±5.37) %, they had no significant difference ($P>0.05$), the percent of apoptosis in experimental group was (45.45±11.36)% (Figure 3), compared with the other two groups, the difference was obvious ($P<0.05$).

Cell invasion

The invaded cell number in blank group, control group and experimental group were 54.7±5.5, 60.5±10.6 and 120.8±15.8 respectively (Figure 4); comparing with

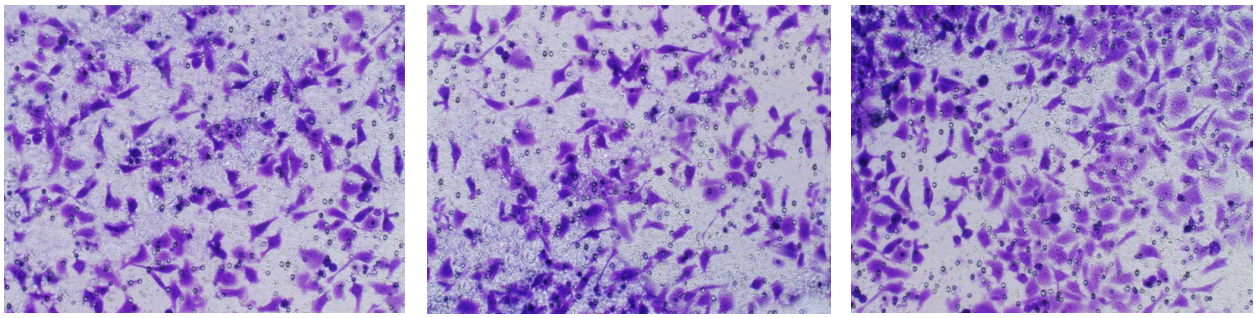

Figure 3. The Invaded Cells (crystal violet stainingx200). A: blank group; B: control group; C: experimental group

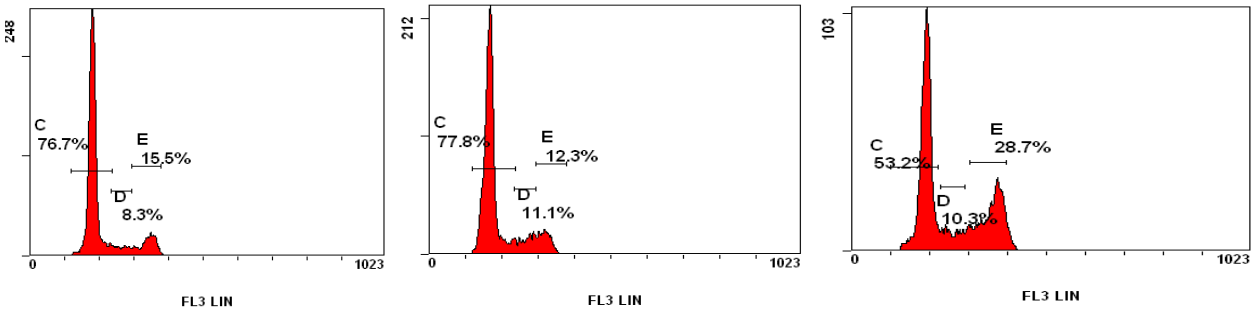

Figure 4. The Percent of Apoptosis in Blank Group after UV Irradiation. A: blank group; B: control group; C: experimental group

blank group and control group, the invaded cell number in experimental group significantly increased, difference between them was significant ($P<0.05$). The invaded cell number in blank group and control group had no significant difference ($P<0.05$).

Discussion

The previous study was mainly concentrated in the anti proliferative effect of SMP30 on normal rat liver cells.Ishigami found that, after induced by Carbon Tetrachloride, the liver cell mitosis of SMP30 Gene Knockout rats were significantly increased than heterozygous donor rats (Ishigami et al., 2001), other study also confirmed the anti proliferative effect of SMP30 on rat liver cells and kidney cells (Nakagawa et al., 2005; Yamaguchi et al., 2005). TsurusakiY determined the [3H] UTP in hepatocytes nuclei RNA, and found that putting SMP30 into the reaction basic matter could inhibit the enhancement of CaC12 on RNA synthesis (Tsurusaki . 2002). Missal H transformed the recombinant of SMP30 into rat cells, after culture, the number of cells with positive recombinants was lower than control group, their DNA synthetic activity also decreased (Misawa et al., 2001). All the analysis showed that SMP30can inhibit the rat cell proliferation at the protein level, DNA level and RNA level.in our Cell viability assay, The level of cell growth in experiment group (with siRNA transfection) was higher than the other two. It suggested that the inhibition of SMP30can promote the proliferation of Hep G2 cells. In other words, SMP30 can inhibit the proliferation of human hepatoma cell and be seen as a cancer suppressive factor.

It is well known that, to a great extent, the occurrence of tumor is due to the fact that the converted cells can not undergo a normal process of apoptosis (Pulaski et al., 2004; Su et al., 2006). In this experiment, the percent of apoptosis in three groups had no difference, it means that SMP30 can not promote tumor growth by inhibiting apoptosis.

Matrigel is a soluble basement-membrane matrix extracted from EHS mice Sarcoma. at room temperature, it polymerized into biologically active three-dimensional matrix structure.which is similar with mammalian cell-basement membrane, and often be used to simulate the cell-basement membrane during the process of tumor cell invasion and metastasis.In our Transwell invasion assay, before the Hep G2 cells through polycarbonate membrane into the lower chamber of Transwell, they have to secret MMPs (Matrix metalloproteinases) to degrade matrix.The cell number in the lower chamber can reflect the invasion capacity of Hep G2.comparing with blank group and control group, the invaded cell number in experimental group significantly increased, difference between them was significant, it suggested that the inhibition of SMP30 can promote the invasion ability of Hep G2 cells. In means SMP30 maybe inhibit the secretion of MMPs.

The research shows that endogenous SMP30 can strongly inhibit the secretion of many enzymes which participate in protein metabolism and signal transduction. such as protein kinase (Inagaki et al., 2001), protein tyrosine phosphatase (Tsurusaki et al., 2003), protein phosphatase (Ichikawa et al., 2004), nitric oxide syntheses (Ma et al., 2003;Tobias et al., 2003), so it maybe also can inhibit the secretion of MMPs.but this needs future study to confirm.In addition, according to some relevant papers (Izumi et al., 2004; Tsurusaki et al., 2004; Nakagawa et al., 2005), we inferred that SMP30 possibly inhibit the expression of MMPs by down-regulation of TNF-a, c-myc, c-src, bcl-2.

From our apoptosis assay, we included that SMP30 can inhibit the apoptosis of Hep G2 cells induced by UV irradiation, but has no effect on the apoptosis of Hep G2 cells under normal condition. It is well known that the occurrence of tumor to a great extent is due to the fact that the converted cells can not undergo a normal process of apoptosis.the transformed cells. Therefore, SMP30 will not promote tumor-genesis and progression of tumor by inhibiting apoptosis.

In a word, SMP30 can inhibit the proliferation and invasion of human hepatoma cells and be seen as a cancer suppressive factor.

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