The Effect of SOCS3 on the Epithelial Stromal Transformation of Human Hepatoma MHCC97-H Cells

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Abstract

Objective: To study the effect of suppressor of cytokine signaling 3 (SOCS3) on the epithelial mesenchymal transition (EMT) of human hepatoma cell line MHCC97-H.

Methods: Human hepatocarcinoma cell line MHCC97-H was cultured in vitro. The cells were transiently transfected with 25 nmol / L SOCS3 siRNA (positive transfection group) and transfected with empty plasmid (negative control group). After 24 hours, the fluorescent expression of the transfected cells was observed by fluorescence microscope. 48 hours later, the morphological changes of the cells were observed by inverted microscope. The expression of E-cadherin, an epithelial marker of EMT, and α-SMA, an interstitial marker in MHCC97-H cells were detected by immunofluorescence staining.

Results: Compared with the negative control group, MHCC97-H cells successfully transfected with SOCS3 siRNA showed green fluorescence. After transient transfection of SOCS3 siRNA, the hepatoma cells changed from cobblestone shape with epithelioid characteristics to spindle and spindle shape with interstitial cell morphology. The expression of E-cadherin and α-SMA was detected by immunofluorescence. The results showed that the expression of e-cadherin, the epithelial marker of SOCS3 siRNA positive transfection group, decreased significantly, while the expression of a-SMA, the interstitial marker of cells, increased significantly.

Conclusion: This study shows that the down-regulation of SOCS3 expression in hepatoma cells can promote the proliferation of hepatoma cells by changing their EMT phenotype, molecular and phenotypic characteristics, suggesting that SOCS3 may play an important role in the EMT of hepatoma cells. Regulating the expression level of SOCS3 can inhibit the occurrence and development of liver cancer, and provide a new idea for clinical prevention and treatment of liver cancer.

Keywords: Liver cancer; Epithelial mesenchymal transition (EMT); Suppressor of cytokine signaling 3 (SOCS3).

1. INTRODUCTION

Liver cancer is one of the common malignant tumors in our country, which has the characteristics of high invasion, easy metastasis and recurrence. These characteristics are also the key links leading to poor prognosis and poor clinical efficacy. At present, there is no ideal prevention and treatment method for liver cancer, one of the important factors is that the mechanism of its occurrence and development is still unclear. In the process of occurrence and development of malignant tumors, epithelial mesenchymal transition (EMT) will appear, that is to say, it will gradually lose the epithelioid characteristics and show the interstitial characteristics of increased invasion and motility, and a variety of signal pathways participate in this process. Inhibition of related signal pathways can inhibit EMT and the occurrence of malignant tumors. EMT also plays an important role in the occurrence and development of liver cancer, but the specific mechanism is still unclear [1]. As a tumor suppressor gene, suppressor of cytokine signaling 3 (SOCS3) is closely related to the
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Development of liver cancer. However, the relationship between SOCS3 and EMT of liver cancer is not clear [2]. Therefore, this study intends to explore the relationship between SOCS3 and EMT, which is expected to provide new ideas and approaches for the prevention and treatment of liver cancer [3-5].

2. MATERIALS AND METHODS

2.1. Materials

The high metastasis cell line MHCC97-H was provided by the Institute of liver cancer of Central South University; fetal bovine serum (FBS) and DMEM high sugar medium were purchased from hyclone company of the United States; Rabbit anti human multi antibody E-cadherin, SOCS3, a-SMA were purchased from Santa Cruz of the United States. ShRNA lentivirus was purchased from Shanghai Jikai gene. All primers were synthesized by Invitrogen company of the United States. The specific small interfering RNA (siRNA) interference fragment (genolmesmartp001. M-004299-08-0005) of SOCS3 and the negative control siRNA fragment (sienome non targeting siRNA pool, d-001206-13-05) were designed. Dharma fect 4 (t-2002-04), Siglo green fluorescence (61FAM) transfection indicator d-001630-01-05 was purchased from dharmacon company of the United States.

2.2. Hepatoma Cell Culture

The human hepatocarcinoma cell line MHCC97-H was inoculated in DMEM high glucose complete medium (100ml / L FBS, 1 million U / L penicillin and 100ms / L streptomycin), cultured in a cell incubator at 37 ℃ and 50mvl Co: and passed o

2.3. siRNA Experiment of SOCS3 in Hepatoma Cells

MHCC97-H cells (1 × 10^5) were inoculated into the 6-well plate, and the cells grew to 60% - 80% for experiment. According to the instructions of Dharma fect4 transfection, 25 nmol / L SOCS3 siRNA was used to transfect cells instantaneously (positive transfection group) and empty plasmid was used to transfect cells (negative control group). After 24 hours, the morphology of the transfected cells was observed by fluorescence microscope (13 bennikon). All the experiments received 60% - 70% transfection efficiency.

2.4. Morphological Observation of Hepatoma Cells

The morphological changes of MHCC97-H cells transfected with SOCS3 siRNA and empty plasmid were observed with inverted microscope (Nikon, Japan) at 100 and 200 times.

2.5. Immunofluorescence of Hepatoma Cells

MHCC97-H cells in the logarithmic growth stage were inoculated into 24 well plates containing glass plates. The cells were transiently transfected with 25 nmol / L SOCS3 siRNA (positive transfection group) and the cells were transfected with empty plasmids (negative control group) for 48 hours. The expression of EMT markers E-cadherin and a-SMA in MHCC97-H cells was detected by immunofluorescence staining. The steps were as follows: cells were fixed by 40 g / L polyformaldehyde solution after climbing tablets, i.e. treated with 2.5 ml / L Triton X-100 at room temperature for 10 min, washed with PBS for 5 × 3Rain, and sealed with normal goat serum at room temperature for 30 min Rabbit anti human E-cadherin (1:loo) and a-SMA (1:100) were incubated overnight at 4 ℃, PBS was used to wash 5 × 3 rain, and FITC was used to label Sheep anti rabbit II anti light and normal temperature for 2 hours. The experiment was repeated three times, and each group had three multiple holes. Glycerin was used to seal the film, laser confocal microscope (Nikon, Japan) was used to observe and take photos, and the fluorescent staining results were analyzed quantitatively.

2.6. Statistical Analysis

All data were expressed as mean ± standard deviation (x ± s), and analyzed by prism 5.0 statistical software. The data were first examined for normal distribution and homogeneity of variance, and then compared between the two groups by Student t test. The difference was statistically significant (P < 0.05).
3. RESULTS

3.1. Fluorescence Detection of Transfected Cells

After 24 h transient transfection of MHCC97-H cells with SOCS3 siRNA (25 nmol / L) and Siglo green transfection indicator (50 nmol / L), the fluorescent expression of MHCC97-H cells was observed by fluorescence microscopy. Compared with the negative control group, MHCC97-H cells transfected with SOCS3 siRNA showed green fluorescence (Figure 1). It is suggested that SOCS3 siRNA has been successfully transfected into MHCC97-H cells, which can be used for further experimental study.

![Figure 1. Fluorescent detection of transfected MHCC97-H cells](image1)

3.2. Morphological Changes after Transfection

In order to study the role of SOCS3 in the morphological changes of MHCC97-H cells, cells were transiently transfected with empty plasmid siRNA and SOCS3 siRNA for 48 hours. As shown in the phase contrast microscope in Figure 2, compared with the negative control group, SOCS3 siRNA knocks down its expression level, causing significant changes in cell morphology. Liver cancer cells changed from cobblestone shaped epithelial cells to spindle and spindle shaped stromal cells. It is suggested that siRNA down regulating the expression of SOCS3 can promote the transformation of hepatoma cells to EMT characteristics and promote their development.

![Figure 2. Effect of SOCS3 siRNA on morphological changes of cells](image2)
3.3. Immunofluorescence Expression of Epithelial Markers and Stromal Markers

In order to detect the effect of down-regulation of SOCS3 on the expression of E-cadherin and α-SMA in MHCC97-H cells, the cells were transiently transfected with empty plasmid siRNA (negative control group) and SOCS3 siRNA (positive control group) for 48 h, respectively, and the expression of E-cadherin and α-SMA was detected by immunofluorescence. The results showed that compared with the negative control group, SOCS3 siRNA significantly reduced the expression of E-cadherin, the epithelial marker of MHCC97-H cells, and significantly induced the expression of α-SMA, the interstitial marker of MHCC97-H cells (Figure 3). These results suggest that siRNA down regulating the expression of SOCS3 can promote the transformation of HCC cells to EMT characteristics, and promote the occurrence of HCC. The mechanism is related to down-regulation of E-cadherin and up regulation of α-SMA, and the expression level of SOCS3 is negatively related to the development of EMT.

![Figure 3](image1.png)

**Figure 3.** Effect of SOCS3 siRNA Oil immunofluorescent expressions of E-cadhefin and α-SMA in cells

3.4. Protein Expression of Epithelial Markers and Stromal Markers

To investigate the effect of down-regulation of SOCS3 on the protein expression of E-cadherin and α-SMA in MHCC97-H cells. The protein levels of E-cadherin and α-SMA were significantly higher than those in the control group(Figure 4).

![Figure 4](image2.png)

**Figure 4.** Western-blot detection of E-cadhefin and α-SMA expression in lung tissues of each group. *P<0.01 VS control group.
4. DISCUSSION

In this study, we found that siRNA transfection down regulated the expression of SOCS3, and the morphology of MHCC97-H cells changed from cobblestone with epithelioid characteristics to stromal cells with spindle and spindle characteristics, indicating that down regulated SOCS3 can promote the EMT of liver cancer and promote the development of liver cancer. Further studies showed that siRNA transfection interfered with the expression level of SOCS3 in MHCC97-H cells, which could reduce the expression of E-cadherin, an epithelial marker with EMT characteristics, and increase the expression of α-SMA, an interstitial marker.

SOCS3, as a negative regulator of JAK / STAT signaling pathway, has been described as a tumor suppressor in human cancer. Recent studies show that the expression of SOCS3 also plays an important role in the occurrence and development of liver cancer, and its expression level is negatively related to the survival period and prognosis of liver cancer patients, which can be used as a prognostic indicator of liver cancer [6]. The results of cell culture in vitro show that inhibition of SOCS3 can promote the growth and proliferation of hepatoma cells, similar to the results of this study. However, in vivo studies have shown that the lack of SOCS3 expression can promote the growth, invasion and metastasis of HCC. So far, the relationship between SOCS3 and EMT in HCC is still unclear [7]. Therefore, this study focuses on the effect of down-regulation of SOCS3 expression on EMT of MHCC97-H cells, and preliminarily discusses the relationship between them. Our study found that the down-regulation of SOCS3 expression by siRNA can promote the EMT process of MHCC97-H cells, suggesting that there is a negative correlation between the two, that is, the down-regulation of SOCS3 can promote the EMT process of liver cancer, so as to promote the occurrence and development of liver cancer [8].

EMT is one of the key and dynamic processes to promote tumor development, invasion and metastasis, including liver cancer. In addition to the phenotypic changes, hepatoma cells also have the molecular characteristics of EMT, including the down-regulation of e-cadherin and the up-regulation of α-SMA [9-11]. The absence of E-cadherin and the up-regulation of α-SMA make tumor cells develop EMT, which is also one of the most important molecular phenotypes to promote tumor cell development, invasion and metastasis. In this study, we further showed that the expression of e-cadherin and α-SMA were down regulated by transfection of SOCS3 siRNA into MHCC97-H cells, which promoted the formation of EMT and the development of liver cancer.

In conclusion, this study shows that siRNA transfection can down regulate the expression of SOCS3 and promote the development of liver cancer by changing the phenotype of EMT. This study suggests that SOCS3 may play an important role in EMT of liver cancer cells, which provides a new idea for clinical prevention and treatment of liver cancer. However, its specific role and mechanism still need further study.

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DISCLOSURE OF CONFLICT OF INTEREST

None

REFERENCES


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