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Effects of CHOP on Epithelial Mesenchymal Transition, Migration and Invasion of Liver Cancer

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Abstract:

Objective: *Objective to investigate the effect of CHOP on epithelial mesenchymal transition, migration and invasion of liver cancer.*

Methods: *Western blotting was used to detect the expression of CHOP in colorectal cancer cell lines, and cell lines with CHOP overexpression and knockdown were constructed; Transwell chamber test was used to detect the migration and invasion ability of cells, Western blotting was used to detect the expression of EMT related molecular marker E-cadherin; Cellular immunofluorescence was used to detect the effect of CHOP expression on the expression of E-cadherin in hepatoma cells.*

Results: *The expression of CHOP protein was the highest in SMMC-7721 cells, followed by HepG2 and Hep3B cells, and the lowest in MHCC-97 cells. Overexpression of CHOP inhibited the migration and invasion of MHCC-97 cells and increased the expression of E-cadherin, while knockdown of CHOP significantly enhanced the migration and invasion of SMMC-7721 cells and inhibited the expression of E-cadherin. Under fluorescence microscope, overexpression of CHOP increased the expression of E-caherin, while knockdown of CHOP inhibited the expression of E-caherin.*

Conclusion: *CHOP regulates the migration and invasion of liver cancer cells by participating in epithelial mesenchymal transition.*

Keywords: *Liver cancer; Epithelial mesenchymal transition (EMT); CHOP.*

1. INTRODUCTION

Liver cancer is a common gastrointestinal cancer[1]. The early invasion and metastasis of tumor cells are the main causes of death in patients with liver cancer. Therefore, it is of great significance to study the molecular mechanism of invasion and metastasis of liver cancer. CHOP is a transcription factor,

which is involved in regulating cell proliferation, differentiation and metastasis[2-5]. Recent studies have shown that CHOP plays an important role in the development and occurrence of many kinds of tumors[6,7]. The recent results suggest that the expression of CHOP in liver cancer is abnormal and is significantly related to the occurrence, development and prognosis of the patients[8-11]. However, the role and molecular mechanism of CHOP in the epithelial mesenchymal transition (EMT) and invasion and metastasis of liver cancer are not clear. Therefore, we studied the effect of CHOP on EMT regulation, migration and invasion of hepatoma cells, and explored possible molecular mechanisms.

2. Materials and Methods

2.1. Human hepatoma cancer cell line and its culture. Human hepatoma cell lines SMMC-7721, HepG2, Hep3B and MHCC-97 were purchased from the Institute of Chinese Academy of Sciences. RPMI 1640 medium containing 10% fetal bovine serum was used to culture at 37 ℃ and 5% CO2.

2.2. Plasmid construction and cell transfection. Western blot was used to detect the expression of CHOP in SMMC-7721, HepG2, Hep3B and MHCC-97 cells. The cells with high expression of endogenous CHOP were used for knockdown experiment, and the cells with low expression were used for overexpression experiment. The specific CHOP silencing siRNA (siRNA CHOP) and negative control siRNA (siRNA control) were designed and synthesized by Guangzhou saize company. The siRNA was transfected into SMMC-7721 cells with high endogenous CHOP expression by lipofectamin 2000 (Invitrogen company). The overexpression plasmid of CHOP (pcdna3.1-znf545) and control plasmid (pcdna3.1-control) were purchased from Guangzhou saize company. The plasmid was transfected into MHCC-97 cells with low expression of endogenous CHOP by lipofectamin 2000.

2.3. The influence of CHOP on the migration and invasion of hepatoma cells. In transwell chamber experiment, the cells in each group were starved for 12 h, digested and centrifuged, and then resuspended in 200 μ l serum-free medium with a cell density of 1 × 105 cells / ml. the cells were evenly spread in the upper chamber of Transwell chamber, and 400 μ l medium containing 10% fetal bovine serum was added in the lower chamber. After 48 h of culture, Transwell was fixed with methanol The cells in the lower layer of the chamber were stained with crystal violet and rinsed with water. Finally, the upper layer of cells were gently wiped off with a cotton swab and observed and photographed under a microscope. In addition to Matrigel coated Transwell cell, the other steps were the same as migration experiment.

2.4. Detection of EMT related molecular marker protein expression. Western blotting was used to extract total protein from logarithmic growth phase cells by adding appropriate amount of Ripa containing protease inhibitor (PMSF), and the supernatant was centrifuged. The protein concentration was determined by BCA method. The sample buffer was added and then boiled for denaturation. A sample of 50 g protein was taken for sample preparation, 10% polyacrylamide gel electrophoresis was carried out, and then transferred to PVDF membrane. Then 5% skim milk powder was sealed at room temperature for 2h, then added to CHOP (1: 1000, UK Abcam company), E-cadherin (Ecadherin) (1: 1000, British Abcam company), zinc phosphate finger transcription factor (slug, 1: 1000, British Abcam). The antibody was incubated at 4 ℃ overnight. After washing the membrane with tbst buffer, anti rabbit or anti mouse antibody labeled with peroxidase (1 ∶ 1000, Abcam company, UK) was added and incubated at room temperature for 2 hours. After washing the film with tbst buffer, chemiluminescence reagent was used to develop the film.

2.5. Effect of CHOP expression on E-cadherin expression in hepatocellular carcinoma cells. The cell immunofluorescence was used to detect. After the cell successfully climbed, formaldehyde was fixed

(room temperature, 30min); drop the anti-inflammatory solution of CHOP, put the glass flat in the wet box, and overnight at 4 ℃; add fluorescein isothiocyanate Rabbit anti mouse immunoglobulin G antibody, incubate at room temperature for 2 hours; then dye again with DAPI, rinse the film, observe and take photos under the high magnification of fluorescence microscope. 1.6 SPSS 25.0 statistical software was used for statistical treatment. The measurement data were expressed by single factor variance analysis, LSD-t test was used between the two groups, and the difference was statistically significant ($P < 0.05$).

3. RESULTS

3.1. Expression of CHOP in hepatocellular carcinoma cell lines. The expression of endogenous CHOP protein was the highest in SMMC-7721 cells, followed by HepG2 and Hep3B cells, and the lowest in MHCC-97 cells, as shown in Figure 1. Therefore, SMMC-7721 cells with high expression of endogenous CHOP were selected for low knockdown test and MHCC-97 cells with low expression were used in overexpression experiment.

3.2. The effect of CHOP on the migration and invasion of hepatoma cells. After overexpression of CHOP, the migration and invasion ability of MHCC-97 cells were significantly reduced, and the average number of cells passing through the basement membrane of the small chamber was significantly reduced ($P < 0.01$), as shown in Figure 2A; after the knockdown of CHOP expression, the migration and invasion ability of SMMC-7721 cells were significantly enhanced, and the average number of cells passing through the basement membrane of the small chamber was significantly increased ($P < 0.01$), as shown in Figure 2B.

3.3. The effect of CHOP on EMT related molecular markers. Overexpression of CHOP significantly increased the expression of E-cadherin, a key tumor suppressor gene related to tumor invasion and metastasis, with statistical significance ($P < 0.01$). The expression of E-cadherin was inhibited after knockdown of CHOP, and the difference was statistically significant ($P < 0.01$).

3.4. The effect of CHOP expression on the expression of E-cadherin in hepatoma cells. Compared with the control group (MHCC-97) and the negative control group (NC group), the expression of E-cadherin in the over expression group (CHOP group) of MHCC-97 was significantly increased. Compared with the control group (SMMC-7721) and the negative control group (Si-NC group), the expression of E-cadherin in the group of SMMC-7721 knockdown CHOP (Si-CHOP group) was significantly reduced.

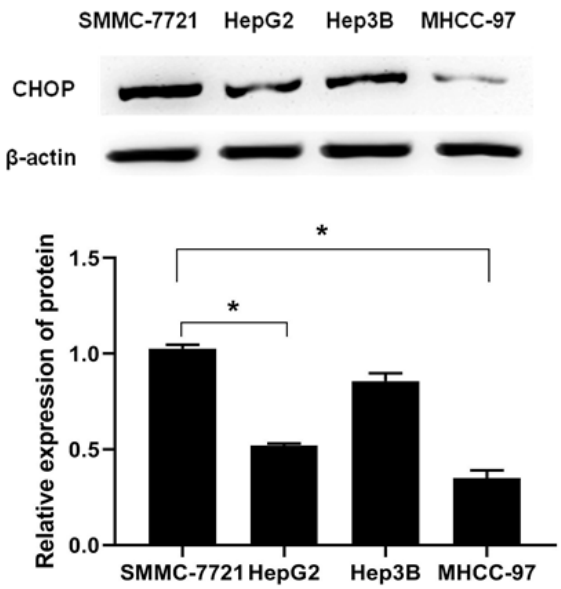

Figure1. *Electrophoretic map of CHOP protein expression in SMMC-7721, HepG2, Hep3B and MHCC-97. *P<0.01 VS control group.*

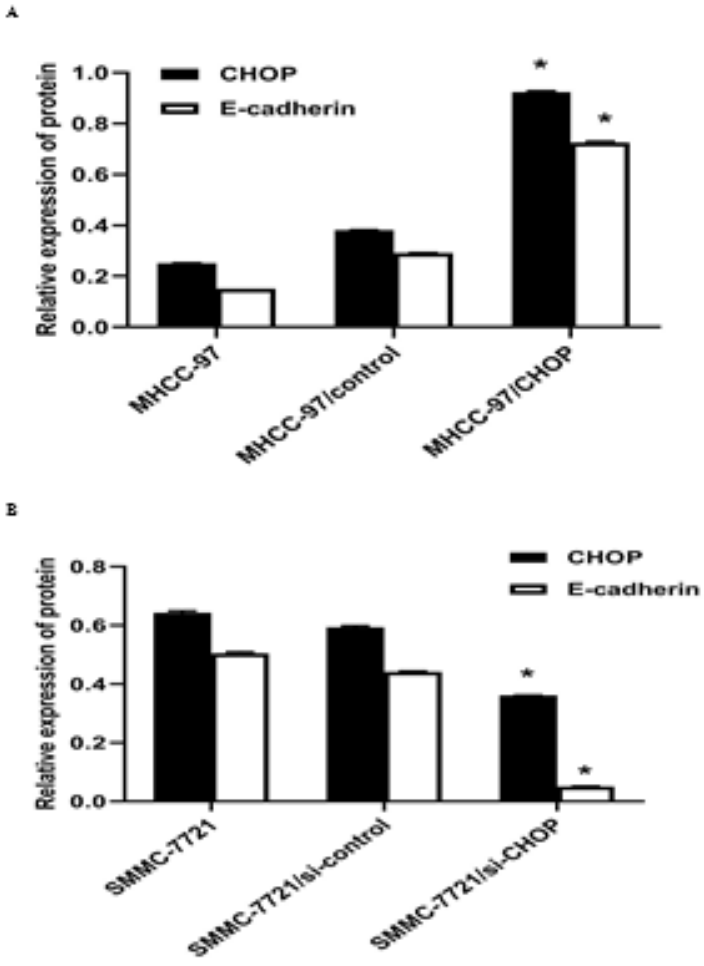

Figure2. *Effects of CHOP on EMT related molecular markers. A: Overexpression of CHOP increased the expression of E-cadherin. B: Knockdown of CHOP inhibited the expression of E-cadherin. *P<0.01 VS control group.*

4. DISCUSSION

Epithelial mesenchymal transition(EMT) behavior of malignant tumor is a biological process which has been confirmed in recent years and is closely related to tumor proliferation, invasion and metastasis[12-15]. Through EMT, epithelial cells can be transformed into mesenchymal cells with the characteristics of anti-apoptosis, degradation of extracellular matrix, loss of polarity, and promotion of invasion and metastasis. In recent years, the relationship between EMT and invasion and metastasis of hepatoma cells has increased[16]. Related studies have found that the loss of E-cadherin expression and the increase of vimentin and N-cadherin expression are common in colorectal cancer[17]. The loss of function of key tumor suppressor genes caused by gene mutation and loss of expression results in the expression changes of key EMT molecules, which play an important role in the invasion and metastasis of colorectal cancer[18].

CHOP is a newly discovered transcription factor. Studies have shown that CHOP is widely expressed in human normal tissues, but in liver cancer, gastric cancer, breast cancer and colorectal cancer, the abnormal methylation of CpG island in its promoter region leads to the loss of CHOP expression[19-21]. However, there is no report about the role of CHOP in EMT, invasion and metastasis of liver cancer In this study, we first detected the expression of CHOP in various hepatoma cell lines, then selected SMMC-7721 cells with high endogenous CHOP expression for knockdown experiment, and MHCC-97 cells with low endogenous CHOP expression for over expression experiment. The effect of CHOP on hepatoma cells was detected by Transwell chamber experiment. It was found that CHOP silencing could significantly enhance the migration and invasion ability of hepatoma cells, while CHOP overexpression could significantly reduce the migration and invasion ability of hepatoma cells. Western blotting showed that CHOP silencing could increase the expression of stromal marker protein and inhibit the expression of epithelial marker E-cadherin; overexpression of CHOP could increase the expression of E-cadherin. Cell immunofluorescence assay further confirmed that CHOP could regulate the expression of E-cadherin. These results suggest that CHOP may promote the migration and invasion of colorectal cancer cells by inducing EMT. In liver cancer tissues, abnormal methylation of CHOP promoter leads to low expression of CHOP, which in turn activates EMT transformation of colorectal cancer cells and enhances the metastatic ability of tumor cells. Therefore, CHOP plays an important role in the occurrence and development of liver cancer.

5. CONCLUSION

In conclusion, CHOP plays a role as a tumor suppressor gene in the progression of liver cancer. It can reduce the migration and invasion ability of liver cancer cells by inhibiting EMT. Targeted regulation of CHOP expression may provide a new direction for the prevention and treatment of liver cancer.

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