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Influences of siRNA on biology of hepatocellular carcinoma by specific silencing protein of PI3K/AKt signal pathway

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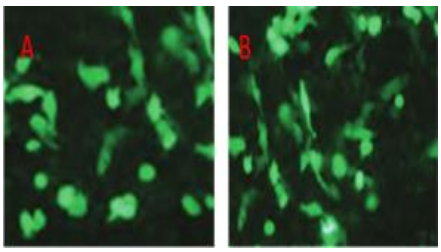
Abstract: **To study the Influences of siRNA on Biology of hepatocellular carcinoma by specific silencing protein in PI3K/AKt signal pathway, further to explore the molecular mechanism of PI3K/AKt signal pathway in the development of hepatocellular carcinoma. PI3K-siRNA and AKT-siRNA were transfected into hepatocellular carcinoma cells line MHCC-97H respectively by lipofectermine 3000, and the PI3K and Akt protein expression level in liver cancer MHCC-97H cells were detected with Western blotting method, while the Liver cancer cell proliferation and cell cycle change were determined by Transwell invasion and flow cytometry. After transfection, PI3K, and Akt protein level were significantly decreased. siRNA can significantly decrease levels of PI3K and Akt protein in MHCC-97H cell lines, and can significantly inhibit the MHCC-97H cell proliferation and the process of cell cycle. It was also can promoted apoptosis (P< 0.05). siRNA can specific silencing protein of PI3K/AKt signal pathway, and to control the malignant biological behavior of tumors. PI3K/AKt signal pathway can become a new target for clinical treatment.**



Keywords: siRNA; PI3K/AKt signaling pathway; Hepatocellular carcinoma

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1. Introduction

Hepatocellular carcinoma (HCC) is a kind of invasive malignant tumor and lack of effective treatments [1]. Many factors affect the prognosis of liver cancer patients, including clinical factors, treatment, and molecular biology, etc., which the metastasis of tumor is closely related to the prognosis [2]. Hepatocellular carcinoma is one of the common malignant tumors in the world, and China is a high incidence of hepatocellular carcinoma (HCC). In recent years, the treatment of liver cancer is increasing, but the treatment effect is not ideal, because of tumor recurrence and metastasis, patients survival rate reduce [3]. The occurrence of liver cancer, invasion and metastasis are the result of multifactor comprehensive. Research on liver cancer cells about the process of the mechanism and design corresponding intervention measures is particularly important. Academics have long argued that the tumor is not only composed of tumor cell mass lesions, but by the tumor cells, fibroblast cells, immune cells and inflammatory cells, glial cells and other cells to construct heterogeneous mixture [4-5], Further study the molecular mechanism of PI3K/AKt signaling pathway in promoting malignant biological behavior of liver cancer, it can improve the effect of the treatment of liver cancer and guide clinical application.

2. Material and methods

2.1. Material and Cell culture

Hepatocellular carcinoma cell line of MHCC-97H bought from Shanghai cell bank of Chinese academy of sciences. rabbit anti PI3K, and Akt antibodies bought from Abcam company, β -actin antibodies bought from the Cell Signal Corpo-ration; PBS liquid cushion, PCR primers bought from Invitrogen Company. Lipopolysaccharide and western blot kits purchase from Jinqiao Company. Cells culture in 1640 completely medium containing 10% fetal bovine serum, 2% glutamine, 100U/L streptomycin and100 U/m L penicillin at 37℃, 5% CO_2 incubator. Experiment is set to 3 groups, siPI3K group, the siAkt, Control group.

2.2. Western blot

Detecte PI3K, and Akt protein expression with Western blot before and after transfection. Collection of each experiment cell, protein extraction and purification using protein extraction kit (Beijing Pulitzer kit) and determination of protein concentration with BCA method. Making up 10% of SDS separation adhesive, and put it on the sample, electrophoresis, transfer to nitrocellulose membrane, sealing 2 hours at room temperature, rabbit anti PI3K antibodies and rabbit anti Akt antibodies (Santa Cruz) were diluted to 1:150 and incubated at 4℃ for the night. horseradish peroxidase rabbit anti sheep IgG (second resistance, Jinqiao Beijing company) were diluted to 1:5000 and proceeding immune response. Washing, exposuring, development and fixing, filming pictures and analyzing purposes banding using Bander Leader software.

2.3. Transwell invasion experiment

Transwell invasion experimental analyzes longitudinal migration ability for hepatocellular carcinoma. In upper Transwell Chambers (24 hole, Diameter: 8 microns, Corning), 10μ l fiber link protein (40 mg/l) were Packaged at the bottom of the membrane surface and placed in 4℃ for 48 h. The 50μl Matrigel (1:3 dilution with serum-free DMEM broth) join in Transwell nesting incubation 37℃ for 30 min. EDTA-Trypsin digest and collect different activated macrophages cells, mading from cell suspension using serum-free culture medium (DMEM-F12 join 2% B27, insulin 140 U/100 ml and 0.4% BSA). Hep G2 cells of liver cancer were inoculated on the Transwell room (1×106 cells/hole), and different activated macrophages were inoculated in t lower chamber, which joining 20% fetal bovine serum. Cultivation in 37 ℃ for 20h, matrix in transwell indoor were removed by swab and with fixed Invading cells in the Transwell upper chamber membrane with 5% paraformaldehyde for 10 min, staining 20 min with 1% crystal violet, The experimental results were analyzing by Image J software. The experiment repeated three times.

2.4. Cell cycle detection

Digesting and collecting normal group and the transfection cells by excluding EDTA pancreatic enzyme, washing three times with PBS, cells were suspended with 100μl RNase A and incubated in 37℃ water bath solution for 30min. Joining 400μl PI dying liquid, thoroughly incorporated, cells were incubated for 30min at 4℃ avoid light condition and detected the cell cycle with Flow cytometry instrument. The experiment repeated three times.

2.5. Statistical methods

Date analyzed with SPSS 16. 0 statistical software, using variance analysis for multiple groups comparison, and using least significant difference methods between groups. The difference had statistically significant when P<0. 05.

3. Results

3.1. Detection of transfection efficiency

After transfection for 24 hours, Green fluorescent protein expression in siPI3K group and si-Akt group. It suggests that it had successful transfection. There were observed under fluorescence microscope (Figure1).

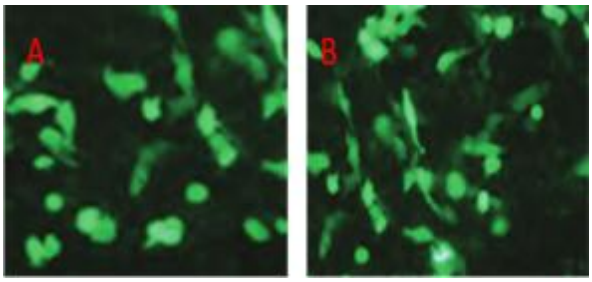

Figure 1. Green fluorescent protein expression of MHCC-97H cells transfected with siPI3K and si-Akt after 24h detected by fluorescent microscope (×200) A: siPI3K group B: si-Akt group.

3.2.Detection of PI3K, and Akt protein expression in Hep G2 cell lines with Western blot

Akt and PI3K protein expression levels in experimental group were significantly lower than control group (Figure 2).

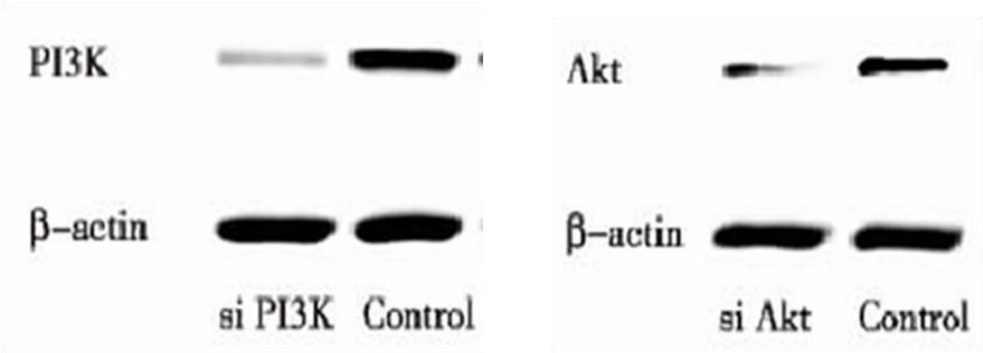

Figure 2. PI3K and Akt protein expressed can be inhibited in MHCC-97H cells after transfection with siRNA. Note: Compared with Control group, siPI3K protein and Akt protein levels were significantly lower (P=0.001).

3.3. Transwell invasion experiment

Compared with the Control group, the si-PI3K and si-Akt small molecules interference fragment can significantly inhibit MHCC-97H cells vertical infiltration capacity (Figure 3).

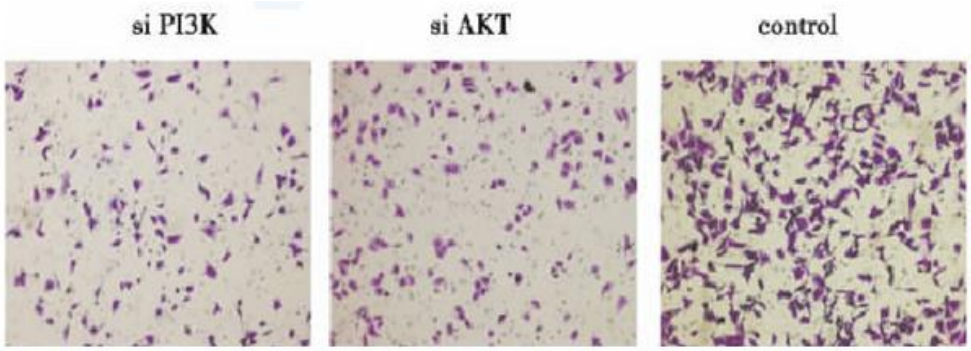

Figure 3. Selective macrophages can promote MHCC-97H cells of vertical infiltration capacity. Note: * * P < 0.01.

3.4. Detection of cell cycle

Cell cycle detection results show that S, G2 period cells increased significantly after the interference of si PI3K and siAkt (P=0.001), and G1 phase cell ratio decreases. It shows that cells cycle arrest in G2 / M phase (Figure 4).

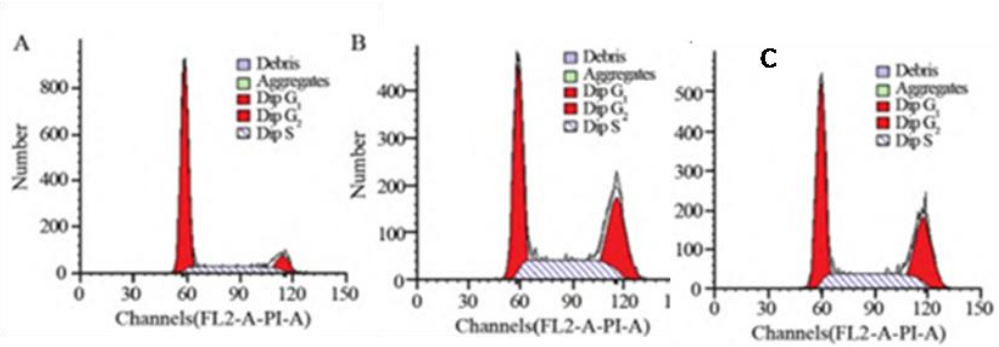

Figure 4. Detection of cell cycle A: Control group B: si PI3K group C: siAkt group

4. Discussion

Hepatocellular carcinoma (HCC) is one of malignant tumor, as well as the cells in the vicinity of including interstitial, microvascular and infiltration of many biological molecules [6]. Previous studies showed that many malignant tumors such as pancreatic cancer, prostate cancer, breast cancer around the tumor is present a very obvious interstitial reaction [7]. As early as in 1889, Stephen Paget based on organ specificity of breast cancer metastasis in clinical observation, put forward the concept of the famous "seed and soil" [8].

Invasion adjacent normal tissue and distant metastasis is one of important biological characteristics of tumor [9]. Research shows that in liver cancer, stomach cancer, colon cancer, breast cancer, pancreatic cancer and other tumor tissues, there were excessive expression and activation of PI3K/Akt signal pathway, It both play an important role in the development of malignant tumor, invasion, migration, proliferation, apoptosis and treatment outcome [10]. Related present research shows that the Akt-2 express in hepatocellular carcinoma and being with 46% of Akt phosphorylation-2 phenomenon [11]. The activation of PI3K/Akt signal pathway may cause liver cancer patients with loss of chromosome 10 protein phosphatase and tension of the homologous genes abnormal expression [12]. In addition, with the combination of different growth factor and tyrosine kinase receptor, Akt phosphorylation increase, and then affect the adhesion and proliferation of tumor cells. At the same time, the downstream effect of Akt molecular mTOR also plays an important role in the process of the evolution of liver cancer. In the mouse model of liver cancer, application of mTOR inhibitors rapamycin can reduce tumor growth and metastasis [13]. Experiment combined with siRNA transfection to silence Akt or PI3K effects, detected pathways of PI3Kand Akt expression level changes, and explore the molecular mechanism of TAMs in tumor.

The experimental results showed that si-PI3K and si-Akt small molecules transfection separately can obviously inhibit liver cancer cell invasion ability to migrate. Compared with negative control group,It can significantly decrease the PI3K, and Akt protein expression level in liver cancer cells. The results fully show that behavior of invasion and migration in liver cancer cell were inhibited by PI3K/Akt signaling pathway. Results show that Akt was the downstream target of PI3k.Liver cancer was controlled by evaluating PI3k/Akt pathway.

5. Conclusion

To sum up, this study focuses on the influences of PI3K/Akt signaling pathway on liver cancer cells of migrating and infiltrating ability. PI3K, and Akt may become the new target of diagnosis and treatment for anti-tumor invasion and migration.

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