Influence of PI3K p85α expressing deletion on invasion and metastasis of colorectal cancer cells

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Abstract Objective: To investigate the influence of RNA interference targeting PI3K p85α on the invasion and metastasis of human colorectal cancer LoVo cells. Method: Lentiviral PI3K p85α interference vectors were constructed and stably transfected into LoVo cells. Wound healing assay and Transwell assay were used to determine the influence of PI3K p85α expression on invasion and metastasis of colorectal cancer cells. Results: Wound healing assay showed that the healing ability of PI3K p85α-depleted cells declined significantly; results of Transwell assay indicated that a decrease in PI3K p85α expression resulted in a significant decrease in invasive potential and motility. Conclusion: Depletion of PI3K p85α protein expression can obviously inhibit the migration of LoVo cells. PI3K p85α may be a new therapeutic target for treatment of colorectal cancer metastasis.

Keywords: PI3K p85α; RNA interference; colorectal neoplasms; neoplasm metastasis

The initiation and progression of colorectal cancer often involves genetic abnormality, such as the over-expression of oncogene and the mutation or deletion of tumor suppressor gene. Distant metastasis is often a main reason leading to the death of patients with colorectal cancer [1]. The recent researchers have found that Phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT), as a major signal pathway of cell growth and proliferation, plays an important role in cell proliferation, differentiation, invasion and migration [2]. IA Type PI3K/Akt activated by tyrosine kinase receptors on the surface of cells is probably the most extensively studied in the PI3K family. It is composed of an 85-kDa regulatory subunit (p85) and a 110-kDa catalytic subunit (p110). The regulatory subunits p85α encoded by the PIK3R1 gene is the most abundantly expressed regulatory isoform of PI3K and is essential for the stability and aggregation of the p110 catalytic subunit and for the activation of PI3K, furthermore, its mutation or amplification can lead to activation of PI3K[3]. In a previous study, we have demonstrated that the loss expression of regulatory subunits p85α can inhibit the proliferation of colorectal cancer cells and increase their sensitivity to chemotherapeutic drugs [4]. In this study, to investigate the influence of PI3K p85α expressing deletion on the invasion and metastasis of human colorectal cancer cells, lentiviral PI3K p85α interference vectors were constructed and stably transfected into LoVo cells. The results might provide a potential therapeutic strategy for colorectal carcinoma.

1 Materials and methods

1.1 Cell culture and transfection

The human colorectal cancer cell lines LoVo (ATCC, USA) were grown in RPMI-1640 supplemented with 10% fetal bovine serum under standard cell culture conditions (37°C, 5% CO₂ in a humidified incubator). Lentiviral PI3K p85α interference vectors were supplied by Forevergen Bioscience Co., Ltd. The pLL-PI3K p85α-shRNA lentiviral vectors were prepared, and then the lentiviral packaging plasmids(pGag/Pol; pRev; pVSV-G) were transfected into 293T cells using Lipofectamine 2000. At 48 h after transfection, fluorescence microscope was used to measure the proportion of green fluorescent protein (GFP) positive lentivirus to determine whether the lentiviral vector plasmids were packaged. At 72 h after transfection, the lysates were collected by scraping from the plates and centrifuging at 4000G for 120 min and western blot assay were employed to measure the protein infected with LoVo cell line.

1.2 Wound healing assay

LoVo cells and cells of control group were seeded in 12-well plates at a density of 2×10⁵ per well. While monolayer of cells covered the bottom of the hole, scratch with tip (10ul) vertically, and take pictures to observe the scratches healing ability at 0h, 24h, 48h and 72 h, respectively.

1.3 Transwell migration assay

Cells of Control group and interference group in logarithmic growth phase were treated with serum-free RPMI1640 starvation overnight. The cell suspension(4 × 10⁵ cells/ml, 100μl) was added into each upper chamber. Each lower chamber was added into 600μl of RPMI1640 medium containing 10% FBS. Three-parallel-well cells were cultured at 37 °C in a humidified environment with 5% CO₂. At 12h after incubation, filter membrane was taken out, and the number of cell permeating septum was finally determined in 200 times under the microscope vision after Giemsa's staining.
1.4 Transwell invasion assay

Polycarbonate membrane of the Transwell chamber was coated with 40μl of Matrigel (500μg/ml), and dried overnight in clean bench. Cells of control group and interference group in logarithmic growth phase were treated with serum-free RPMI1640 starvation overnight. The cell suspension (4×10⁵ cells/ml, 150μl) was added into each upper chamber. Each lower chamber was added into 600μl of RPMI1640 medium containing 10% FBS. All the three-parallel-well cells were cultured at 37 °C in a humidified environment with 5% CO₂. At 48h after incubation, filter membrane was taken out, and the number of cell permeating septum was finally determined in 200 times under the microscope vision after Giemsa's staining.

1.5 Statistical analyses

Statistical analysis was performed by using SPSS13.0 Software. The comparisons among different group were done with Student's t-test. All experiments were performed three separate times. Data are expressed as means ± SD. P<0.05 was considered as statistically significant.

2 Results

2.1 Results of Western blot assay to validate PI3K p85α protein expression changes after transfection

As shown in Fig. 1 and Table 1, Western blot assay revealed that the protein levels of PI3K p85α were decreased 83 % in LoVo cells transfected with p85α siRNA compared to those of control cells. The effect has significant difference compared with that of the control group (P<0.05).

![Figure 1. Effect of RNA interference on PI3K p85α protein expression](image1)

Table 1. Effect of RNA interference on PI3K p85α protein expression

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Relative density (mean±SD)</th>
<th>RNAi effect</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>825.13±116.92</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNAi</td>
<td>3</td>
<td>298.39±56.04</td>
<td>74%</td>
<td>5.704</td>
<td>0.005</td>
</tr>
</tbody>
</table>

2.2 Results of wound healing assay

As shown in Fig. 2, the cellular migration ability of interference group significantly reduced compared to that of the control group. The "wound" of control group has been close to healing after 72 hours, while migrations of interference cells are not very noticeable. The spacing difference measurement of three sites between 0h and 72h was taken as cell migration distance. As shown in Table 2, the effect of interference group has significant difference compared with that of the control group (P<0.05).

![Figure 2. Effect of PI3K p85α depletion on wound closure of LoVo cells](image2)
### Table 2 Effect of PI3K p85α depletion on wound closure of LoVo cells

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>±s</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3</td>
<td>0.43±0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RNAi</td>
<td>3</td>
<td>0.14±0.01</td>
<td>28.256</td>
<td>0.001</td>
</tr>
</tbody>
</table>

2.3 Results of Transwell assay

As shown in Fig. 3 and Table 3, results of Transwell migration assay showed that the number of cell permeating septum of interference group at 12h after transfection were obviously decreased compared to those of control cells \((P<0.05)\); As shown in Fig. 4 and Table 3, results of Transwell invasion assay showed that the number of cell permeating septum of interference group at 48h after transfection were obviously decreased compared to those of control cells \((P<0.05)\).

### Table 3. Effect of PI3K p85α depletion on transwell migration and invasion LoVo cells

<table>
<thead>
<tr>
<th>LoVo</th>
<th>Control</th>
<th>RNAi</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration</td>
<td>34.67±5.50</td>
<td>9.67±1.15</td>
<td>7.695</td>
<td>0.002</td>
</tr>
<tr>
<td>Invasion</td>
<td>550.13±26.15</td>
<td>79.05±39.15</td>
<td>17.326</td>
<td>0.001</td>
</tr>
</tbody>
</table>

### Figure 3. Effect of PI3K p85α depletion on transwell migration LoVo cells

### Figure 4. Effect of PI3K p85α depletion on transwell invasion LoVo cells

3 Discussion

Obtaining migration and invasion phenotype is the critical process in tumor progression and metastasis, as well as an extremely complex pathological process which has a close relationship with tumor development and prognosis of the patient's condition \([5]\). The mechanism of metastasis is extremely complex, while recent years’ studies found that for many human tumors, such as ovarian cancer, pancreatic cancer, breast cancer, non-small cell lung cancer and oral squamous cell carcinoma, the PI3K/Akt pathway ingredients were often over expression \([6-8]\). Basically, the mechanism of PI3K/Akt signal transduction pathway contributing to tumor metastasis includes several aspects as follows: (1) Activation of PI3K/Akt signal transduction pathway increases the athletic ability of the tumor cells; (2) PI3K/Akt signal transduction pathway promotes tumor metastasis by modulating the growth factor receptor; (3) PI3K/Akt signal transduction pathway promotes tumor metastasis by reducing the cell adhesion; (4) PI3K/Akt signal transduction pathway promotes tumor metastasis by virtue of the effect on extracellular matrix; (5) PI3K/Akt signal transduction pathway promotes tumor metastasis by increasing the activity of nuclear transcription factors; (6) PI3K/Akt signal transduction...
pathway promotes tumor metastasis by phosphorylating related enzymes; (7) Activation of PI3K/Akt signal transduction pathway promotes membrane translocation, thereby facilitating the invasion. Therefore, direct inhibition of the PI3K/Akt signal transduction pathway or interference of its upstream material is expected to inhibit the invasion and metastasis of malignant tumors and to become a highly targeted mechanism for delivering cancer-fighting gene therapy.

The regulatory subunits PI3K p85α protein is the most abundantly expressed regulatory isoform of PI3K. In our previous studies, we have demonstrated that the loss expression of regulatory subunits p85α can inhibit the proliferation of colorectal cancer cells and increase their sensitivity to chemotherapeutic drugs and RNA interference specifically knocking out or turning off the expression of specific genes can be used to explore the function of specific genes. Therefore, in the present paper we constructed lentiviral PI3K p85α interference vectors and stably transfected them into LoVo cells. The results showed that PI3K p85α protein used for subsequent experiments can be obviously inhibited.

In this study, after the transfected lentivirus interference vectors knocked out the expression of PI3K p85α protein, Wound healing assay was used to study the correlation between the PI3K p85α expression levels and migration ability of colorectal cancer cell. The results showed that the healing ability of PI3K p85α-depleted cells declined significantly. The "wound" of control cells have been close to healing after 72 hours, while migrations of interference cells were not very noticeable. Results of Transwell migration and invasion assay showed that the number of cell permeating septum of interference group at 12h and 48h after transfection was obviously decreased compared to those of control cells. Heterogeneous adhesion experiment also suggests that cell adhesion capacity of interference cells was obviously decreased. These survey results indicate that the improvement of invasion ability and athletic ability of tumor cells caused by PI3K p85α protein abnormalities may be one of the mechanisms that lead to colorectal cancer metastasis. It can inhibit the biological behavior of malignant cells to reduce PI3K p85α protein expression levels and metastasis.

All in all, This research proved in many ways that depletion of PI3K p85α protein can reduce the athletic ability of colorectal cancer cells. Moreover, intensive research on the mechanism of molecular biology of affecting tumor metastasis was not only conducive to elucidate the molecular mechanisms of colorectal cancer metastasis, can also deepen the understanding of the function of PI3K p85α and search effective targeted and anti-angiogenic therapy to tumors.

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**References**


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