Up-regulation of miR-149 by E-cadherin inhibits breast cancer cell invasion and migration

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ARTICLE INFO ABSTRACT

Article history:
Received 4 Aug 2016
Received in revised form 17 Aug 2016
Accepted 15 Aug 2016
Available online 24 Aug 2016

Keywords:
Micro RNA-194
Breast cancer
E-cadherin
Cell invasion
Cell migration

Objective: To investigate the effects and possible mechanism of MicroRNA (miR)-149 expression and over-expression in breast cancer cells on their invasion and migration.

Methods: Relative transcript levels of miR-149 in 60 cases of breast cancer tissues and para-carcinoma tissues, 50 cases of normal breast tissues were detected by real-time PCR. Transient transfection of breast cancer MDA-MB-231 was conducted by miR-149 mimics (MiR-149 transfection group), transient transfection by empty vector was set as control group and culture solution group was set, relative transcript levels of miR-149 in each group were detected by real-time-PCR, cell invasion and migration abilities in each group were detected by transwell chambers method, protein expression of E-cadherin was detected by western-blot. Results: Relative transcript levels of miR-149 in breast cancer tissues were significantly lower than that in para-carcinoma tissues and normal breast tissue, and it was considered to be statistically significant. Relative transcript levels of miR-149 in para-carcinoma tissues and normal breast tissue were not statistically significant. Relative transcript levels of miR-149 in different time points after transfection (MiR-149 transfection group) were significantly higher than that in transient transfection by empty vector and culture solution group, and it was considered to be statistically significant. Comparison of control group and culture solution group was not statistically significant. Invasive cell number and migration cell number in miR-149 transfection group were both significantly lower than that in control group and culture solution group, and it was considered to be statistically significant. Comparison of control group and culture solution group was not statistically significant. Protein expression of E-cadherin in miR-149 transfection group was significantly higher than that in control group and culture solution group, and it was considered to be statistically significant. Comparison of control group and culture solution group was not statistically significant. Conclusion: miR-149 was low expression in breast cancer tissues, over-expression of miR-149 may inhibit breast cancer cell invasion and migration by up-regulation of E-cadherin.

1. Introduction

Breast cancer is a malignant tumor with relatively good prognosis in women, but tumor recurrence and distant metastasis is the important cause of death in patients with breast cancer[1]. Micro RNA (microRNA, miRNA) is a kind of non-coding RNA, and it plays an important role in the occurrence and development of tumor, and plays a regulatory role of a wide variety in tumor biological behaviors[2-4]. Luo et al[5] reported that miR-14 may play the role of tumor suppressor genes and could inhibit hepatocellular carcinoma metastasis. There are few researches about miR-149 in the breast cancer at home and abroad, therefore, this study investigate the effects and possible mechanism of MicroRNA (miR)-149 expression and over-expression in breast cancer cells on their invasion and migration.

2. Clinical data and methods
2.1. Specimen tissues

A total of 60 cases of breast cancer tissue samples were from July 2014 to February 2016 in the General Surgery Department of Shanghai Jing’an District Central Hospital. 60 cases of patients were all female with the age from 25 to 72, and the median age was 44. Patients have not been accepted preoperative radiotherapy and chemotherapy. Para-carcinoma tissues were removed from 5 cm in the edge of the tumor tissues, and there was no cancer cell invasion by pathological examination. In addition, 50 cases of normal breast tissues were from mammary gland excision during the same period of hyperplasia, and there was no cancer cell invasion by pathological examination. All tissue samples were immediately preserved in liquid nitrogen after being selected. This study was approved by the Ethics Committee, all patients volunteered for the study and signed the informed consent.

2.2. Cell culture

Breast cancer cells MDA-MB-231 were cultured in RPM11640 medium with 10% fetal calf serum, in the condition of 37 °C, 5% CO2 and saturation humidity. Adherent growth cells in the exponential phase after passage steady for 3 times were selected for follow-up experiments.

2.3. Expression of miR-149 by real–time–PCR

Total RNA was extracted from cells and tissues. The reverse transcription reaction system were prepared and cDNA was produced according to manufacturer’s protocols. 2 μL of cDNA were taken and 25 μL of real-time PCR mixture were prepared. According to the temperature-time profile as following: pre-denaturation of 95 °C for 5 min, denaturation of 94 °C for 1 min, 30 cycles of 55 °C for 15 s, 72 °C for 1 min, then extend of 72 °C for 10 min. The dissolution curve was made after reaction. RNU6B was selected as the internal control and the relative expression levels of miR-149 were calculated.

2.4. Transfection of breast cancer cells MDA–MB–231 by miR–149mimics

Breast cancer cells MDA-MB-231 were inoculated in 96-well plates, and cultured until the cells at high confluence with about 50%, 100 pmol/L miR-149mimics and 5 μL LipofectamineTM2000 were added in miR-149 transfection group according to the kit instruction, FAM-mimics NC was added in control group, isovolume culture medium was added in culture solution group, and placed in 50 mL/L CO2 and saturation humidity, and cultured at 72 °C for 6 h, then the medium was replaced and the cells were continued to culture.

2.5. Cell invasion and migration abilities detected by transwell chambers

The cells after 72 h transfection were prepared, and resuspended by serum DMEM to adjust the cell density. Isovolum cell suspensions were added in upper embedded culture room without Matrigel coating (migration test) or with Matrigel coating (matrigel test), 600 μL DMEM containing 10% FBS were added in bottom of each well, 3 repetitive wells were set in each group, and dyed by 1 g/L crystal violet after culture in 5% CO2 and at 37 °C for 48 h. Microscopic examination was conducted after flushing and air drying, 6 horizons were randomly selected in each well under light microscope and average was calculated. The experiment repeated 3 times, the data averaged.

2.6. Protein expression of E-cadherin detected by Western–blot

Breast cancer cells after transfection were cracked for 30 min, supernatant was got after centrifugation, 12% polyacrylamide gel electrophoresis was selected, trasmembran was conducted for 12 h in the condition of 23 V and 4 °C, the second antibody was added after TBST rinse, incubation at indoor temperature for 1 h, coloration after TBST rinse, film exposure. β -action was selected as the internal control and the relative protein expression of E-cadherin was calculated.

2.7. Statistics

Measurement data were described as mean ± standard deviation, pairwise comparison was conducted by analysis of variance and SNK-q test, SAS8.1 statistical software was adopted for data analysis. Values of \( P < 0.05 \) were considered to be statistically significant.

3. Results

3.1. Low expression of miR–149 in breast cancer tissues

Real-time PCR results showed that the relative transcript level of miR-149 in breast cancer tissues was (2.67±0.73), which was significantly lower than that in para-carcinoma tissues (4.92±1.43) and normal breast tissue (5.08±1.36), and it was considered to be statistically significant (\( P < 0.05 \)). Relative transcript levels of miR-149 in para-carcinoma tissues and normal breast tissue were not statistically significant (\( P > 0.05 \)).

3.2. Increased expression of miR–149 after miR–149mimics transfection in breast cancer cells MDA–MB–231

Relative transcript levels of miR-149 in different time points after transfection were significantly higher than that in transient transfection by empty vector and culture solution group, and it was considered to be statistically significant (\( P < 0.05 \)). Comparison of control group and culture solution group was not statistically significant (\( P > 0.05 \)). See Table 1.
Obstacle to breast cancer was due to cancer metastasis, so the cancer metastasis is a major cause of patient mortality through targeted regulatory role and clarifying the molecular mechanisms of invasion and metastasis breast cancer, and improve the life quality of the patients. miRNA is a kind of RNA molecules with coding protein functions, an increasing number of studies have found that abnormal expression of miRNA plays an important role in breast cancer[7-9]. Understanding the biology function and mechanism of miRNA in breast cancer could provide help for early diagnosis and treatment of breast cancer.

3.3. Over-expression of miR-149 significantly inhibit breast cancer cells invasion and migration

After transfection for 72 h, cell invasion and migration abilities in each group were detected by transwell chambers method, invasive cell number was detected with Matrigel coating and migration cell number was detected without Matrigel coating, the results showed that invasive cell number and migration cell number in miR-149 transfection group were both significantly lower than that in control group and culture solution group, and it was considered to be statistically significant (P<0.05). Comparison of control group and culture solution group was not statistically significant (P>0.05). See Table 2.

Table 2. Breast cancer cells invasion and migration detected by transwell chamber.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Invasive cell number (each/horizon)</th>
<th>Migration cell number (each/horizon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-149 transfection</td>
<td>6.25±1.53</td>
<td>6.92±1.76</td>
</tr>
<tr>
<td>Control</td>
<td>9.33±2.64</td>
<td>9.94±2.79</td>
</tr>
<tr>
<td>Culture solution</td>
<td>10.16±2.58</td>
<td>10.68±2.68</td>
</tr>
</tbody>
</table>

Note: Compared with miR-149 transfection group, P<0.05.

3.4. Over-expression of miR-149 enhance protein expression of E-cadherin

Western blot results showed that protein expression of E-cadherin in miR-149 transfection group was significantly higher than that in control group and culture solution group, and it was considered to be statistically significant (P<0.05). Comparison of control group and culture solution group was not statistically significant (P>0.05). See Table 3.

Table 3. Relative protein expression of E-cadherin detected by Western blot.

<table>
<thead>
<tr>
<th>Groups</th>
<th>E-cadherin/β-actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>miR-149 transfection</td>
<td>0.75±0.08</td>
</tr>
<tr>
<td>Control</td>
<td>0.30±0.05</td>
</tr>
<tr>
<td>Culture solution</td>
<td>0.28±0.06</td>
</tr>
</tbody>
</table>

Note: Compared with miR-149 transfection group, P<0.05.

4. Discussion

It was reported that the death of exceed 90% breast cancer patients was due to cancer metastasis, so the cancer metastasis is a major obstacle to breast cancer[6]. Invasive and mobility of mammary gland epithelial cells significantly increased after canceration, and caused widely spread of primary tumors. Therefore, it is expected to reduce patient mortality through targeted regulatory role and clarifying the molecular mechanisms of invasion and metastasis breast cancer, and improve the life quality of the patients. miRNA is a kind of RNA molecules with coding protein functions, an increasing number of studies have found that abnormal expression of miRNA plays an important role in breast cancer[7-9]. Understanding the biology function and mechanism of miRNA in breast cancer could provide help for early diagnosis and treatment of breast cancer.

miRNA mainly play a variety of biological functions through combining with the 3’-end non-coding regions, including angiogenesis, embryonic development and cell growth etc[10-12]. Besides, miRNA also could act as oncogenes or tumor suppressor genes, and play an important role in a series of activities including the occurrence, development, invasion and metastasis of cancer[13,14]. miR-149 was reported in a wide variety of tumor, Oster et al[15] found that down-regulation of miR-149 may regulate the transcription of SRPX2 in colorectal cancer which was involved in the initiation and progression of colorectal cancer. Xue et al[16] found that low expression of miR-149 is closely related to the prognosis of patients in glioma, it could be involved in the occurrence and development of glioma through the activation of Akt/mTOR. Low expression of miR-149 can increase the risk of stomach cancer according to a meta-analysis data[17]. As Normally accepted, miR-149 is a kind of tumor suppressor genes and its expression is related to maintain normal cell mobility.

There are few researches about miR-149 in breast cancer at home and abroad, the relative expression levels of miR-149 in 60 cases of breast cancer tissues and para-carcinoma tissues, 50 cases of normal breast tissues were detected by real-time PCR. The results showed that relative transcript levels of miR-149 in breast cancer tissues were significantly lower than that in para-carcinoma tissues and normal breast tissue, but relative transcript levels of miR-149 in para-carcinoma tissues and normal breast tissue were not statistically significant (P>0.05). The results indicated that miR-149 was low-expression in breast cancer. Transient transfection of breast cancer cells was further conducted by miR-149 mimics, and the results showed that relative transcript levels of miR-149 in different time points after transfection were significantly higher than that in transient transfection by empty vector and culture solution group, invasive cell number and migration cell number in miR-149 transfection group were both significantly lower than that in control group and culture solution group, and it was considered to be statistically significant. The results indicated that over-expression of miR-149 could significantly inhibit the invasion and metastasis of breast cancer cells.

E-cadherin is a kind of cell adhesion molecules, which is involved in tumor invasion and migration[18]. Low expression of E-cadherin could enhance the epithelial cell migration and cause original extremely disappear of cells, and the ability of cells invade...
surrounding tissues is enhanced [19]. Zhang et al. [20] found that metastasis-associated protein 2 (MTA2) could promote non-small cell lung cancer metastasis by inhibiting E-cadherin expression. To further investigate the mechanism of miR-149 regulating invasion and migration of breast cancer cells, protein expression of E-cadherin was detected by western-blot. The results showed that protein expression of E-cadherin in miR-149 transfection group was significantly higher than that in control group and culture solution group, and over-expression of miR-149 may inhibit breast cancer cell invasion and migration by up-regulation of E-cadherin.

In conclusion, this study found that miR-149 was low-expression in breast cancer cells, and over-expression of miR-149 may inhibit breast cancer cell invasion and migration by up-regulation of E-cadherin, but its specific function and mechanism still needs further discussion. In addition, miRNA has broad biological functions, and it still needs further research to investigate whether it could be involved in the regulation of breast cancer cell migration and invasion by other functions.

References

[16] Xue L, Wang Y, Yue S. Low MiR-149 expression is associated with unfavorable prognosis and enhanced Akt/mTOR signaling in glioma. Int J Clin Exp Pathol 2015; 8(9): 11178-84.