Serum midkine expression in breast cancer patients and its clinical significance
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ABSTRACT
Objective: To study serum midkine expression in breast cancer patients and its clinical significance. Methods: A total of 45 cases of patients with breast cancer and 45 cases of patients with benign breast tumor were selected for study, breast tumor specimens were collected to detect mRNA content of MK and serum was collected to detect protein content of MK; breast cancer MCF-7 cell lines were cultured and transfected with varying concentrations of MK expression plasmid, and then cell proliferation and apoptosis, VEGF expression in media as well as MMPs and TIMPs expression in cells was detected. Results: MK expression in breast tissue and serum MK content of breast cancer patients were higher than those of benign breast tumor patients, and MK expression in breast tissue and serum MK content of breast cancer patients with TNM Ⅰ/Ⅱ stage, low/un-differentiation and lymph node metastasis were higher than those of breast cancer patients with TNM Ⅲ/Ⅳ stage, medium/high differentiation and without lymph node metastasis; MK expression plasmid could dose-dependently increase mRNA content and protein content of MK in breast cancer cell lines, increase cell viability and decrease apoptosis percentage; VEGFA, VEGFB and VEGFC contents in media as well as MMP2 and MMP9 contents in cells of 100.0 μg/mL plasmid group were significantly higher than those of 0 μg/mL plasmid group, and contents of TIMP1 and TIMP2 in cells were significantly lower than those of 0 μg/mL plasmid group. Conclusion: Serum midkine content in breast cancer patients abnormally rises, and high expression of MK can induce breast cancer cell proliferation, inhibit breast cancer cell apoptosis and promote angiogenesis and cell invasion.

1. Introduction
Breast cancer is one of the common malignant tumors in women and its mechanism is complex. Breast cancer cells have strong proliferation and anti-apoptosis ability, in the development process, cancer cells display infiltrative growth and a variety of cytokines are associated with the above biological behavior of cancer cells. Midkine (MK) is a multifunctional cytokine newly discovered in recent years, belongs to heparin-binding factor family and is related to the regulation of cell proliferation and invasion, angiogenesis and other processes[1,2]. Studies have reported that high expression of MK is related to the occurrence of a variety of malignant tumors[3,4], and thus it is considered that high expression of MK in local tumor tissue may cause elevated MK level in serum and then have reference value in the assessment of breast cancer. In the following research, serum midkine expression in breast cancer patients and its clinical significance were analyzed.

2. Research subjects
2.1. Case information
Breast tumor patients receiving surgical treatment in our hospital from May 2012 to September 2014 were selected for study, tumor nature was determined by postoperative pathological results, and 45 cases of patients with breast cancer and 45 cases of patients with benign breast tumor were selected for study.
2.2. Research materials

Breast cancer cell lines were MCF-7 cell lines provided by the cell bank of Chinese Academy of Sciences and MK expression plasmids were synthesized by Generay Company; cell viability kits were CCK-8 Cell Counting kits of Vazyme Company, cell apoptosis kits were Annexin V-FITC kits of Vazyme Company, PCR kits were 2 × AceTaq Master Mix (Dye Plus) kits of Vazyme Company and Elisa kits were human MK, VEGFA, VEGFB and VEGFC kits of Roche Company.

2.3. Research methods

2.3.1. Experimental sample collection

Breast tumor tissue was collected during operation, washed with normal saline, rapidly frozen with liquid nitrogen and then used for follow-up test; MCF-7 cell lines were recovered, cultured with media containing 5% fetal bovine serum and digested with trypsin, and 24 h after MK expression plasmid with final concentrations of 0 μg/mL, 0.1 μg/mL, 1.0 μg/mL, 10.0 μg/mL and 100.0 μg/mL were transfected into cells, cells and media were collected and used for follow-up test.

2.3.2. Elisa detection

Serum specimens and cell culture medium specimens were taken, processed according to kit instructions and then added to Elisa reaction plates, meanwhile preconfigured concentration gradient protein standards were added to Elisa reaction plates, after incubation for appropriate time, kit instructions were followed for plate wash, secondary antibody incubation, color development and other operations, finally OD values at 490 nm wavelength were read in ELIASA, and corresponding protein contents in test specimens were calculated according to the OD values of protein standards.

2.3.3. PCR detection

Breast tissue and cell specimens were taken, added to Trizol lysate and extracted through chloroform, isopropyl alcohol and 75% alcohol in turn to get RNA, after reverse transcription into cDNA, PCR amplification was carried out according to 2 × AceTaq Master Mix (Dye Plus) kit instructions, and β-actin amplification was used as internal reference to calculate RNA contents of MK, MMP2, MMP9, TIMP1 and TIMP2.

2.3.4. Cell proliferation and apoptosis detection

Cell specimens were taken, CCK-8 Cell Counting kits were used for experiment and OD values were detected to reflect cell viability; Annexin V-FITC kits were used for experiment and apoptosis cell percentage was detected.

2.4. Statistical methods

SPSS 22.0 software was used for statistical processing, comparison between two groups was by t test, comparison among groups was by variance analysis and differences were considered to be statistically significant at a level of \( P<0.05 \).

3. Research results

3.1. MK expression in breast tissue and serum MK content

MK expression in breast tissue and serum MK content of breast cancer patients were higher than those of benign breast tumor patients; analysis of MK expression in breast tissue and serum MK content of breast cancer patients with different clinical pathological features was as follows: MK expression in breast tissue and serum MK content of breast cancer patients with TNM I/II stage, low/un-differentiation and lymph node metastasis were higher than those of breast cancer patients with TNM III/IV stage, medium/high differentiation and without lymph node metastasis.

3.2. MK expression and corresponding biological behavior in breast cancer cell lines after plasmid transfection

After transfection of different concentrations of plasmids, detection and analysis results of MK expression were as follows: MK expression plasmid could dose-dependently increase mRNA content and protein content of MK in breast cancer cell lines; detection and analysis results of cell viability and apoptosis percentage were as follows: MK expression plasmid could dose-dependently increase cell viability and decrease apoptosis percentage.

Table 1.

<table>
<thead>
<tr>
<th>Clinical pathological feature</th>
<th>Grouping</th>
<th>n</th>
<th>MK expression in breast tissue</th>
<th>Serum MK content (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue type</td>
<td>Benign breast tumor</td>
<td>45</td>
<td>100.00±14.52</td>
<td>3.82±0.45</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>45</td>
<td>214.85±25.96</td>
<td>0.62±0.08</td>
<td></td>
</tr>
<tr>
<td>TNM stage</td>
<td>I/II stage</td>
<td>25</td>
<td>153.48±17.54</td>
<td>1.14±0.15</td>
</tr>
<tr>
<td>III/IV stage</td>
<td>20</td>
<td>275.27±31.25</td>
<td>5.81±0.64</td>
<td></td>
</tr>
<tr>
<td>Differentiation degree</td>
<td>High/medium differentiation</td>
<td>27</td>
<td>132.23±16.92</td>
<td>1.87±0.20</td>
</tr>
<tr>
<td>Low/un-differentiation</td>
<td>18</td>
<td>327.18±35.68</td>
<td>4.68±0.52</td>
<td></td>
</tr>
<tr>
<td>Lymph node metastasis</td>
<td>Without</td>
<td>28</td>
<td>140.22±16.68</td>
<td>1.44±0.17</td>
</tr>
<tr>
<td>With</td>
<td>17</td>
<td>309.29±41.28</td>
<td>5.10±0.62</td>
<td></td>
</tr>
</tbody>
</table>

a: compared with benign breast tumor patients, there were differences; b: compared with breast cancer patients with TNM I/II stage, there were differences; c: compared with breast cancer patients with high/medium differentiation, there were differences; d: compared with breast cancer patients with lymph node metastasis, there were differences;
3.3. Angiogenesis-related molecules

24 h after transfection of 0 μg/mL MK expression plasmid and 100.0 μg/mL MK expression plasmid, cell media were collected, VEGFA, VEGFB and VEGFC contents in media were detected and analyzed, and results were shown in Figure 1: VEGFA, VEGFB and VEGFC contents in media of 100.0 μg/mL plasmid group were significantly higher than those of 0 μg/mL plasmid group.

3.4. Extracellular matrix degradation-related molecules

24 h after transfection of 0 μg/mL MK expression plasmid and 100.0 μg/mL MK expression plasmid, cells were collected, MMP2, MMP9, TIMP1 and TIMP2 contents in cells were detected and analyzed, and results were shown in Figure 2: MMP2 and MMP9 contents in cells of 100.0 μg/mL plasmid group were significantly higher than those of 0 μg/mL plasmid group, and contents of TIMP1 and TIMP2 were significantly lower than those of 0 μg/mL plasmid group.

4. Discussion

Midkine (MK) is a heparin-binding growth factor, has complex biological function and is involved in cell differentiation and proliferation, angiogenesis and extracellular matrix degradation process in multiple tissues of the body. In the formation of malignant tumors, cell proliferation, invasion and angiogenesis are the important biological characteristics, and studies have reported that in thyroid cancer, gastric cancer and other malignant tumor tissues, MK expression abnormally increases and is closely associated with tumor malignancy[5,6]. It indicates that abnormally high expression of MK is related to the occurrence and development of malignant tumors. Breast cancer is the malignant tumor with highest incidence in women, and our analysis of MK expression in breast cancer tissue showed that MK expression in breast tissue of breast cancer patients was higher than that of benign breast tumor patients, and the worse the TNM stage of breast cancer, the lower the differentiation degree, with lymph node metastasis, the higher the MK expression, which confirmed that high expression of MK was related to the occurrence of breast cancer and disease development.

The key to clinical diagnosis and treatment of breast cancer is early diagnosis of disease and accurate determination of the condition, and the detection of related molecules in tumor tissue can provide accurate evidence for the judgment of pathological stage and tumor malignancy[7], but tumor tissue is obtained through biopsy, on the one hand, it may cause a certain degree of trauma to patients, belongs to the category of invasive exanimation and is not suitable for long-term monitoring and assessment of disease[8]; on the other hand,
biopsy can only be carried out after making clear tumor lesion by imaging examination, at this moment, the diameter of tumor lesion is bigger and it’s less difficulty to obtain tumor tissue by biopsy, but the disease has mostly developed, clinical prognosis is poor and tumor cannot be detected and diagnosed in the early stage. Serum tumor marker has been the commonly used indicator for clinical diagnosis of malignant tumor and judgment of its condition, but there has been lacking specific tumor maker for breast cancer[9,10]. As has been noted, MK is abnormally highly expressed in breast cancer tissue, and based on this, serum MK content was analyzed, results showing that serum MK content of breast cancer patients was significantly higher than that of benign breast tumor patients and associated with pathological process of the tumor.

Above analysis preliminarily showed that serum MK content could provide evidence for the diagnosis of breast cancer and disease evaluation, and in order to further clarify the role that MK played in the occurrence and progression of breast cancer, breast cancer cell lines were selected for in vitro experiment study. In order to simulate the pathological state of high MK expression in breast cancer tissue, plasmid transfection was used to increase MK expression in breast cancer cell lines and then corresponding biological behavior of cells was detected. After plasmids that could express MK were constructed, they were transfected into breast cancer cell lines, and it was detected that MK plasmid could dose-dependently promote MK expression in breast cancer cell lines, increase cell viability and inhibit cell apoptosis, and the effect of MK overexpression of 100 μg/mL group was the most significant, which indicated that transfection of MK expression plasmid could increase MK expression in breast cancer cell lines, induce breast cancer cell proliferation and inhibit breast cancer cell apoptosis.

In the development process of breast cancer, local angiogenesis as well as cancer cell invasion and breaking through basement membrane are important pathological links of the disease. MK is a cytokine with a variety of biological functions, not only related to the regulation of cell proliferation, but also involved in angiogenesis and cell invasion. Vascular endothelial growth factor (VEGF) is the most important cytokine mediating local tumor angiogenesis, tumor itself can synthesize VEGF and increase the number of local angiogenesis[11,12], and after transfecting MK expression plasmid, it was observed that VEGFA, VEGFB and VEGFC contents in media of 100.0 μg/mL plasmid group were significantly higher than those of 0 μg/mL plasmid group, which indicated that transfection of MK expression plasmid could increase VEGF isoforms display distinct colonisation characteristics. As has been noted, MK is abnormally highly expressed in breast cancer tissue, and based on this, serum MK content was analyzed, results showing that serum MK content of breast cancer patients was significantly higher than that of benign breast tumor patients and associated with pathological process of the tumor.

To sum up, it is believed that serum midkine content in breast cancer patients abnormally rises, and high expression of MK can induce breast cancer cell proliferation, inhibit breast cancer cell apoptosis and promote angiogenesis and cell invasion.

References