Diagnostic value of MicroPure imaging for malignant thyroid nodule microcalcification and its correlation with the oncogene expression in nodules

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ABSTRACT

Objective: To study the diagnostic value of MicroPure imaging for malignant thyroid nodule microcalcification and its correlation with the oncogene expression in nodules. Methods: Patients with thyroid nodules confirmed by ultrasound in Dongtai People's Hospital between June 2014 and October 2016 were selected and divided into those with malignant thyroid nodules and benign thyroid nodules according to the pathological results, and MicroPure imaging technology was used to judge the microcalcification and further divide the malignant thyroid nodules into microcalcification (+) and microcalcification (-). The biopsy tissue was collected to detect the expression of cyclin, cell invasion molecules and angiogenesis molecules. Results: CyclinD1, CyclinE, MCM7, MMP2, MMP13, Vimentin, N-cadherin, Twist, HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in malignant thyroid nodules of microcalcification (+) group and microcalcification (-) group were significantly higher than those of benign group while CyclinG2 and P53 expression were significantly lower than those of benign group; CyclinD1, CyclinE, MCM7, MMP2, MMP13, Vimentin, N-cadherin, Twist, HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in malignant thyroid nodules of microcalcification (+) group were significantly higher than those of microcalcification (-) group while CyclinG2 and P53 expression were significantly lower than those of microcalcification (-) group. Conclusion: Malignant thyroid nodule microcalcification detected by MicroPure imaging has a good correlation with cancer cell proliferation, invasion and angiogenesis.

1. Introduction

Malignant thyroid nodule is one of the most common malignant tumors in the head and neck. Early detection and treatment of disease is of great significance to improve the prognosis[1,2]. Calcification is the important basis of benign and malignant thyroid nodules, microcalcification, coarse calcification, annular calcification and egg-shell calcification are four common kinds of calcification in thyroid nodules, and the occurrence of microcalcification with diameter < 2 mm is closely related to malignant thyroid nodules[3]. Conventional ultrasonography is difficult to detect the microcalcification with diameter < 2 mm in the thyroid nodule lesions, but the newly developed MicroPure imaging can detect the microcalcification with diameter less than 0.5 mm[4]. The formation of microcalcifications is closely related to the psammoma bodies in the cancer tissue, and the mechanism of the psammoma bodies is related to the rapid proliferation and necrosis of tumor cells in the lesion. Therefore, early detection of microcalcifications in thyroid nodules is not only conducive to the judgment of nodules, but also provides evidence for the evaluation of malignancy. In the following studies, we analyzed the diagnostic value of MicroPure imaging for microcalcification of malignant thyroid nodules and its correlation with the expression of oncogene in nodules.
2. Case information and research methods

2.1 General case information

Patients with thyroid nodules confirmed by ultrasound in Dongtai People’s Hospital between June 2014 and October 2016 were selected. All patients received thyroid fine needle aspiration and pathology. They were divided into those with malignant thyroid nodules and benign thyroid nodules according to the pathological results, and MicroPure imaging technology was used to detect the microcalcification and further divide the malignant thyroid nodules into microcalcification (+) and microcalcification (-). There were 62 cases in microcalcification (+) group, including 33 men and 29 women that were 38-59 years old; there were 41 cases in microcalcification (-) group, including 22 men and 19 women that were 36-60 years old; there were 50 cases in benign group, including 28 men and 22 women that were 35-60 years old. There was no significant difference in the general data among the three groups (p>0.05).

2.2 MicroPure imaging test

Toshiba Aplio XG color Doppler ultrasound was used for ultrasonography of thyroid. Patients were put in supine position with head back, the probe with frequency of 10-12 MHz was used to scan the lateral lobe and isthmus of thyroid gland, the location, number, size, shape, edges and other features of the thyroid nodules were confirmed at first, then MicroPure imaging was selected to observe the calcification within each nodule, and the size of the calcification was referred to judge whether there was a microcalcification.

2.3 Gene protein expression test

The right amount of ultrasound biopsy thyroid nodule tissue was collected, added in PBS buffer and then fully grinded, the obtained tissue suspension was centrifuged in 4 ℃ for 15 min at a speed of 12 000 r/min to separate the supernatant liquid, then BCA kit was used for total protein quantification, and the enzyme-linked immunosorbent assay kit was used to detect Cyclin1, CyclinE, MCM7, CyclinG2, P53, MMP2, MMP13, Vimentin, N-cadherin, Twist, HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 protein expression.

2.4 Statistical methods

SPSS 17.0 statistical software was used for variance analysis of the gene expression data among three groups and P<0.05 indicated statistical significance in differences in analysis results.

3. Results

3.1 Cyclin expression in thyroid nodule tissue

Analysis of cyclin CyclinD1, CyclinE, MCM7, CyclinG2 and P53 expression in thyroid nodule tissue was as follows: CyclinD1, CyclinE and MCM7 expression in malignant thyroid nodules of microcalcification (+) group and microcalcification (-) group were significantly higher than those of benign group while CyclinG2 and P53 expression were significantly lower than those of benign group; CyclinD1, CyclinE and MCM7 expression in malignant thyroid nodules of microcalcification (+) group were significantly higher than those of microcalcification (-) group. Differences in pair-wise comparison of CyclinD1, CyclinE, MCM7, CyclinG2 and P53 expression in thyroid nodule tissue were statistically significant among the three groups (P<0.05).

3.2 Cell invasion molecule expression in thyroid nodule tissue

Analysis of cell invasion molecules MMP2 (ng/mg protein), MMP13 (ng/mg protein), Vimentin (pg/mg protein), N-cadherin (pg/mg protein) and Twist (ng/mg protein) expression in thyroid nodule tissue was as follows: MMP2, MMP13, Vimentin, N-cadherin and Twist expression in malignant thyroid nodules of microcalcification (+) group and microcalcification (-) group were significantly higher than those of benign group; MMP2, MMP13, Vimentin, N-cadherin and Twist expression in malignant thyroid nodules of microcalcification (+) group were significantly higher than those of microcalcification (-) group. Differences in pair-wise comparison of MMP2, MMP13, Vimentin, N-cadherin and Twist expression in thyroid nodule tissue were statistically significant among the three groups (P<0.05).

Table 1.

Comparison of cyclin expression in thyroid nodule tissue (ng/mg protein).

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>CyclinD1</th>
<th>CyclinE</th>
<th>MCM7</th>
<th>CyclinG2</th>
<th>P53</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcalcification (+) group</td>
<td>62</td>
<td>2.31±0.29</td>
<td>1.75±0.20</td>
<td>0.82±0.08</td>
<td>0.58±0.08</td>
<td>0.32±0.05</td>
</tr>
<tr>
<td>Microcalcification (-) group</td>
<td>41</td>
<td>1.42±0.18</td>
<td>1.03±0.14</td>
<td>0.54±0.07</td>
<td>0.83±0.11</td>
<td>0.61±0.08</td>
</tr>
<tr>
<td>Benign group</td>
<td>50</td>
<td>0.77±0.09</td>
<td>0.65±0.08</td>
<td>0.33±0.05</td>
<td>1.42±0.18</td>
<td>0.94±0.10</td>
</tr>
</tbody>
</table>

*: compared with index expression of benign group, differences were statistically significant, P<0.05; #: compared with index expression of microcalcification (-) group, differences were statistically significant, P<0.05.

Table 2.

Comparison of cell invasion molecule expression in thyroid nodule tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>MMP2</th>
<th>MMP13</th>
<th>Vimentin</th>
<th>N-cadherin</th>
<th>Twist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcalcification (+) group</td>
<td>62</td>
<td>5.12±0.76</td>
<td>2.44±0.34</td>
<td>136.4±16.7</td>
<td>224.5±29.5</td>
<td>1.52±0.18</td>
</tr>
<tr>
<td>Microcalcification (-) group</td>
<td>41</td>
<td>3.32±0.52</td>
<td>1.51±0.18</td>
<td>70.5±9.3</td>
<td>107.5±14.5</td>
<td>0.93±0.11</td>
</tr>
<tr>
<td>Benign group</td>
<td>50</td>
<td>1.28±0.14</td>
<td>0.77±0.09</td>
<td>42.6±5.9</td>
<td>65.4±8.3</td>
<td>0.65±0.08</td>
</tr>
</tbody>
</table>

*: compared with index expression of benign group, differences were statistically significant, P<0.05; #: compared with index expression of microcalcification (-) group, differences were statistically significant, P<0.05.
Table 3.
Comparison of angiogenesis molecule expression in thyroid nodule tissue.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>HIF-1α</th>
<th>VEGF-C</th>
<th>VEGF-2</th>
<th>VEGF-3</th>
<th>Ang-2</th>
<th>Tie-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcalcification (+) group</td>
<td>62</td>
<td>1.98±0.22*</td>
<td>2.65±0.32*</td>
<td>105.6±12.8*</td>
<td>178.5±20.3*</td>
<td>1.03±0.15*</td>
<td>1.86±0.20*</td>
</tr>
<tr>
<td>Microcalcification (-) group</td>
<td>41</td>
<td>1.05±0.14*</td>
<td>1.42±0.18*</td>
<td>67.2±8.4*</td>
<td>116.2±14.6*</td>
<td>0.78±0.09*</td>
<td>1.13±0.14*</td>
</tr>
<tr>
<td>Benign group</td>
<td>50</td>
<td>0.77±0.08</td>
<td>0.89±0.11</td>
<td>40.4±6.2</td>
<td>70.3±8.9</td>
<td>0.35±0.06</td>
<td>0.67±0.08</td>
</tr>
</tbody>
</table>

*: compared with index expression of benign group, differences were statistically significant, \( P<0.05; \) *#*: compared with index expression of microcalcification (-) group, differences were statistically significant, \( P<0.05.\)

3.3 Angiogenesis molecule expression in thyroid nodule tissue

Analysis of angiogenesis molecules HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in thyroid nodule tissue was as follows: HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in malignant thyroid nodules of microcalcification (+) group and microcalcification (-) group were significantly higher than those of benign group; HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in malignant thyroid nodules of microcalcification (+) group were significantly higher than those of microcalcification (-) group. Differences in pair-wise comparison of HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in thyroid nodule tissue were statistically significant among the three groups (\( P<0.05).\)

4. Discussion

Malignant thyroid nodule is a common malignant tumor of the head and neck, its incidence is increasing year by year, the microcalcification with diameter < 2 mm is the important characteristic of malignant thyroid nodules, there is microcalcification in about 30%–50% of malignant thyroid nodules, and the microcalcification specificity in the diagnosis of thyroid cancer has reached more than 90%.[5] Microcalcification in thyroid nodules is closely related to formation of psammoma bodies in carcinoma tissues, and its mechanism may be the local tissue hyperplasia and necrosis as well as calcium deposit after rapid tumor cell proliferation within the lesions. Thyroid ultrasound is an important auxiliary examination method for clinical diagnosis of thyroid nodules and preliminary evaluation of the nodule property, and the sonographic features of microcalcification are the tufted or scattered pinpoint-like echogenic foci accompanied by the posterior no acoustic shadow or weak acoustic shadow.[6] But due to the influence of surrounding tissue occlusion, conventional ultrasonography can not accurately detect the microcalcification in thyroid nodules, thus affecting the early detection of nodule property.[7] MicroPure imaging technology is the new means of ultrasonography developed in recent years, which can highlight some microcalcification points that cannot be displayed in routine ultrasonography, help discover the microcalcifications in thyroid nodules, and then provide the basis for judging the thyroid nodule properties and evaluating the malignant degree.[8]

The occurrence of microcalcification in malignant thyroid nodules is closely related to the proliferation of cancer cells, and the cell cycle is an important mechanism for regulating cell proliferation. CyclinD1 and CyclinE are important positive regulatory proteins of cell cycle, which can form complexes with CDK2, CDK4, CDK6 and other kinases to accelerate the development of cell cycle and thereby promote cell proliferation.[9] MCM7 is an important member of the MCM family involved in cell cycle regulation, which initiates DNA replication and promotes cell proliferation. CyclinG2 is a protein that has negative regulation effect on cell cycle and can hinder cell cycle progression and inhibit cell proliferation by up-regulating the expression of tumor suppressor gene P53.[10] In order to define the correlation of microcalcification in malignant thyroid nodules with cell proliferation and cell cycle progression, the expression levels of cyclin within the thyroid nodule were analyzed in the study, and the results showed that CyclinD1, CyclinE and MCM7 expression in malignant thyroid nodules were significantly higher than those in benign group while CyclinG2 and P53 expression were significantly lower than those of benign group, and the CyclinD1, CyclinE and MCM7 expression were higher while CyclinG2 and P53 expression were lower in malignant thyroid nodules with microcalcification. This indicates that the cyclin expression changes and the cell cycle is significantly accelerated in malignant thyroid nodules, and the emergence of microcalcification in malignant nodules is closely related to the changes in cyclin expression and the acceleration of cell cycle.

The cancer cells in malignant thyroid nodules will infiltrate to the surrounding tissue on the basis of the continuous proliferation, and the enhancement of cell movement and invasion is an important pathological link in mediating the invasive growth of cancer cells. Extracellular matrix and basement membrane degradation is an important biological link for cancer cells to break away from the primary lesion and infiltrate to the adjacent tissue, and the MMP2 and MMP13 in MMPs family can hydrolyze the collagen, laminin and multiple other ingredients in extracellular matrix and basement membrane, and then promote the cancer cells to invade the neighboring tissues.[11–13] Epithelial mesenchymal transition is an important biological link causing the decline of intercellular polarity and the enhancement of movement capacity, Twist is the key transcription factor regulating the process, and it can directly inhibit epithelial phenotype marker molecule expression, make cellular epithelial phenotype transit to mesenchymal phenotype, increase
the expression of Vimentin, N-cadherin and other mesenchymal phenotype marker molecules and promote cell movement and invasio[14,15]. In the study, analysis of cell invasion molecule expression in thyroid nodule showed that MMP2, MMP13, Vimentin, N-cadherin and Twist expression in malignant thyroid nodules were significantly higher than those in benign group, and MMP2, MMP13, Vimentin, N-cadherin and Twist expression were higher in malignant thyroid nodules with microcalcification. This indicates that the cell invasion is significantly enhanced in malignant thyroid nodules and the occurrence of microcalcifications in malignant nodules is closely related to the high MMPs expression and the excessive epithelial mesenchymal transition.

Thyroid cancer cell proliferation and invasion are both active energy dissipation processes and rely on the nutrients provided by the new blood vessels within lesions, and the angiogenesis mediated by VEGF family and Ang family has played an important role in the pathologic process of malignant thyroid nodules. VEGF-C is the VEGF family member promoting angiogenesis, which is continuously expressed under the action of transcription factor HIF-1α and acts on the membrane receptors VEGFR-2 and VEGFR-3 to promote the proliferation of endothelial cells and the formation of vascular structures[16,17]; Ang-2 in the Ang family is a type of secreted glycoprotein, which can initiate the process of angiogenesis by combining with tyrosine kinase receptor Tie-2[18]. In the study, analysis of the expression of the angiogenesis molecules in the thyroid nodules showed that HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression in malignant thyroid nodules were significantly higher than those in benign group, and HIF-1α, VEGF-C, VEGFR-2, VEGFR-3, Ang-2 and Tie-2 expression were higher in malignant thyroid nodules with microcalcification. This indicates that there is a significant increase in the expression of angiogenesis molecules in malignant thyroid nodules and the presence of microcalcifications in malignant nodules is closely related to the high expression of angiogenesis molecules.

Accelerated cell cycle, enhanced cell invasion and increased angiogenesis are closely related to the occurrence of malignant thyroid nodule; the microcalcification detected by MicroPure imaging is related to the abnormal expression of cyclin, cell invasion molecules and angiogenesis molecules.

References