Stem cell characteristics of early colon cancer tissue and their relationship with cell proliferation and invasion

Tian-Ming Xu, Rui-Ping Li*, Hong Pang, Qiang Chen, Hui-Ling Yuan

1. Introduction

Colon cancer is a common gastrointestinal malignancy in China, and its incidence is second only to gastric cancer and has been rising year by year[1]. Colon cancer development has certain pathological characteristics, and it develops from normal mucosa to adenoma, and then to adenocarcinoma, the expression of a variety of proliferation and invasion genes is significantly abnormal in the process, but the specific regulatory mechanism is still not clear. In recent years, it has been confirmed that tumor stem cell theory is closely related with a variety of malignant tumors, tumor stem cells are with the potentials of self-renewal, infinite proliferation and multi-directional differentiation, and they can become the seed and source of malignant tumors[2]. Ubiquitin specific peptidase 22 (USP22), Nanog, leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5) and CD44 are marker genes of tumor stem cells and can maintain the characteristics of tumor stem cells and participate in regulating cell proliferation, invasion and other biological behaviors. In the following study, we aim to take the surgically removed early colon cancer tissues as the research object, and specifically analyze the stem cell characteristics of colon cancer tissues and their relationship with cell proliferation and invasion.

2. Clinical information and research methods

2.1 General information of clinical samples

Colon cancer tissues and adjacent tissues surgically removed in Dongguan People’s Hospital between January 2010 and October 2017 were chosen as the clinical samples of this study, the protein was extracted to determine the protein expression of tumor stem cell genes USP22, Nanog, Lgr5 and CD44, and RNA was extracted to determine the mRNA expression of cell proliferation genes and cell invasion genes. Results: USP22, Nanog, Lgr5 and CD44 protein expression as well as Rab5A, TBX2, MDM2, TGF-β 1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression in colon cancer tissues were significantly higher than those in adjacent tissues while Bad, Bax and Fas mRNA expression were significantly lower than those in adjacent tissues; USP22, Nanog, Lgr5 and CD44 protein expression in colon cancer tissues were positively correlated with Rab5A, TBX2, MDM2, TGF-β 1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression, and negatively correlated with Bad, Bax and Fas mRNA expression. Conclusion: The activation of stem cell characteristics in early colon cancer can promote the proliferation and invasion of cancer cells.

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chemoradiotherapy or immunotherapy. A total of 50 cases were enrolled, there were 28 males and 22 females, they were 42-65 years old, 21 cases were at Duke Stage A and 29 cases were at Stage B.

2.2 Laboratory research methods

2.2.1 Protein expression detection
Right amount of surgically removed colon cancer tissues and adjacent tissues were taken, RIPA lysate was adopted to separate and extract total protein from the tissue, and then Elisa kit was used to measure the USP22, Nanog, Lgr5 and CD44 protein expression in tissue protein extracting solution.

2.2.2 mRNA expression detection
Right amount of surgically removed colon cancer tissues and adjacent tissues were taken, Trizol lysate was adopted to separate and extract total RNA from the tissue, and then cDNA synthesis kit was used for reverse transcription of mRNA in total RNA into cDNA; cDNA was taken, the PCR kit instructions were referred to configure PCR reaction system, the Rab5A, TBX2, MDM2, Bad, Bax, Fas, TGF-β1, Smad2/3, Vimentin, Rac1 and VEGF were amplified, and the mRNA expression of the corresponding genes were determined.

2.3 Statistical methods
Software SPSS 23.0 was used to input the data, the measurement data between groups were analyzed by paired t test, the correlation was analyzed by Pearson test and \( P < 0.05 \) showed statistical significance in the differences.

3. Results

3.1 Stem cell marker gene expression in colon cancer tissues
Analysis of stem cell marker genes USP22, Nanog, Lgr5 and CD44 protein expression in colon cancer tissues and adjacent tissues was as follows: USP22, Nanog, Lgr5 and CD44 protein expression in colon cancer tissues were significantly higher than those in adjacent tissues. Differences were statistically significant in USP22, Nanog, Lgr5 and CD44 protein expression in different origins of tissues (\( P < 0.05 \)).

3.2 Cell proliferation gene expression in colon cancer tissues
Analysis of cell proliferation genes Rab5A, TBX2, MDM2, Bad, Bax and Fas mRNA expression in colon cancer tissues and adjacent tissues was as follows: Rab5A, TBX2 and MDM2 mRNA expression in colon cancer tissues were significantly higher than those in adjacent tissues while Bad, Bax and Fas mRNA expression were significantly lower than those in adjacent tissues (\( P < 0.05 \)). Pearson test showed that USP22, Nanog, Lgr5 and CD44 protein expression in colon cancer tissues were positively correlated with Rab5A, TBX2 and MDM2 mRNA expression, and negatively correlated with Bad, Bax and Fas mRNA expression.

3.3 Cell invasion gene expression in colon cancer tissues
Analysis of cell invasion genes TGF-β1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression in colon cancer tissues and adjacent tissues was as follows: TGF-β1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression in colon cancer tissues were significantly higher than those in adjacent tissues (\( P < 0.05 \)). Pearson test showed that USP22, Nanog, Lgr5 and CD44 protein expression in colon cancer tissues were positively correlated with TGF-β1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression.

4. Discussion
The pathological features of colon cancer are the development from normal mucosa to adenoma, and then to adenocarcinoma, and the expression of a variety of proliferation genes and invasion genes has changed in the adenoma or early adenocarcinoma stage, which will mediate the abnormal proliferation and invasion of the cells[3,4]. However, it is still unclear about the regulatory mechanism of proliferation and invasion gene expression in the occurrence and development of colon cancer. The activation and enhancement of the stem cell characteristic are the malignant tumor-related biological

Table 1.
Comparison of cell proliferation genes in colon cancer tissues and adjacent tissues.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>Rab5A</th>
<th>TBX2</th>
<th>MDM2</th>
<th>Bad</th>
<th>Bax</th>
<th>Fas</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer tissue</td>
<td>50</td>
<td>2.31±0.35</td>
<td>1.89±0.25</td>
<td>2.15±0.33</td>
<td>0.65±0.08</td>
<td>0.56±0.06</td>
<td>0.46±0.07</td>
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<tr>
<td>Adjacent tissue</td>
<td>50</td>
<td>1.03±0.16</td>
<td>1.01±0.14</td>
<td>0.97±0.16</td>
<td>1.05±0.12</td>
<td>1.04±0.17</td>
<td>0.96±0.11</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</table>

Table 2.
Comparison of stem cell marker genes in colon cancer tissues and adjacent tissues.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>USP22</th>
<th>Nanog</th>
<th>Lgr5</th>
<th>CD44</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer tissue</td>
<td>50</td>
<td>4.95±0.62</td>
<td>335.75±42.83</td>
<td>528.69±63.27</td>
<td>3.48±0.52</td>
</tr>
<tr>
<td>Adjacent tissue</td>
<td>50</td>
<td>2.37±0.35</td>
<td>173.32±22.41</td>
<td>203.84±28.83</td>
<td>1.93±0.31</td>
</tr>
<tr>
<td>t</td>
<td></td>
<td>22.172</td>
<td>20.182</td>
<td>28.598</td>
<td>15.675</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>
links discovered in recent years, which can promote the occurrence and development of tumor through its self-renewal, infinite proliferation and multi-directional differentiation potential in the prophase or early stages of the malignant tumor[5,6]. USP22, Nanog, Lgr5 and CD44 are currently known as tumor stem cell marker genes. USP22 is the necessary molecule for activator regulatory gene expression, for instance, the gene transcription regulation mediated by Myc gene family is highly dependent on USP22, and the c-Myc, n-Myc and other molecules can regulate the expression of a variety of downstream genes and promote cell proliferation under the assist of USP22[7]. Nanog is a transcription factor directly involved in the maintenance of stem cell characteristics, which can enhance the self-renewal and multidirectional differentiation ability of the cells[8,9]. Lgr5 is a transmembrane protein with 7 -helix structures, which can be activated after receiving the biological signals from the Wnt pathway, and then promote the abnormal differentiation of cells[10]. CD44 is the adhesion molecule located on the cell surface, which mediates the adhesion between cells and extracellular matrix, and can enhance the migration and invasion characteristics of stem cells[11,12]. In the study, analysis of the changes in above tumor stem cell marker gene expression in early colon cancer tissues showed that USP22, Nanog, Lgr5 and CD44 protein expression in colon cancer tissues were significantly higher than those in adjacent tissues. This means that the tumor stem cell activity has been excessively activated in the early stages of colon cancer, and it is further speculated based on the biological characteristics of tumor stem cells that the enhanced tumor stem cell activity can maintain the strong proliferation and invasion ability of tumor cells to promote the occurrence and development of colon cancer.

The self-renewal and multidirectional differentiation of tumor stem cells can induce malignant transformation of cells and make them obtain strong growth activity. Cell proliferation is an important pathologic link to promote the growth of tumor cells, and the abnormal expression of multiple genes is closely related to it. Rab5A is a new member of the Rab GTPase family and can interact with the EGF signaling pathway to promote cell cycle transition and cell growth[13]; TBX2 is a member of the T-BOX family, which can on the one hand, work together with Ras, Myc and other genes to promote the excessive proliferation of the cells, and on the other hand, inhibit the activity of tumor suppressor gene p21, prevent apoptosis and facilitate cell growth[14]; MDM2 is a negative regulatory molecule of tumor suppressor gene p53, which blocks the cell cycle inhibitory activity mediated by p53 to relieve the inhibitory effect of p53 on cell cycle, and accelerate the cell cycle development and cell growth[15]. Bad and Bax are Bcl-2 family members with negative inhibitory activity on proliferation, which can promote the cytochrome C in mitochondria to enter the cytoplasm, and then activate the cascade apoptosis process mediated by caspase[16,17]; Fas is a member of the tumor necrosis factor receptor superfamily, and after identifying the ligand FasL, it can recruit caspase by downstream FADD and activate cell apoptosis[18]. Analysis of the changes in proliferation gene expression in colon cancer tissue showed that Rab5A, TBX2 and MDM2 mRNA expression in colon cancer tissues were significantly higher than those in adjacent tissues whereas Bad, Bax and Fas mRNA expression were significantly lower than those in adjacent tissues. This indicates that the expression of pro-proliferation genes is significantly up-regulated and the expression of anti-proliferation genes is significantly down-regulated in the early stage of colon cancer. Further analysis of the correlation between stem cells characteristics in colon cancer tissue and cell proliferation indicated that USP22, Nanog, Lgr5 and CD44 protein expression were positively correlated with Rab5A, TBX2 and MDM2 mRNA expression, and negatively correlated with Bad, Bax and Fas mRNA expression. This indicates that the enhancement of tumor stem cell activity in the early stage of colon cancer can regulate the expression of proliferation genes to promote the proliferation of cancer cells.

The malignant cell transformation caused by the tumor stem cell characteristics within colon cancer lesions can not only promote cancer cell generation through cell proliferation, but also promote cancer cell invasion through cell invasion. Epithelial mesenchymal transition is the precondition of tumor cell invasion to the surrounding tissues and can make the tumor cells obtain extremely strong movement performance[19]. TGF-β 1 is the upstream cytokine that regulates the epithelial mesenchymal transition, which is combined with the receptor on the cell membrane to conduct signal transduction via downstream Smad2/3 and increase the expression of mesenchymal gene Vimentin; the highly expressed Vimentin may weaken the intercellular polarity and adhesion to facilitate the cell movement and migration to surrounding tissues[20,21]. Rac1 is the Rac gene family member, which can on the one hand, directly activate the uPA through the downstream Cdc42, Limk1 and other molecules to hydrolyze the proteins in the extracellular matrix of tumor and promote the tumor cells to break through the limit of the extracellular matrix and invade the surrounding area[22,23], and on the other hand, start the expression of angiogenesis molecule VEGF through the downstream ERK/JNK, NF-κ B and other pathways to increase the number of new blood vessels within the tumor lesion and provide nutrients for the tumor cell invasion[24]. Analysis of the changes in invasion gene expression in colon cancer tissues showed that TGF-β 1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression in colon cancer tissues were significantly higher than those in adjacent tissues. It indicates the significant up-regulation of invasion gene expression in the early stages of colon cancer. Further analysis of the correlation between stem cell characteristics in colon cancer tissue and cells invasion showed that USP22, Nanog, Lgr5

### Table 3.

Comparison of cell invasion genes in colon cancer tissues and adjacent tissues.

<table>
<thead>
<tr>
<th>Tissue origin</th>
<th>n</th>
<th>TGF-β 1</th>
<th>Smad2/3</th>
<th>Vimentin</th>
<th>Rac1</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon cancer tissue</td>
<td>50</td>
<td>2.12±0.32</td>
<td>1.88±0.27</td>
<td>1.96±0.22</td>
<td>2.37±0.37</td>
<td>2.72±0.38</td>
</tr>
<tr>
<td>Adjacent tissue</td>
<td>50</td>
<td>1.06±0.12</td>
<td>0.97±0.15</td>
<td>1.03±0.14</td>
<td>1.01±0.16</td>
<td>0.96±0.14</td>
</tr>
<tr>
<td>t</td>
<td>22.383</td>
<td>18.938</td>
<td>20.318</td>
<td>25.546</td>
<td>32.374</td>
<td></td>
</tr>
<tr>
<td>P</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>
and CD44 protein expression were positively correlated with TGF-β1, Smad2/3, Vimentin, Rac1 and VEGF mRNA expression. This indicates that the enhancement of tumor stem cell activity in the early stage of colon cancer can regulate the expression of invasion genes to promote the invasion of cancer cells.

To sum up, it can be concluded that the stem cell characteristics are significantly activated in the early colon cancer tissue, and many stem cell marker genes are highly expressed; the enhanced stem cell characteristics can regulate the expression of proliferation and invasion genes to promote the proliferation and invasion of cancer cells.

References


