Pancreatic CSC-like cells: a new entity of CTCs

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Background
Pancreatic cancer is one of the most aggressive types of cancer. It spreads quickly making new metastatic sides. It is usually diagnosed in advanced stages. For this reason, the need to improve the current panel of treatment becomes imperative. According to the scientific community, cancer stem cells (CSCs) may be a new target to cancer therapy. Capability of self-renewal, differentiation into multiple cell types, asymmetric and rapid cell division are the basic hallmarks of them. On the other hand, circulating tumor cells (CTCs) represent the main population of cells in a tumor mass. In conclusion, identifying the entity of CSCs in a population of CTCs is the key to cancer prognosis and diagnosis.

Methods
In order to prove the above hypothesis molecular and cellular based methods were used. In the first panel of the test, it was tested the gene expression of five molecular cancer stem cell markers (nanog, nestin, oct3/4, sox2 and CD34) which are a strong evidence of the existence of CSCs, by using Reverse Transcription (RT) PCR analysis. In the second panel of the experiment, it was examined the sphere formation that the CSCs get when are cultivated in semi-suspension. In addition, it was compared the different growth rates between pancreatic cancer stem cell-like cells and pancreatic cancer cells (PANC-1 cell line) obtained from the European Collection of Cell Cultures (ECACC). CTCs were isolated from blood sample of three patients who suffered from pancreatic carcinoma.

Results
In the first panel of the experiment, almost all the CSCs markers were expressed in the three populations of CTCs (patients a, b & c) (fig. 1). Specially, nanog, nestin, oct3/4 and sox2 gene were expressed in patient a, b as well as in patient c. Concerning CD34 gene, it was expressed in patient a and b but not in c. The second panel of the test represented data concerning the growth rate of PANC-1 cell line and one cancer stem cell line becoming from CTCs which was isolated from patient a. It is obvious that the cellular division rate in pancreatic CSC-like is more rapid when compared with PANC-1 cell line (table1 & 2). Finally, the sphere formation of the CSC-like cells could be identified in semi-suspension with the appropriate growth medium in specific conditions (fig. 2).

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Table 1. Growth curve analysis. Number of cells in a time window of ten days.

<table>
<thead>
<tr>
<th>Number of Cells</th>
<th>Day 0</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANC-1</td>
<td>5x10^5</td>
<td>1x10^6</td>
<td>14,4x10^5</td>
<td>61,5x10^6</td>
<td>12x10^6</td>
<td>19,5x10^6</td>
<td>24x10^6</td>
<td>18x10^6</td>
<td>13,5x10^6</td>
<td>10,8x10^6</td>
</tr>
<tr>
<td>Patient A</td>
<td>5x10^5</td>
<td>3x10^6</td>
<td>4x10^6</td>
<td>261x10^6</td>
<td>462x10^6</td>
<td>36x10^6</td>
<td>88x10^6</td>
<td>64x10^6</td>
<td>110x10^6</td>
<td>55x10^6</td>
</tr>
</tbody>
</table>

Table 2. Increase fold values day to day and from day 0 respectively.

<table>
<thead>
<tr>
<th>Increase Fold / day</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 5</th>
<th>Day 7</th>
<th>Day 8</th>
<th>Day 9</th>
<th>Day 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANC-1</td>
<td>0,20</td>
<td>14,40</td>
<td>4,27</td>
<td>1,95</td>
<td>1,63</td>
<td>1,23</td>
<td>0,75</td>
<td>0,75</td>
<td>0,80</td>
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<tr>
<td>Patient A</td>
<td>60,00</td>
<td>13,33</td>
<td>65,25</td>
<td>1,77</td>
<td>0,08</td>
<td>2,44</td>
<td>0,73</td>
<td>1,72</td>
<td>0,50</td>
</tr>
</tbody>
</table>

Figure 1. Gene expression analysis of five CSCs molecular markers using RT–PCR. The bands were detected by using agarose gel 3%.

Figure 2. Pancreatic CSCs. Spheres formed in semi-suspension.

Conclusion
From the experiments that were performed, in this particular case, it was proven that the entity of CSC-like cells may be included in the population of CTCs in pancreatic carcinomas.

References