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 .

Number of Cells	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 8	Day 9	Day 10
PANC-1	$5x10^5$	$1x10^5$	$14,4x10^5$	$61,5x10^5$	$12x10^6$	$19,5x10^6$	$24x10^6$	$18x10^6$	$13,5x10^6$	$10,8x10^6$
Patient A	$5x10^5$	$3x10^6$	$4x10^8$	$261x10^8$	$462x10^8$	$36x10^8$	$88x10^8$	$64x10^8$	$110x10^8$	$55x10^8$

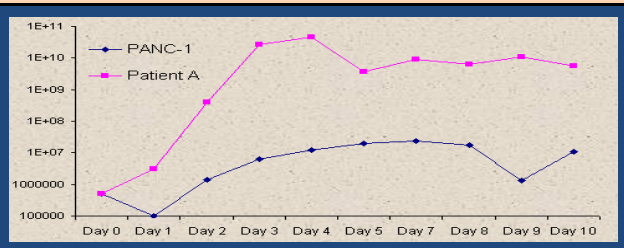

Table 1. Growth curve analysis. Number of cells in a time window of ten days.

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

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).

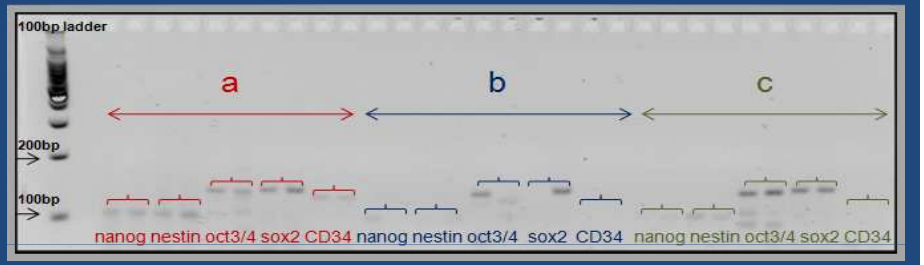

Figure 1. Gene expression analysis of five CSCs molecular markers using RT– PCR. The bands were detected by using agarose gel 3%. Expected base pairs : nanog (104bp), nestin (132bp), oct3/4 (138bp), sox2 (140bp), CD34 (130bp)

Increase Fold / day	Day 1	Day 2	Day 3	Day 4	Day 5	Day 7	Day 8	Day 9	Day 10
PANC-1	0,20	14,40	4,27	1,95	1,63	1,23	0,75	0,75	0,80
Patient A	60,00	13,33	65,25	1,77	0,08	2,44	0,73	1,72	0,50
Increase fold from day 0	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
PANC-1	0,2	2,9	12,3	24,0	39,0	48,0	36,0	27,0	21,6
Patient A	60	800	52200	92400	7200	17600	12800	22000	11000

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

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

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