Comparative study of Nanog, Oct3/4 and Sox2 gene expression following c-Met gene knockdown in Colon Cancer Stem Cells

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Hypothesis

Cancer Stem Cells (CSCs) are cancer cells that have the ability to generate tumors through the stem cell processes of self-renewal and differentiation into multiple cancer cell types. Recently, the interest of scientists focuses in finding therapies that target these cells as they could be included into the subpopulation of the Circulating Tumor Cells (CTCs) that is responsible for the invasion and metastasis. The purpose of the present study is to determine whether there is any relationship between the expression of c-Met (or C-MET or HGF-R) and the expression of the stem cell markers Nanog, Oct3/4 and Sox2. *c-Met was chosen as the knockdown gene because in cancer its abnormal activation triggers tumor growth, angiogenesis and metastasis*. In physiological conditions, stem cells express c-Met and grow invasively in order to generate new tissues in an embryo or regenerate damaged tissues in an adult. In cancer, it is believed that cancer stem cells mimic the ability of normal stem cells to express c-Met and thus become the cause of cancer persistence and spread to other sites in the body.

Materials and methods

Cancer Stem Cells were isolated from patients with Colon cancer and cultivated in medium containing the appropriate growth factors. Knockdown of the c-Met gene was performed with specific siRNA with the use of Lipofectamine® 2000 Reagent. The cells were then analyzed with two different methods. The first panel included flow cytometry for the Nanog, Oct3/4, Sox2 and c-met proteins by using the appropriate antibodies and the second panel included Reverse Transcription and Real-Time PCR with Nanog, Oct3/4, Sox2 and c-met specific primers.

Results

The results show that c-Met gene expression knockdown leads to decreased expression of the stem cell markers Nanog, Oct3/4, Sox2 in Colon Cancer Stem Cells. The rate of decrease for Nanog and Oct3/4 was not as clear as it was for Sox2 gene.

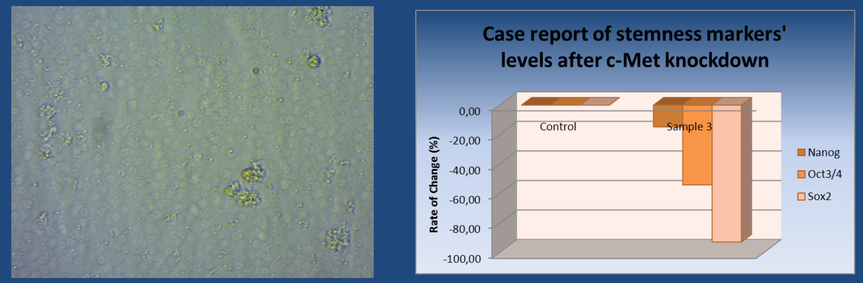

Fig. 1. (left) and Diagram 1 (right) represent Colon CSCs after c-Met knockdown and the following decrease in Nanog, Oct3/4 and Sox2 gene expression respectively from one sample that participated in the experiment.

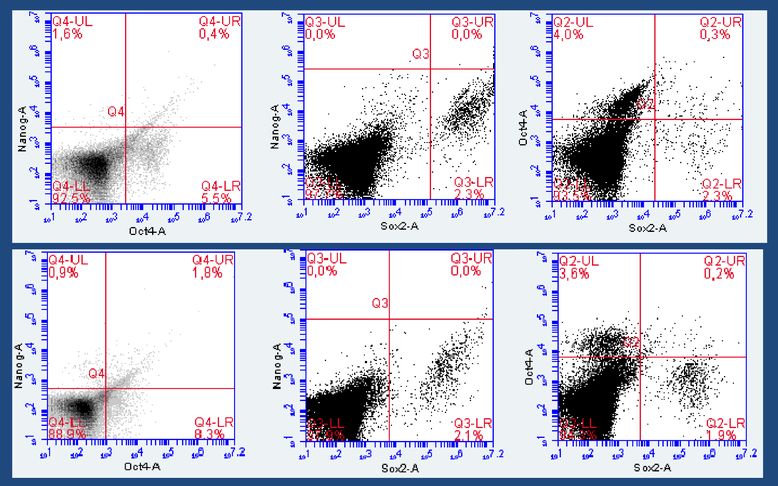

Fig. 2. Flow cytometry diagrams for Nanog, Oct3/4 and Sox2 proteins pre (up) and post (down) c-Met gene knockdown.

Conclusion

c-Met gene expresses the membrane Hepatocyte growth factor receptor (HGF-R) that is essential for embryonic development. The present study made an effort to determine the effect of c-Met gene to the expression of the Nanog, Oct3/4 and Sox2 stem cell markers in gene and protein level. The data show that c-Met knockdown decreases the expression of the three stem cell transcription factors and especially Sox2. Research is in progress for finding an expression pattern among the implicated genes.

References

1.http://en.wikipedia.org/wiki/Cancer_stem_cell

2.Kemper, K., C. Grandela, et al. (2010). "Molecular identification and targeting of colorectal cancer stem cells." Oncotarget 1(6): 387-395.

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