



# Studying the impact of nanog and other transcription factors in stemness pathway

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## BACKGROUND

Recent experimental evidence in a variety of tumors has supported strongly the cancer stem cell hypothesis. According to it, a small population of cancer cells which arises from the initial tumor mass has the ability to cause metastases and relapses. Pluripotency, self-renewal and resistance to chemotherapy are some of the basic hallmarks of them. Regulators in this pathway seems to be transcription factors such as nanog, oct3/4 as well as sox2. A synergistic role between them and stemness cascade may occur. The above hypothesis represents a possible model in the understanding of carcinogenesis and tumor cell biology.

## MATERIALS AND METHODS

In order to prove the above hypothesis three different methods have been chosen. The first panel included the si-RNA based method for repressing nanog gene. For the second panel a flow cytometric protocol as well as a Real-time PCR assay were conducted. For these experiments, breast cancer stem cell-like cells were isolated and cultivated from patients who suffered from breast cancer. Also a commercial breast cancer stem cell line was used.

BREAST CANCER STEM CELLS	PERCENTAGE OF NANOG%
Non-transfected cells	1.63
Transfected cells	0.74

Table 1. Flow cytometric results post nanog's gene knockdown by using an antihuman nanog antibody conjugated with FITC.

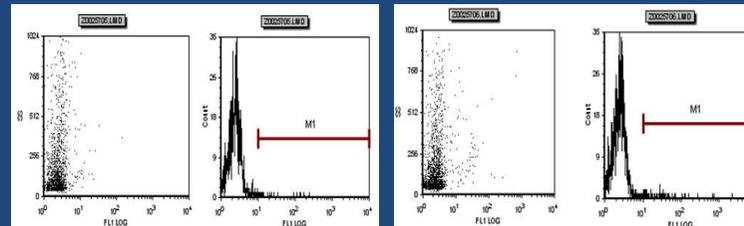


Figure 1. Flow cytometric results for non-transfected breast cancer stem cells.

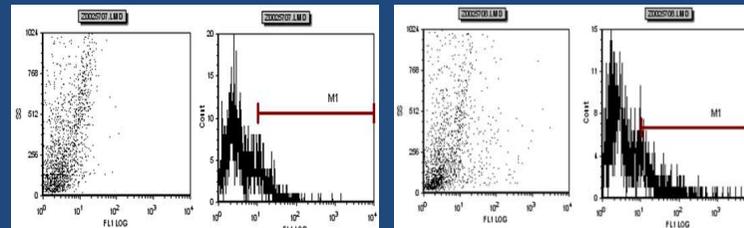


Figure 2. Flow cytometric results for transfected breast cancer stem cells.

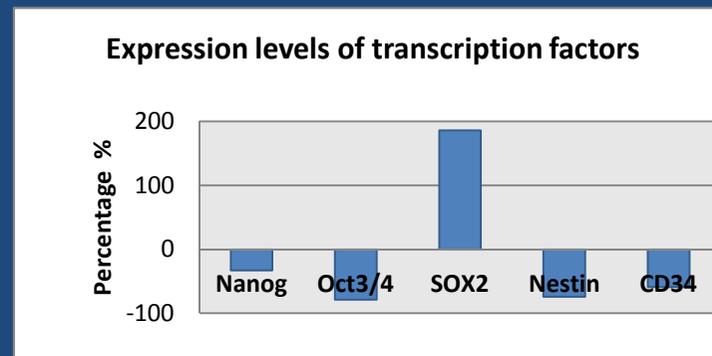


Table 2. Real-time PCR results of the change rate post nanog's gene knockdown (%).

## RESULTS

In the first panel of the test, it has been shown, by using a flow cytometric protocol that there was a reduction in nanog's expression level as it was expected (see table 1 and flow cytometric panels in figures 1 & 2). In the second panel, it has been shown that there was a correlation between nanog and the other transcription factors. The reduction of nanog's expression to 33%, has reduced the expression of Oct3/4, Nestin and CD34 gene to 79%, 84% and 59%, respectively. An exception from this trend was observed for SOX2 gene (see table 2).

## CONCLUSION

The present scientific attempt has indicated that, in this particular case, when nanog gene is downregulated, there is a reducing trend in the four basic transcription factors which are taking place in stemness pathway except of SOX2. Although the present experiments were performed in triplicates in order to avoid incorrect results, further studies need to be conducted in order to prove the above model.

## REFERENCES

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