

Studying the interaction of four receptors of NOTCH signaling pathway in breast cancer

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Background: The Notch signaling pathway might be the most important in cell differentiation. The gene encodes four different transmembrane receptors (Notch1, Notch2, Notch3 and Notch4), which may be linked with different ligands. According to literature and experimental data, the Notch signaling pathway is involved also in cancer. The present study aims to find out the change in gene expression of these four receptors, when the expression in one of them is suppressed.

Materials and Methods: The change of gene expression was studied in MCF-7 human cancer cell line (ECACC), which represents breast carcinoma. The gene suppression was performed with transfection protocol by using complementary RNA sequences of four genes (siRNA). The efficacy of suppression was measured with molecular biology methods and the expression of genes was studied by using Reverse Transcription Quantitative Polymerase Chain Reaction (RT-qPCR). There have been designed specific primers for each receptor and for 18SrRNA, which has been used as housekeeping gene. The analysis was made according to Livak method ($2^{-\Delta\Delta Ct}$), normalized to samples that were not transfected. The reactions were performed in triplicates, to extract statistically reliable data.

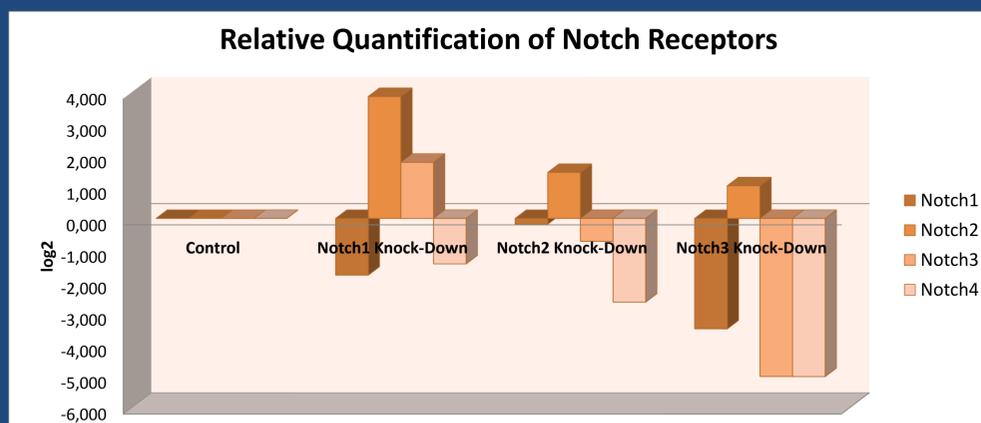


Figure 2: The relative change in gene expressions of Notch pathway receptors

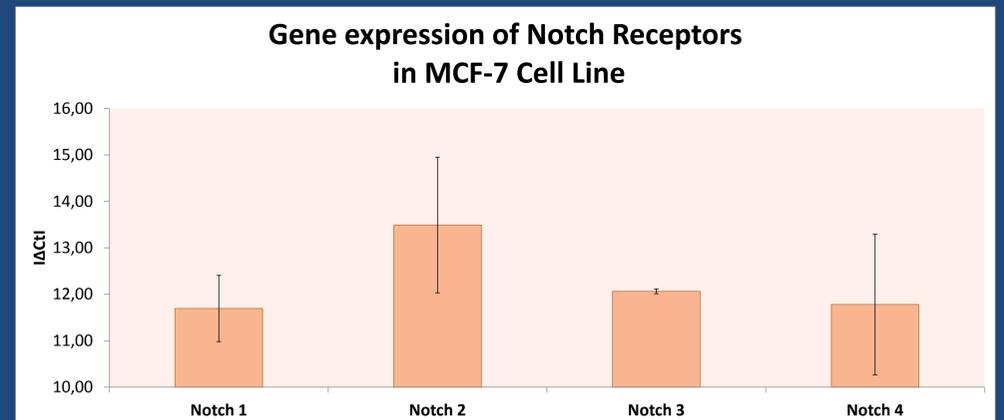


Figure 1: The absolute gene expression of Notch pathway receptors in MCF-7 cell line.

Results: In MCF-7 control cell line, Notch2 is expressed least of all, while the receptors Notch1 and Notch4 have the higher gene expression. When the gene expression of Notch1 is suppressed more than 75%, Notch2 expression is increased, whereas the same is observed for Notch3. The gene expression of Notch4 receptor is decreased in all cases, while a decrease of Notch3 was recorded under the suppression of Notch2.

Conclusions: The above experimental data suggest the possible interaction between the four different receptors of Notch signaling pathway. The pattern of gene expression varies according to the suppressed gene. The expression of Notch2 is probably suppressed by the other receptors in normal conditions, but it may serve as an alternative pathway, when the gene expression of the rest receptors is suppressed. However, it is imperative the study of more samples from different types of cancer, in order to define the relationship between the receptors, so these data to be used in clinical field.

References:

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2. Stylianou S, Clarke RB, Brennan K. Aberrant activation of notch signaling in human breast cancer. *Cancer Res* 2006;66:1517-25.