

Gene expression analysis of Notch signaling pathway receptors in Colon Cancer Stem Cells

P. Apostolou¹, M. Toloudi¹, M. Chatziioannou¹, E. Ioannou¹, I. Papatotiriou¹

¹ Research Genetic Cancer Centre Ltd. (R.G.C.C. Ltd). 115 M.Alexandrou str, 53070 Filotas, Florina, Greece

Abstract Id : 29

Introduction: The Notch signaling pathway is one of the most important during tissue and cell development. It consists of four different receptors (NOTCH-1, NOTCH-2, NOTCH-3 and NOTCH-4). This pathway is also implicated in cancer development. According to recent experimental data, NOTCH-1 activation induces epithelial to mesenchymal transition (EMT) consistent with the cancer stem cell phenotype. ZEB1, CD44, EpCAM and Hes-1 are mesenchymal cell markers, which are activated by Notch-1 over-expression. In order to focus on Notch-1, it would be preferable to study the potential interaction with the other receptors, as the overlap through the pathways is almost a rule. The present study aims to find out the correlation among Notch signaling receptors, under the suppression of Notch-1.

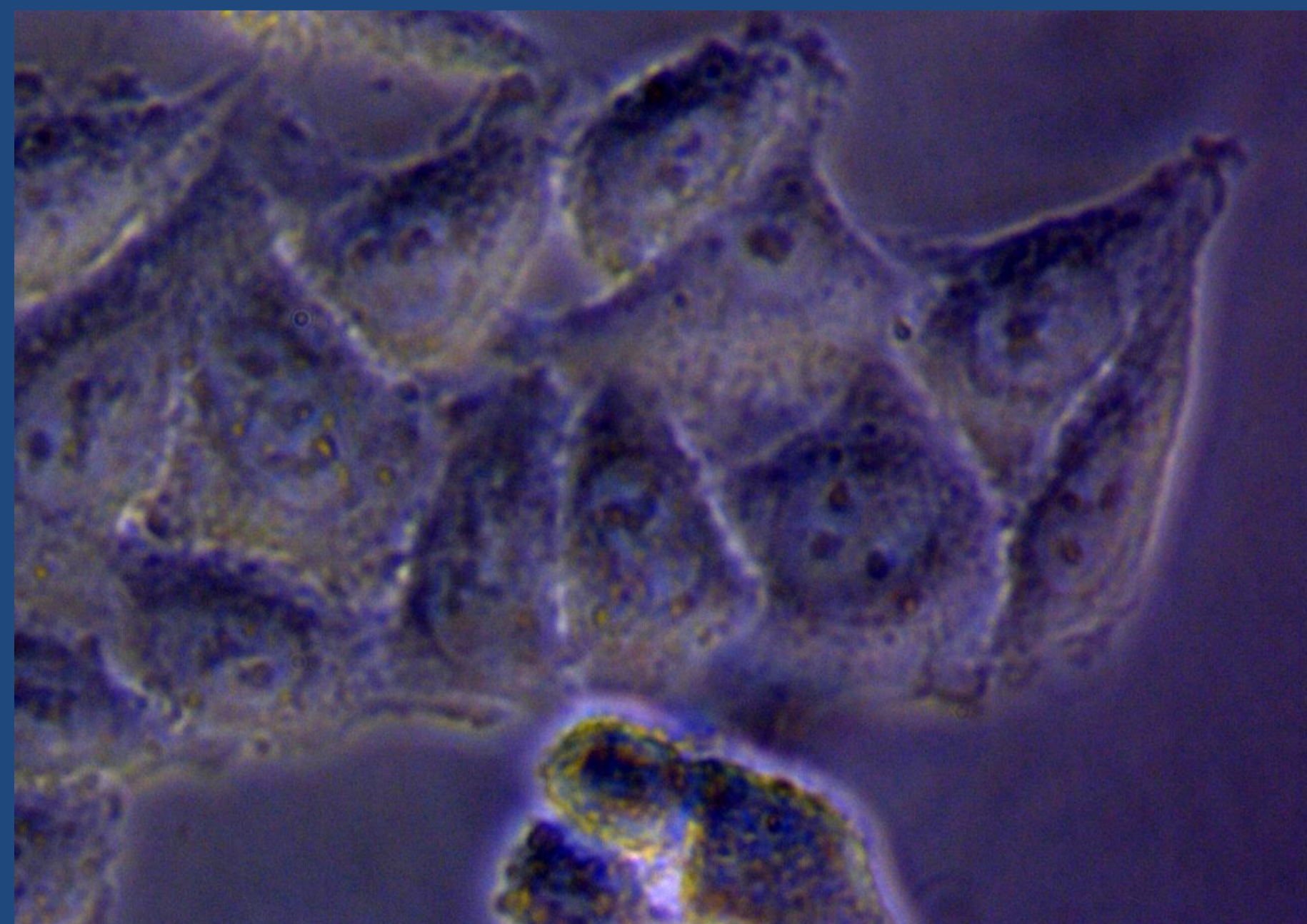


Figure 1: Control Colon CSCs

Materials & Methods: The experiments were performed in established Human Colon Cancer Stem Cells (CSCs). The stemness potential was tested by testing specific transcription markers (Oct4, Sox2, Nanog, CD44). The authenticity of the cell line was tested with STRs assays, compared to the manufacturer's pattern. Notch-1 was suppressed with specific siRNA and the gene expression knock-down was evaluated with qPCR. By using the comparative Ct method ($\Delta\Delta Ct$), gene expression analysis was carried out.

Results: The knock-down of Notch-1 up to 75%, led to a reduction of gene expression in Notch-2 and Notch-3 receptors. No significant difference was observed in Notch-4 (Data now shown). The Notch-2 receptor's gene expression decreased up to 90%

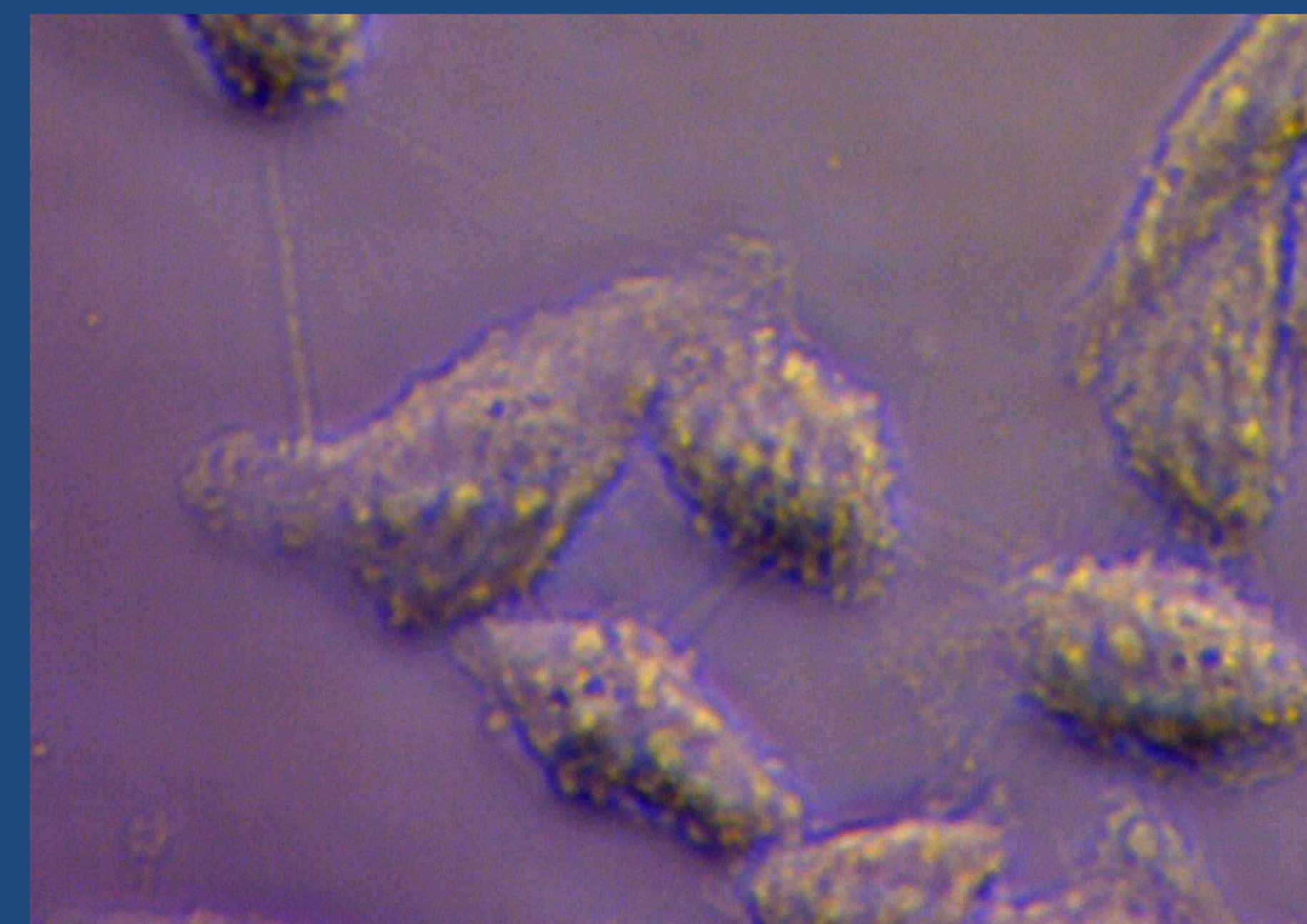


Figure 2 Notch-1 knock-down Colon CSCs

Conclusion: According to the above data, it can be concluded that Notch-1 interacts with Notch-2 and Notch-3, affecting their gene expression. It should be therefore necessary to find out the role of each receptor for EMT capability and then to concentrate to specific transcription factors. Further studies to a greater range of samples need to be performed, in order to use these data at clinical level.

Selected References:

- Dhawan P, Ahmad R, Srivastava AS, Singh AB. Cancer stem cells and colorectal cancer: an overview. *Curr Top Med Chem* 2011;11:1592-8.
- Wang Z, Zhang Y, Banerjee S, Li Y, Sarkar FH. Notch-1 down-regulation by curcumin is associated with the inhibition of cell growth and the induction of apoptosis in pancreatic cancer cells. *Cancer* 2006;106:2503-13.
- Allenspach EJ, Maillard I, Aster JC, Pear WS. Notch signaling in cancer. *Cancer Biol Ther* 2002;1:466-76.
- Bao B, Wang Z, Ali S, et al. Notch-1 induces epithelial-mesenchymal transition consistent with cancer stem cell phenotype in pancreatic cancer cells. *Cancer Lett* 2011;307:26-36.

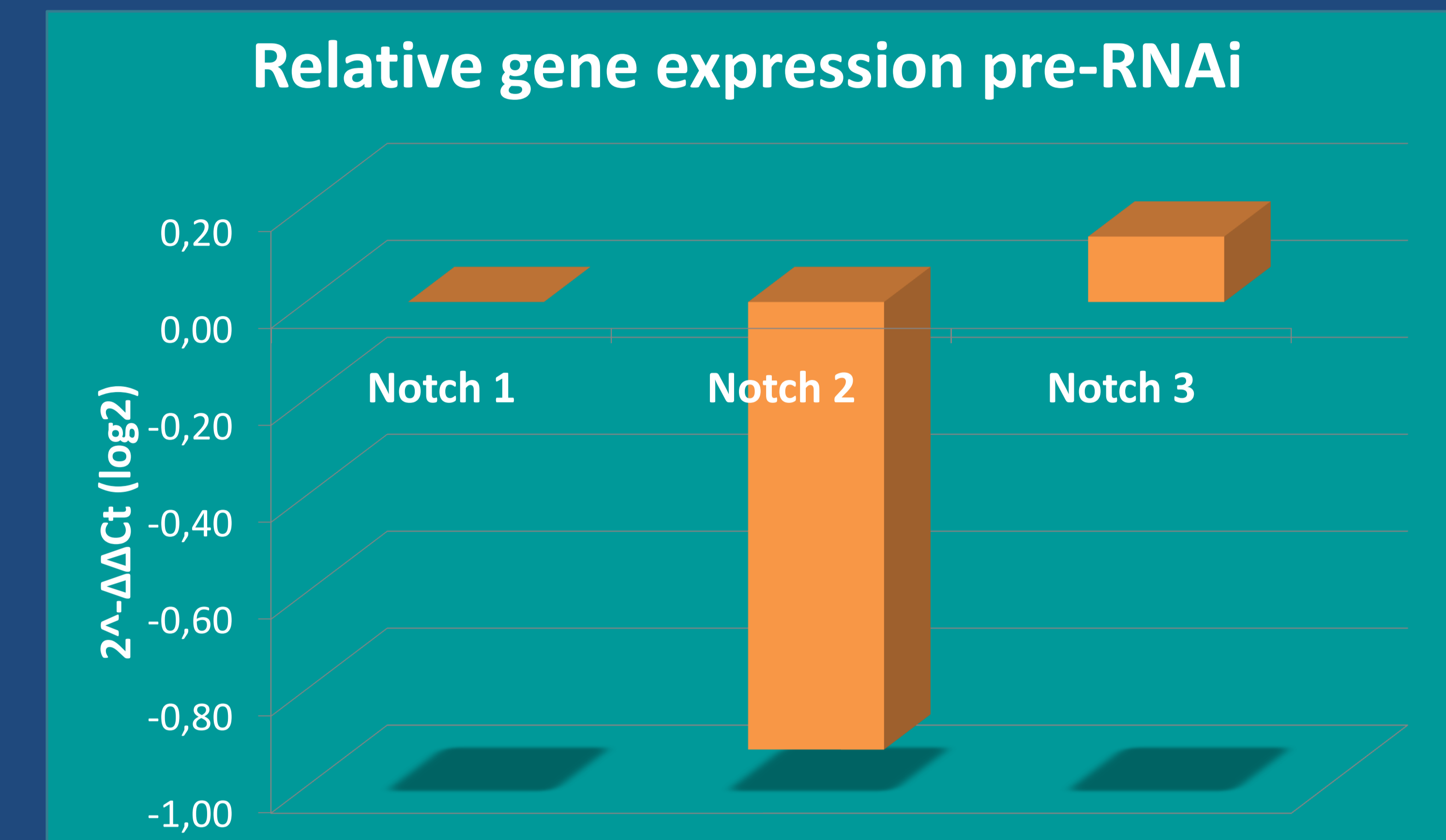


Figure 3: Gene expression among Notch receptors in control Colon CSCs

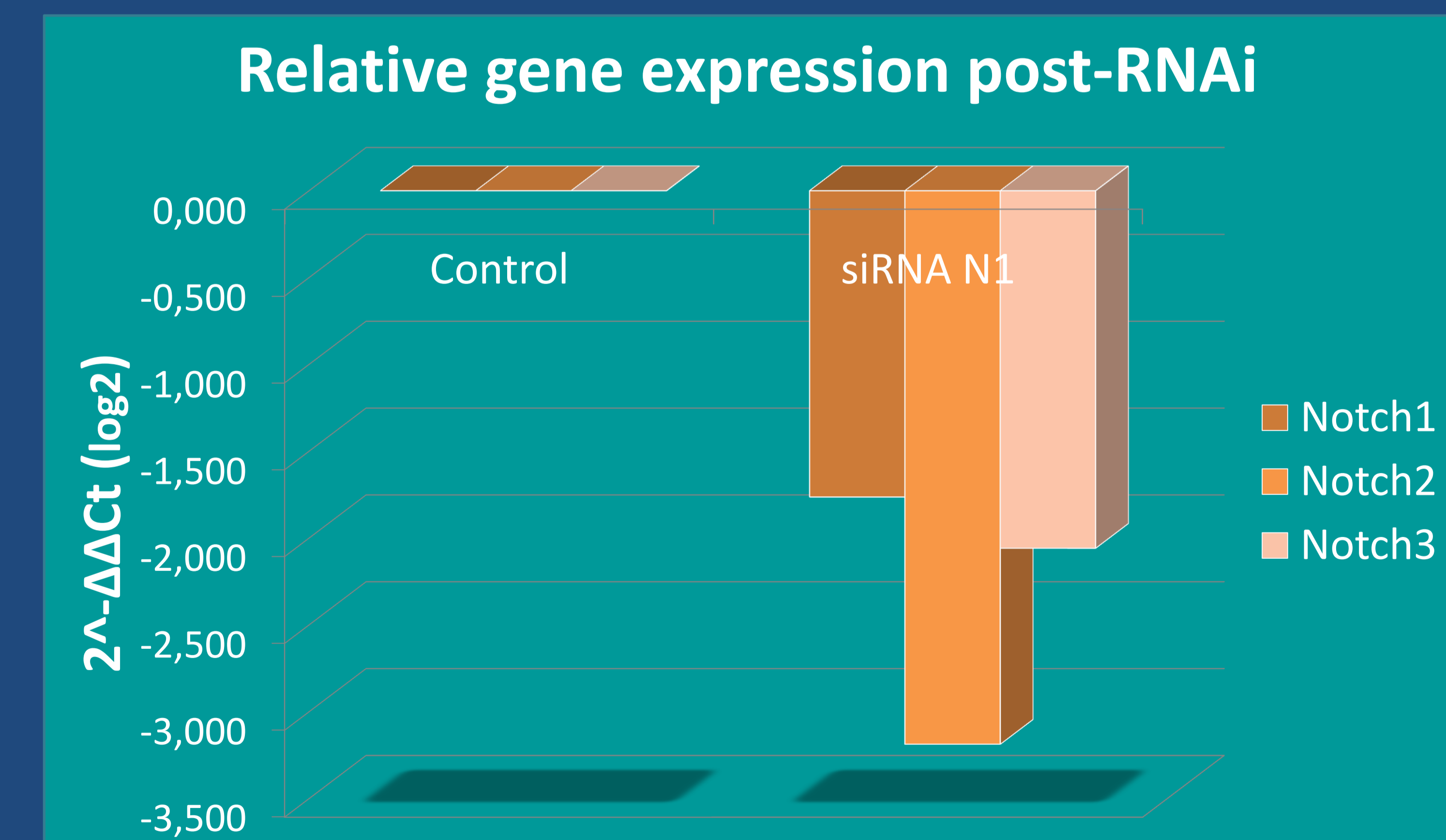


Figure 4: Gene expression among Notch receptors in Colon CSCs after siRNA transfection