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Studies on potential APC/β-catenin target genes in the Notch pathway

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Aim

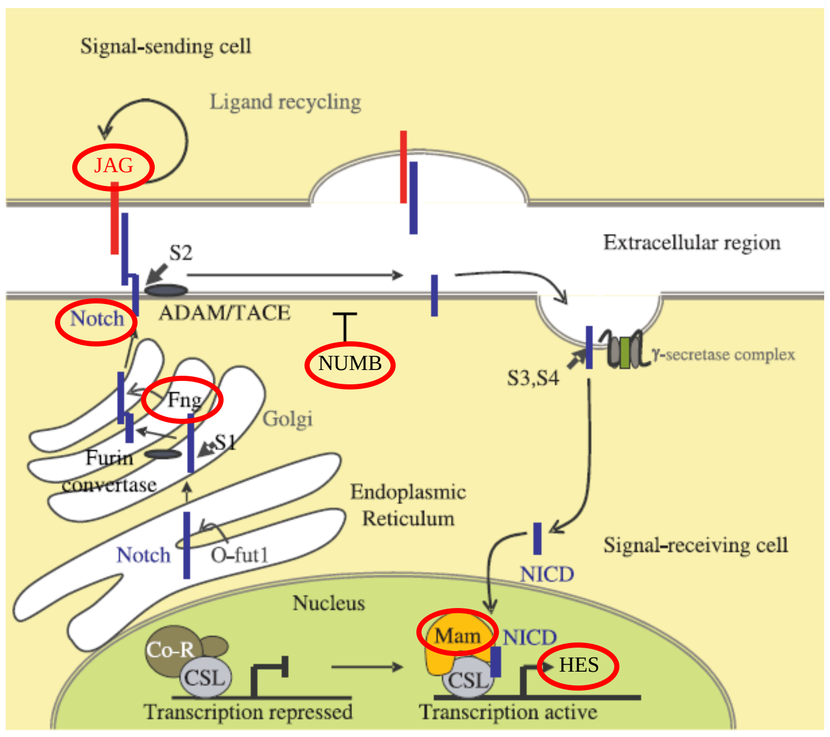
The aim of the study is to investigate the interaction between the Wnt –and Notch-pathways with focus of Wnt pathway regulation of the Notch-pathway, and if this process is important for colorectal cancer development and/or progression.

Method

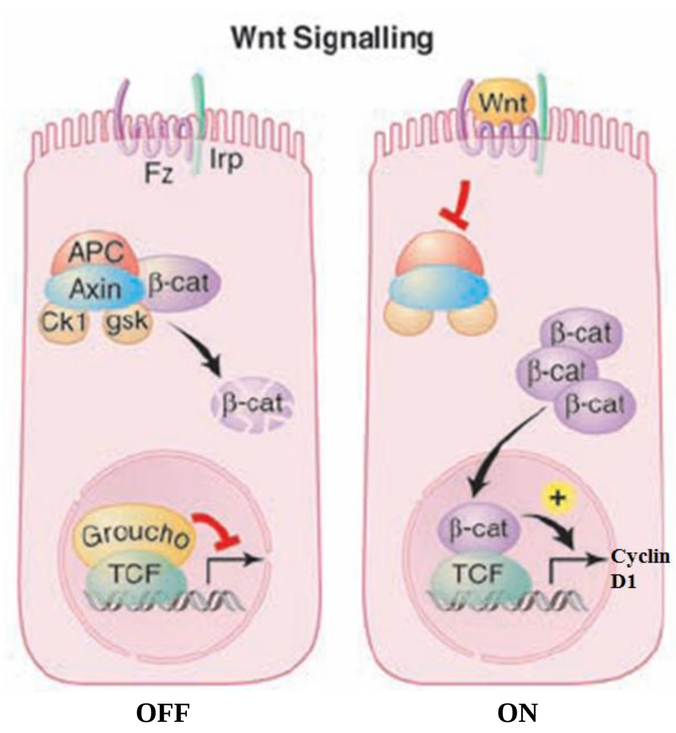
We will use human colorectal cell line HT29 were the protein APC is nonfunctional, leading to continuously Wnt pathway activation. By inserting a vector containing a zinc-inducible APC gene (HT29-APC) makes us control the deactivation of the Wnt pathway.
We will also use siRNA against APC destruction target β-catenin to be sure that we deactivate the Wnt pathway downstream target gene promgram Tcf/Lef.

By treating the HT29 cells with DAPT, a gamma-secretase inhibitor which prevents release of Notch intracellular domain, we will examine what happens with the Wnt/β-catenin signaling when the Notch pathway is inhibited.

Results



The picture[1] is showing the Notch signaling pathway and the circles highlights the genes downregulated when APC is present, functional Wnt inhibition.
Same genes was also downregulated in HT29 cells transfected with siRNA against β-catenin (data not shown).
All experiments were repeated twice.



The picture[2]is showing the the Wnt signaling pathway. Inhibition of the Notch pathway using DAPT was succeful (data not shown), but the preliminary results indicates that there is no regulation of APC/β-catenin(OFF) by the Notch pathway (no Cyclin D1 inhibition).

Conclusion

- By inhibiting the Wnt pathway, our results have shown downregulation of:
  - one ligand (JAG 2)
  - one receptors (Notch 2)
  - one transcriptional activator (MAML 1)
  - two target genes (Hes 1, Hes 7)
  - two inhibitors (NUMB, NUMBL)
  - two Notch glycosyltransferases (LFNG,RFNG)
- This total down regulation of the Notch pathway upon Wnt deactivation shows a correlation between the two signaling pathways in colorectal cancer. It is accomplished by the Tcf/Lef target gene program.
- We did not find any correlation the other way around, between Notch inhibition and Wnt pathway through β-catenin signalling.

References: 1. Fiúza, U.-M., Arias, A.M 2007
Cell and molecular biology of Notch
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2. Radtke, F., Clevers, H 2005
Self-renewal and cancer of the gut: Two sides of a coin
Science 307 (5717), pp. 1904-1909