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 of Notch glycosyltransferases (LFNG, RFNG)

- Is accomplished by the Tcf/Lef target gene pro gram, shown by transfecting the cells with siRNA against β-catenin.

- This total down regulation of the Notch pathway upon Wnt deactivation shows a correlation betwe en the two signaling pathways in colorectal cancer. We did not find any correlation the other way around, between Notch inhibition and Wnt pathway through β-catenin signalling.

- Using gamma-secretase inhibitors may provide a targeted-drug strategy for treating human colorec tal cancer, because of the close correlation betwe en the Notch and Wnt pathway

- Inhibition of Notch signalling gives a decrease in Hes 1 gene expression, leading to halt of cell proli feration and generation of apoptosis.

  Preclinical studies for Alzheimer's disease in ro dents have shown that a side effect of gamma-secretase inhibitors is macroscopic abnormalities i n the GI-tract, where the small and large intestine is distended and with an excess of mucus

- This may be one way of inhibiting the upregulation of the Notch pathway in colorectal cancer.

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

By: John Grünberg
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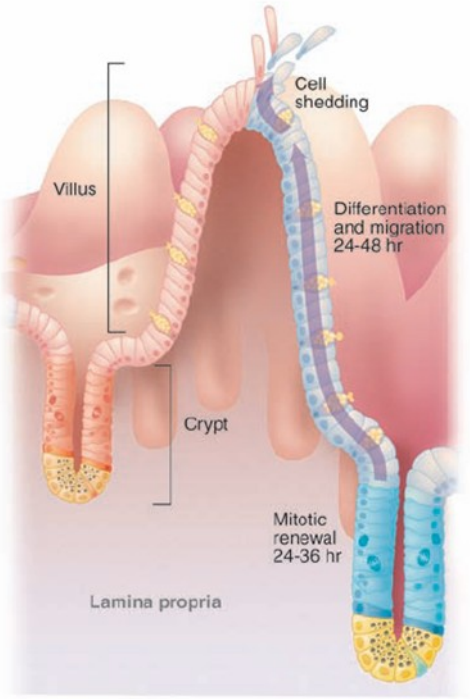

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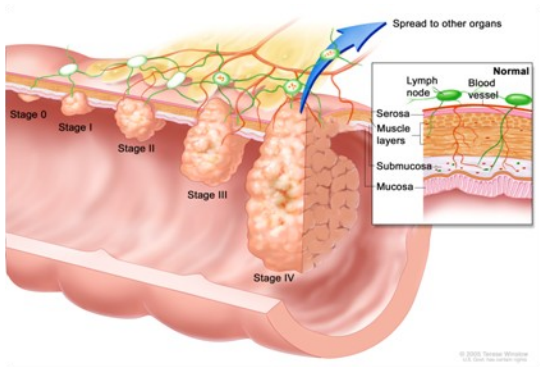
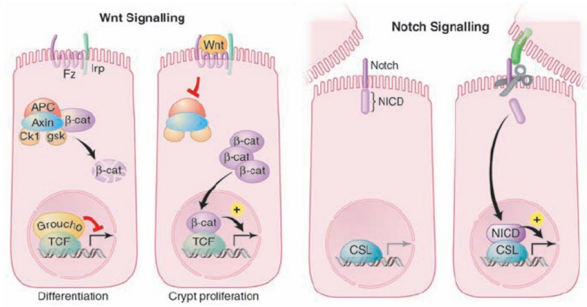
Background

The most common form of cancer in the intestine is colorectal cancer (CRC) and it is the second most common type of cancer in both men and women in Sweden.



Dividing cells of the mammalian intestine are restricted to finger-like invaginations of the epithelium called the crypts of Lieberkühn. The progeny of the stem cells migrates upward, from the cryps towards finger-like outgrows into the lumen called villi. In the villi tips the division stagnates and the differentiation is completed. Cell to cell signalling in the epithelium is mainly carried out by Wnt, Notch and ephyrin pathways.

Both Notch and the Wnt pathways are key regulators in maintaining the homeostasis in the intestine. Defects on the key tumor suppressor adenomatous polyposis coli, APC a gene in the Wnt pathway is most frequently mutated in colorectal cancer.



Aim

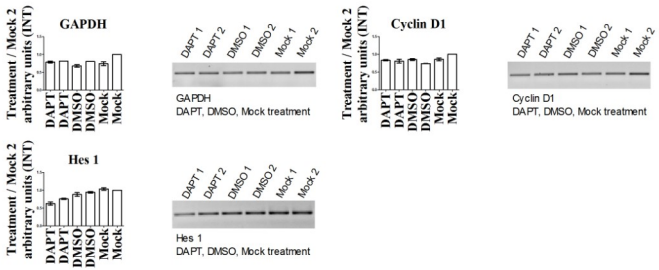
The aim of the study is to investigate the interaction between the Wnt –and Notch-pathways with focus of Wnt pathway regulation of the Notch-pathway, and if this process is important for colorectal cancer development and/or progression.

We will use human colorectal cell line HT29 were both endogenous APC alleles contain truncated mutations, 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 a control cell line containing an analogous inducible lacZ gene (HT29-βgal)

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.

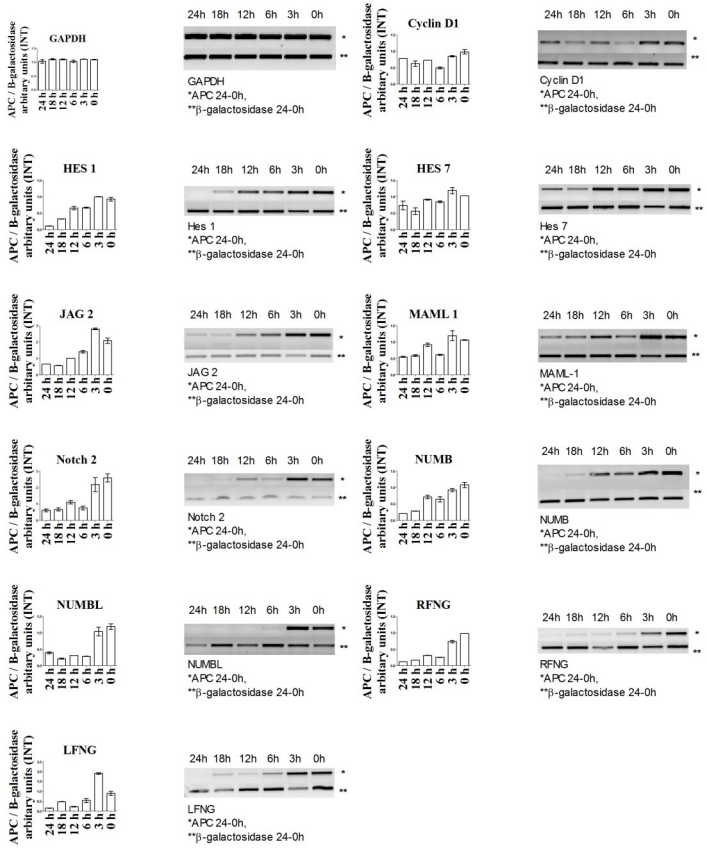
Results

DAPT treated HT29



Results from DAPT,DMSO and Mock treatment of HT29 cells for 48h. Diagrams are showing intensity signal from the different treatments/Mock 2. Experiments were repeated twice.
Western blot against HES1 was preformed and HES1 was downegulated in DAPT treated cells(data not

HT29 APC



Results from semi quantitative PCR with genes from the Notch pathway in HT29-APC and HT29-βgal. Diagrams are showing the intensity signal from HT29APC/HT29-βgal at different time points. There is a clear trend of downregulation, lowering of signal in 24h-6h samples for all genes except GAPDH. Experiments were repeated twice. Western blot against APC was preformed and APC was upregulated in APC 24-6h (data not shown).

Same genes was also downregulated in HT29 cells transfected with siRNA against β-catenin (data not shown).Western blot against β-catenin was preformed and β-catenin was downregulatede all siRNA transfected cells (data not shown).

In the colon the Wnt pathway can regulate the Notch pathway through β-catenin