Stabilization of β-catenin in the Wnt pathway and its implication in gastrointestinal cancers

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Introduction

Signalling by the Wnt family of secreted glycoprotein is one of the fundamental mechanisms that direct cell proliferation, cell polarity, and cell fate determination during embryonic development and tissue homeostasis. As a result, mutations in the Wnt pathway are often linked to human birth defects, cancer, and other diseases.

A critical and heavily studied Wnt pathway is the canonical Wnt pathway, which functions by regulating the amount of the transcriptional coactivator β-catenin. Aberrant canonical Wnt pathway is widely implicated in gastrointestinal cancers. All mechanisms deregulated in these tumour types provide a framework for understanding the complexities faced in attempting leverage this pathway in the clinic.

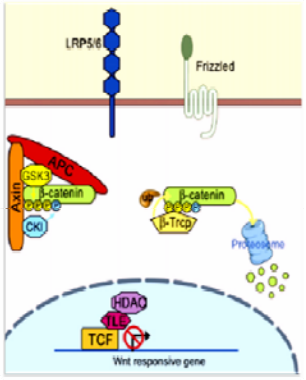
Aims and methodology

The aim of this project was to study the canonical Wnt pathway and its physiological role in the renewal of the intestinal epithelium. This is essential to better understand the pathway alterations that can cause the development of certain intestinal epithelium cancers.

The methodology used was a scientific literature search on PubMed database. Keywords used were "canonical Wnt pathway" and "gastrointestinal cancers". This search was carried out through the selection of recent papers and reviews, according to their quality and date of publication.

The canonical Wnt pathway

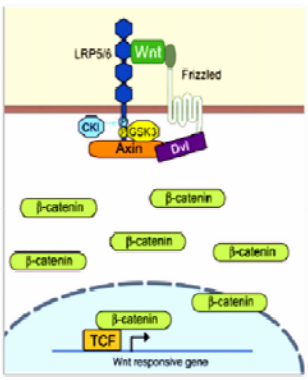
The canonical Wnt pathway controls the stability of β-catenin. **β-catenin** has a dual role in the cell in the cell membrane as part of **adherents junctions** and in the nucleus as a **transcriptional co-activator**



MacDonald BT, Tamal K, He X (2009)

Fig. 1: In the absence of Wnt signals, cytoplasmic β-catenin released from adherents junctions, is constantly degraded by the action of the **multiproteic complex**, which is composed of the scaffolding protein **Axin**, the tumour suppressor *adenomatous polyposis coli gene product* (**APC**), *casein kinase 1* (**CK1**) and *glycogen synthase kinase 3* (**GSK3**).
CK1 and GSK3 sequentially phosphorylate the N-terminal region of β-catenin, resulting in β-catenin recognition by **β-Trcp**, an E3 ubiquitin ligase subunit, and subsequent β-catenin ubiquitination and proteasomal degradation.

This continual elimination of β-catenin prevents β-catenin from reaching the nucleus. Wnt target genes expression is repressed by Groucho/TLE interaction with the DNA-bound *T cell factor/lymphoid enhancer factor* (TCF/LEF) family of proteins.



MacDonald BT, Tamal K, He X (2009)

Fig. 2: Wnt/β-catenin pathway is activated when a Wnt ligand binds Frizzled (**Fz**) receptor and its co-receptor *low-density lipoprotein receptor-related protein 5 or 6* (**LRP5/6**). The formation of the complex Wnt-Fz-LRP5/6, together with the recruitment of the scaffolding protein *Dishevelled* (**Dvl**), results in LRP5/6 phosphorylation and the recruitment of the Axin complex to the receptors.

This event leads to inhibition of the Axin-mediated β-catenin phosphorilation and thereby to the stabilization of β-catenin, which accumulates and translocates to the nucleus to interact with TCF/LEF transcription factors and activates Wnt target gene expression.

Function of the canonical Wnt pathway in the renewal of the intestinal epithelium

The canonical Wnt signalling not only features in many developmental processes; in some self-renewing tissues, such as intestinal epithelium, it remains essential throughout life.

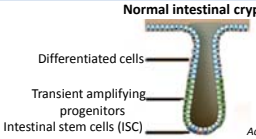


Fig. 3: In the intestinal epithelium, **proliferating crypt precursors** and **differentiated villus cells** form a contiguous sheet of cells that is in perpetual upward motion. **Intestinal stem cells**, which produce the transit-amplifying progenitor cells, reside near the bottom of the crypt and escape this flow.
Evidence suggests that Wnt signalling is required for the establishment of the progenitor compartment in the intestinal epithelium.

Adaptation of Voloshanenko O, Erdmann G, Dubash TD et al. (2013)

Hyperactivation of the canonical Wnt pathway in the development of gastrointestinal tumors

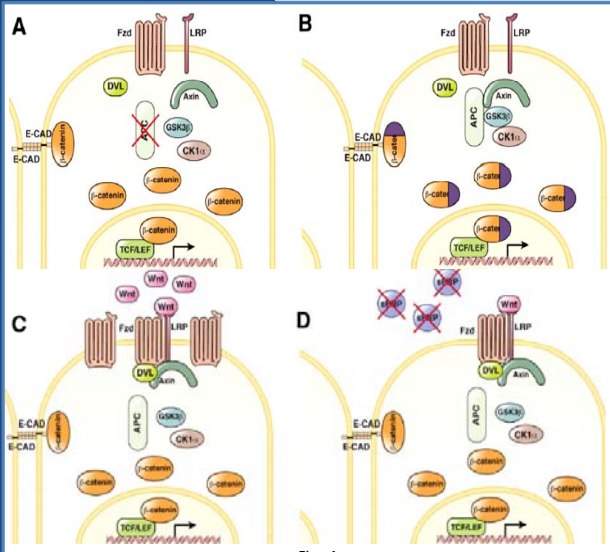


Fig. 4
White B, Chien A and Dawson D (2012)

- There are common mechanisms by which the Wnt/β-catenin pathway is upregulated in gastrointestinal cancer *(Fig. 4)*:
  - **(A) Loss-of-function mutations in APC** lead to a breakdown of the destruction complex and cause a constitutive expression of Wnt/β-catenin-dependent genes.
  - **(B) Gain-of-function mutations in β-catenin**, often occurring in exon 3, prevent its N-terminal phosphorylation thus averting its ubiquitination and degradation.
  - **(C) Overexpression of Fz receptors or Wnt ligands** can lead to increased activation of the pathway.
  - **(D) Underexpression of secreted inhibitors of the pathway** (*secreted frizzled—related proteins*, sFRPs) can also lead to increased sensitivity to Wnt ligands and increased pathway activation.
- Interactions with other signalling pathways influence on the activation of the Wnt/β-catenin pathway, such as Notch, Hedgehog (Hh) and TGF-β.

	CRC	HCC
% of human tumours containing mutations in key members of Wnt/β-catenin pathway	90%	18-44%
Wnt/β-catenin pathway members mutated most often	80% APC, 10% β-catenin	3-44% β-catenin, 5-25% Axin
Crosstalk with other signalling pathways	Notch, Hh	TGF-β, HGF/MET

Table 1: Two types of gastrointestinal cancers with high mutation rate in key members of the canonical Wnt pathway: the colorectal carcinoma (CRC) and the hepatocellular carcinoma (HCC).

Adaptation of White B, Chien A and Dawson D (2012)

❑ **CRC** is the most well studied cancer caused by overactivation of the Wnt/β-catenin. The canonical Wnt pathway is frequently the first altered pathway in this type of gastrointestinal cancer *(Fig. 5)*.

❑ **HCC** is not as well studied as the CRC in the carcinogenesis progression.

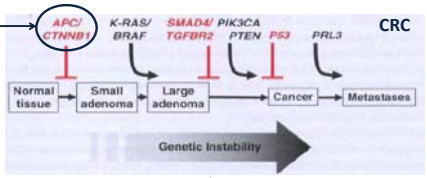


Fig. 5
Adaptation of Fearon and Vogelstein (1990)

Potential therapies

At present there are no inhibitors clinically approved for the Wnt/β-catenin pathway to treat tumors, as example the CRC and the HCC.

There are several possible hypotheses to explain the absence of an effective therapy in these tumors:

1. Involvement of the Wnt/β-catenin pathway in the potential maintaining of stem cells.
2. Heterogeneity of Wnt/β-catenin signalling in normal tissues and in tumors.
3. Uncertainty about its efficacy and toxicity in the individual.

Studies indicate that some phytochemicals and the active form of vitamin D can inhibit or modulate Wnt/β catenin signalling pathway.

Conclusions

- The canonical Wnt pathway is physiologically active in different tissues, such as intestinal epithelium, to renew different cell types throughout life.
- Mutations in APC, β-catenin or overexpression of Wnt ligands are three alterations that cause constitutive activation of the Wnt/β-catenin pathway and originate gastrointestinal cancers.
- Two examples of gastrointestinal cancers are the CRC and the HCC. Within cases of sporadic CRC, most contain mutations in the APC gene, while HCC cases contain mutations mainly in the β-catenin or Axin protein.
- In conclusion, gastrointestinal cancers caused by aberrant activation of the canonical Wnt signalling currently do not have a safe and effective treatment.