DOCUMENT SUMMARY

This foundational 2000 review by Bienz and Clevers details the critical role of the **Wnt signaling pathway** in the development of colorectal cancer. It explains how mutations in the **APC** tumor suppressor gene lead to the stabilization of its target, **β-catenin**. This stabilized β-catenin then forms a complex with **TCF** transcription factors, driving the expression of genes that initiate tumorigenesis. The paper synthesizes insights from developmental biology in model organisms and human cancer genetics to outline the core molecular events that trigger most colorectal tumors.

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METADATA

Category: RESEARCH **Type:** report **Relevance:** Core **Update Frequency:** Static **Tags:** #wnt-signaling #colorectal-cancer #apc #beta-catenin #tumor-suppressor #signal-transduction #tcf #axin **Related Docs:**

- ries_2000_research_report_ras_p53_mdm2_p19arf
- davis_2000_research_report_signal_transduction_jnk_mapk
- cheung_2000_research_report_histone_modification_epigenetics **Supersedes:** N/A

FORMATTED CONTENT

Linking Colorectal Cancer to Wnt Signaling

Introduction

Tumors of the colorectum are amongst the most common human neoplasms. Studies of inherited and sporadic colorectal cancer have demonstrated that in the overwhelming number of cases the primary mutation targets a single signal transduction pathway. A story has rapidly unfolded in which the **Wnt signaling pathway** plays a central role in the etiology of this cancer.

One of the best defined though rare forms of such cancer syndromes is **familial adenomatous polyposis (FAP)**. In 1991, the tumor suppressor **Adenomatous polyposis coli (APC)**

encoded by the FAP locus was identified. It turned out that, not only the tumors from FAP patients, but also most sporadic colorectal tumors have both APC alleles inactivated.

The discovery that APC binds to **β-catenin** provided the first significant clue as to the molecular function of APC. A link between APC and Wnt signaling emerged shortly after this, when the Wnt pathway was discovered by genetic analysis in *Drosophila*. This established that **Armadillo**, the fly homolog of β-catenin, mediates responses to signaling by **Wingless**, the archetypal Wnt ligand in *Drosophila*.

Our review centers on the question of how the deregulation of β-catenin leads to malignant development in the mammalian gut epithelium.

The Canonical Wnt Pathway

Combined work in flies, frogs, and mammals produced the main outline of the canonical Wnt/β-catenin signaling pathway.

- **In unstimulated cells:** Free cytoplasmic β-catenin is destabilized by a multiprotein complex containing **Axin**, **glycogen synthase kinase 3β (GSK3)**, and the **APC** tumor suppressor.
    - Axin acts as a scaffold, bringing the components together.
    - This complex facilitates the phosphorylation of β-catenin by GSK3.
    - This phosphorylation event earmarks β-catenin for ubiquitination and subsequent degradation by the proteasome.
    - As a result, cytoplasmic β-catenin levels are low, and **TCF** (T cell factor) transcription factors in the nucleus are actively repressed.
- **When cells are stimulated by Wnt ligands:**
    - The cytoplasmic protein **Dishevelled** is recruited to the membrane and appears to inhibit the Axin complex.
    - With the degradation complex inhibited, β-catenin is no longer destroyed and begins to accumulate in the cytoplasm.
    - Stabilized β-catenin translocates into the nucleus, where it binds to TCF proteins.
    - The β-catenin/TCF complex then serves as a coactivator to stimulate the transcription of Wnt target genes.

Clearly, the stability of free cytoplasmic β-catenin/Armadillo is at the heart of the canonical Wnt pathway.

It is worth noting that β-catenin has an additional, separate function: it binds to the adhesion molecule **E-cadherin** at the cell membrane, linking it to the actin cytoskeleton to mediate cellular adhesion.

Early Events during Colorectal Tumorigenesis

Some 85% of all sporadic and hereditary colorectal tumors show loss of **APC** function. Colon cancer cells with mutant APC contain high levels of free β-catenin. It is now commonly accepted that the key tumor suppressor function of APC lies in its ability to destabilize free β-catenin.

This was strengthened by the discovery of mutations in other Wnt pathway genes that also cause β-catenin to accumulate:

- Among the 15% of colon carcinomas that retain wild-type APC, point mutations were found in **β-catenin** itself, specifically at the sites targeted by GSK3, making it resistant to degradation.
- Furthermore, a fraction of hepatocellular carcinomas with wild-type β-catenin show **Axin** mutations.

> The evidence is now compelling that stabilized free β-catenin is an early event during, and perhaps even initiates, tumorigenesis in the mammalian intestinal tract.

The direct consequence of APC loss is an increase in the size of the proliferating crypt compartment in the intestinal epithelium. This results in an abnormal tissue architecture which presents as a polyp. It is conceivable that inappropriate activation of **Tcf4** (a TCF family member) by stabilized β-catenin accounts for this expansion of the crypt compartment. The polyp may be built from cells that would normally differentiate but have been misspecified by activated Tcf4 to remain as proliferating crypt cells.

Progression to Malignancy

For colorectal adenomas to progress to malignancy, further genetic changes need to occur.

- One of the best documented is the loss of the response to **TGF-β**, a signaling pathway that can inhibit the growth of epithelial cells.
- Oncogenic activation of **K-ras** and mutations in **p53** are also frequently observed in colorectal tumors.

Interestingly, there appear to be some differences between mice and humans in the factors that are important for tumor progression, possibly because APC loss leads to tumors predominantly in the small intestine of the mouse, but in the colorectum of humans.

The Molecular Function of APC

Almost all APC mutations in colorectal tumors result in protein truncations, clustered in the central **mutation cluster region (MCR)**. Typically, these truncations remove all binding sites for **Axin/Conductin**, and a strong case can be made that APC's ability to bind to Axin is critical for its tumor suppressor function.

Nuclear β-catenin and TCF together bring about changes in gene transcription. Target genes that may contribute to tumor progression include:

- The metalloproteinase **matrilysin**.
- The cell cycle promoting genes **c-myc** and **cyclin D1**.

How does APC destabilize β-catenin? A specific suggestion has been that APC may have a spatial regulatory role, shuttling β-catenin around the cell. This model was inspired by the subcellular distribution of *Drosophila* APC, which is found at cell junctions but also in the cytoplasm and nucleus. Support for this model has come from the discovery that APC contains **nuclear export signals (NES)**.

> Consequently, truncated APC (only retaining the N-terminal NES) is nuclear in cells derived from APC mutant tumors. These cells also show high levels of nuclear β-catenin... It thus appears that the truncated APC typically observed in colon cancers may be unable to export β-catenin efficiently from the nucleus, or may even trap β-catenin in the nuclei of these cells.

Functions of APC in Cell Migration and Adhesion?

It is beginning to emerge that APC may have functions at the cell periphery that are separate from its function effected by nuclear β-catenin.

- In migratory mammalian cells, APC was found to cluster at the plus ends of **microtubules**.
- It was discovered recently that APC binds to **Asef**, an exchange factor that activates the small G protein **Rac**, which in turn controls the actin cytoskeleton and induces migratory activity.
- In *Drosophila*, loss of APC causes cell shape changes and subtle defects in the junctional association of Armadillo (β-catenin).

Such a role could be relevant for APC's function as a tumor suppressor. One of the earliest manifestations of APC loss is an abnormal tissue architecture which may reflect a defect in cell migration or adhesion. Furthermore, it is well established that cadherin-mediated adhesion is typically lost at the invasive front of tumors. This indicates that APC loss may be more powerful than oncogenic activation of β-catenin alone in promoting malignant development.