Wnt breakers in colon cancer

β-catenin and Tcf4 are the downstream effectors of the Wnt signaling cascade. In colorectal cancer, mutations in Wnt cascade genes such as APC lead to the inappropriate formation of β-catenin/Tcf4 complexes. Earlier work has predicted that disruption of the β-catenin/Tcf4 protein-protein interaction could revert the proliferative phenotype of colorectal cancer cells. In this issue of *Cancer Cell*, Shivdasani and colleagues (Lepourcelet et al., 2004) have explored high-throughput screening of compound libraries in a search for small molecule inhibitors of the Wnt cascade. Ultimately, such inhibitors could become a novel class of smart anticancer drugs.

The seminal discovery of the common origin of the *Drosophila* segment polarity gene *Wingless* and the murine protooncogene *Int-1* (Rijsewijk et al., 1987) heralded the start of a remarkable success story of interdisciplinary science. *Drosophila* geneticists, immunologists, frog developmental biologists, cell biologists, and molecular oncologists all provided essential components of a signaling pathway that is now referred to as the canonical Wnt cascade. The term Wnt derives from the contraction of the gene names *Wingless* and *Int-1*. Wnt genes, of which the human genome encodes almost twenty, are found throughout the animal kingdom. Secreted Wnt factors control virtually every developmental decision in the lifetime of an animal. The central player in the cascade is a cytoplasmic protein termed β-catenin, whose stability is regulated by the APC complex. When Wnt receptors are not engaged, kinases in the APC complex phosphorylate β-catenin, thus targeting the latter protein for rapid destruction. When the receptors are activated by their Wnt ligands, the intrinsic kinase activity of the APC complex is inhibited. As a consequence, stable nonphosphorylated β-catenin accumulates and makes its way into the nucleus, where it engages the N terminus of DNA binding proteins of the Tcf/Lef family. The human genome encodes four Tcf/Lef proteins. In the absence of a Wnt signal, these DNA binding proteins occupy target genes to repress their transcription. The interaction with β-catenin transiently converts Tcf factors from repressors into transcriptional activators. A Wnt signal is thus translated into the transient transcription of a Tcf target gene program (Huelsken and Birchmeier, 2001).

The *APC* gene was originally discovered as the culprit in a hereditary cancer syndrome termed familiar adenomatous polyposis (FAP). FAP patients develop large numbers of colon polyps in early adulthood. The patients inherit one defective APC allele. Individual polyps are clonal outgrowths of epithelial cells in which the second APC allele is inactivated. Soon after the discovery of APC in FAP families, it was realized that both APC alleles were also inactivated in up to 80% of sporadic colorectal cancers (Kinzler and Vogelstein, 1996). The loss of APC was subsequently found to induce the inappropriate stabilization and accumulation of β-catenin (Rubinfeld et al., 1996). This observation had two important implications. It assigned to APC a crucial negative regulatory function in the Wnt cascade, and it suggested that the inappropriate activation of the Wnt cascade in the absence of APC somehow transformed epithelial cells. It was subsequently found that Tcf reporter constructs such as pTOPFLASH, normally activated only upon Wnt signaling, were inappropriately active in APC mutant cancer cells (Korinek et al., 1997). Tcf4 was the pertinent Tcf factor in the intestine. In the relatively rare cases of colorectal cancers with wt APC, activating point mutations were found in β-catenin, again leading to the inappropriate formation of active β-catenin/Tcf4 complexes (Morin et al., 1997).

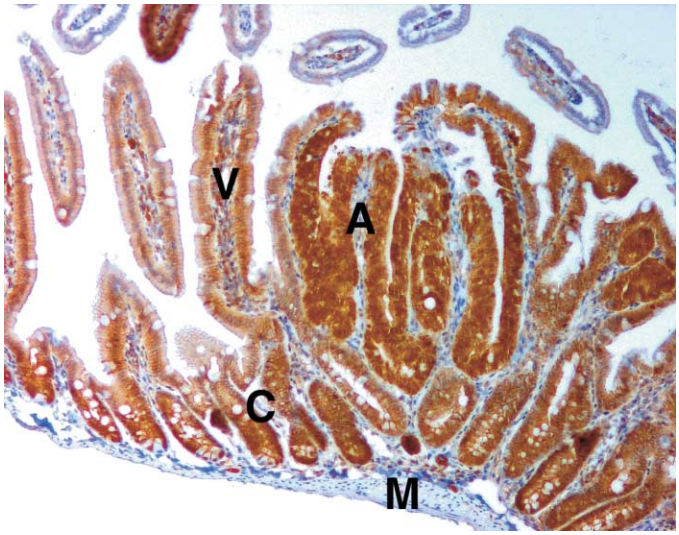

Figure 1. A small adenoma in the intestine of $APC^{+/-}$ mice

A, adenoma; V, villus; C, crypt; M, muscle wall of the small intestine. The section is stained for the presence of β-catenin. Note the accumulation of β-catenin in the adenoma, but also in the crypts. Courtesy of H. Begthel.

Why would the constitutive activity of a transcription factor immortalize intestinal epithelial cells? Tcf4 proved to be of profound physiological importance for the homeostasis of the intestinal epithelium. Proliferative epithelial progenitors occupy the crypts in the wall of the intestine. Cells flow from these crypts onto finger-like epithelial protrusions termed villi. Upon exit from the crypts, proliferation subsides and the cells rapidly differentiate to live for another 3–5 days. Cellular death ensues when the top of the villus is reached. In $Tcf4^{-/-}$ mice, the villus epithelium is intact, but the crypt progenitor compartment is entirely absent at birth. This implies that physiological Wnt signaling is required for maintenance of the crypt progenitor phenotype (Korinek et al., 1998). Indeed, nuclear β-catenin, the hallmark of active Wnt signaling, occurs throughout the crypts of adult intestine (Figure 1).

Multiple studies have addressed the nature of the Tcf4 target gene program in colorectal cancer. Among others, c-Myc (He et al., 1998) and CyclinD1 (Tetsu

and McCormick, 1999) appear to be essential components of this program in transformed intestinal epithelial cells. Recently, a global assessment of the Tcf4 target gene program in colorectal cancer cells was made by DNA arraying (van de Wetering et al., 2002). This study revealed that Tcf4 drives a program in colorectal cancer cells that is shared with that of crypt cells. APC$^{-/-}$ adenoma cells thus represent the transformed counterparts of crypt progenitor cells. Once the Wnt cascade is mutationally activated, the adenoma cells maintain their progenitor outfit indefinitely. This allows the adenomas to persist for many years in the human intestine, providing ample opportunity for the acquisition of further mutations to ultimately become a highly malignant, invasive, and metastasizing carcinoma.

Several studies have found that inhibition of the initiating transforming event in colorectal cancer cell lines, i.e., the inappropriate formation of a β-catenin/Tcf4 complex, drives these cells out of cycle in the face of multiple other acquired mutations in their genome (e.g., Tetsu and McCormick, 1999). Tcf4 inhibition actually induces colorectal cancer cells to differentiate into a villus epithelial phenotype, essentially completing their life cycle after many years' delay (van de Wetering et al., 2002). The accumulated data make a strong argument to consider the β-catenin/Tcf4 complex as a target for the development of small molecule inhibitors. Unfortunately, there is general consensus among drug developers that protein-protein interactions provide a blind alley for small molecule programs. Yet, this is exactly what Shivdasani and colleagues have pursued (Lepourcelet et al., 2004).

The authors set out to perform a high-throughput screen in an enzyme-based protein binding assay, using plate-bound β-catenin and a soluble N-terminal fragment of Tcf4 fused to GST. Of approximately 7,000 natural compounds, eight displayed reproducible and dose-dependent inhibition of the interaction with an IC50 <10 μM. A much larger screen of 45,000 additional compounds did not yield additional compounds. Six compounds were evaluated in a range of secondary assays. One assay explored the inhibition of β-catenin/Tcf4 binding in colorectal cancer cell lysates, measured by pulldown of β-catenin by Tcf4-GST. Inhibition of complex formation in cell lysates was also measured in a gel retardation assay with Tcf binding probes. The compounds were then subjected to a cell-based Tcf reporter gene assay in a colorectal cancer cell line. Most compounds were found to specifically inhibit the reporter assay at high nM concentrations. The colorectal cancer cell line-based studies were extended by expression analysis of two Tcf4 targets, c-*myc* (He et al., 1998) and Cyclin D1 (Tetsu and McCormick, 1999), which were indeed inhibited specifically by some of the compounds.

The compounds were then tested in the classical *Xenopus* assay for Wnt signaling, which exploits the induction of embryonic axis duplication upon β-catenin injection. Three of the compounds inhibited axis duplication induced by β-catenin, but not by the control downstream effector *Siamois*. Lastly, the compounds were analyzed for their antiproliferative effects. Three compounds were found to specifically inhibit proliferation of colorectal cancer cells. In all, three of the compounds, PKF115-584, PKF222-815, and CGP049090, scored consistently in all assays. Significantly, these compounds share a core chemical structure.

The study is very good news for anyone looking at protein-protein interaction targets for drug development. The classical approach of high-throughput screening in a cell-free assay can apparently yield protein interaction inhibitors that retain their effects when tested in vivo. The obvious next step will be the determination of the structural mechanism by which these compounds inhibit the β-catenin/Tcf4 interaction. X-ray structures exist for the β-catenin/Tcf complex. We do not know yet to which of the two proteins the compounds bind. Lepourcelet et al. (2004) note that their best compounds also interfere with the binding of another partner of β-catenin, namely APC. While this may complicate the interpretation of the biological phenomena, the observation implies β-catenin as the direct target of the small molecules.

It will be exciting to see these compounds, or others derived from similar approaches, move into preclinical studies. The efforts of many scientists will guarantee that the success of such studies will not be hindered by a lack of insight into the role of the Wnt signaling cascade in development and cancer.

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Selected reading

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