Winning WNT: Race to Wnt signaling inhibitors

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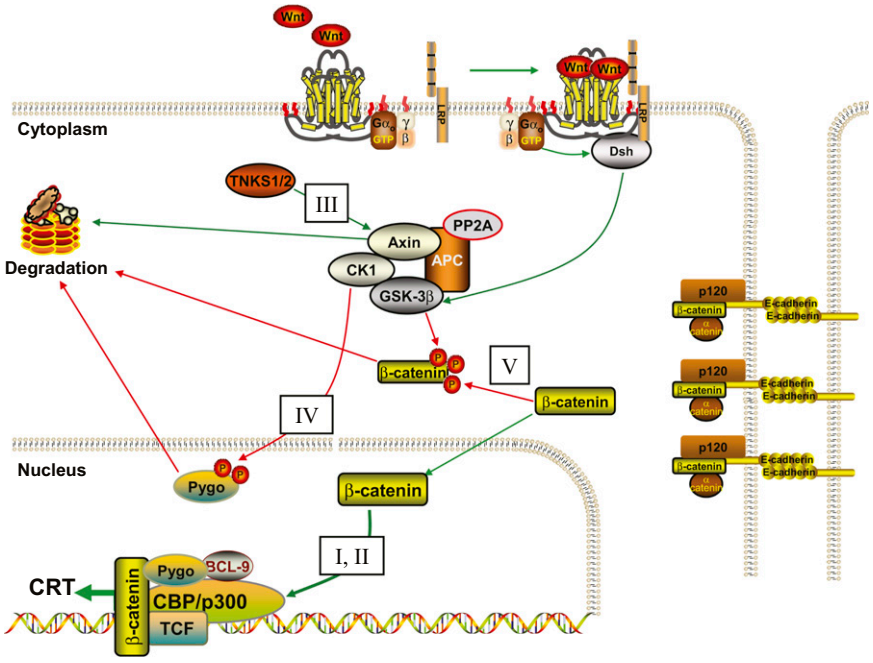
More than a quarter of a century ago, a mammalian homolog of *Drosophila wingless*, *int-1* (*Wnt1*), was identified as an oncogenic viral integration site in mouse breast cancer (1). A decade later, deregulation of canonical Wnt/β-catenin signaling through mutations in *adenomatous polyposis coli* (*APC*) was recognized to be an initiating event in colon carcinogenesis (2). Since that time, Wnt/β-catenin–activating mutations and/or epigenetic alterations have been found in a variety of human malignancies, including that of liver, skin, prostate, and breast (3–5). Thus, this signaling cascade holds excellent potential as a therapeutic target for treating myriad human cancers (6). Despite academic pursuit and industrial investment, clinically applicable inhibitors of the pathway remain elusive, calling for continuous effort and novel approaches. In PNAS, Gonsalves et al. (7) report a unique suppressor screen that combines high-throughput chemical library screening with RNAi technology to identify small molecules that specifically inhibit the β-catenin–responsive transcription (CRT) in the nucleus.

Core to the activation of canonical Wnt signaling is the inhibition of a destruction complex composed of APC, Axin, GSK3β, and other proteins, leading to the stabilization and nuclear translocation of cytoplasmic β-catenin (Fig. 1). A number of inhibitors have been found that act at different steps in the Wnt/β-catenin signal transduction pathway (3, 7–15). However, because Wnt–receptor interaction can trigger both canonical and noncanonical intracellular pathways, interfering with membrane-adjacent events could conceivably have far-reaching effects. Moreover, for cancers where canonical Wnt signaling is activated by mutations in *APC* or *β-catenin*, inhibitors that target upstream events may not be as effective as those that directly target these core components. Although inhibiting β-catenin and its interactions is recognizably an advantageous option, drug design is complicated by the fact that β-catenin also functions in cell adhesion by directly binding to α-catenin and E-cadherin.

How to minimize such unwanted impact on processes like cell adhesion in a chemical screen? Gonsalves et al. (7) take advantage of the *Drosophila* system, which exhibits less genetic redundancy and signaling complexity than mammals. By knocking down Axin, a negative regulator of canonical signaling, Gonsalves et al. (7) aim to find small molecules that interfere with events downstream of β-catenin stabilization without affecting upstream processes or cell adhesion. Thirty-four inhibitors of CRT (iCRT) are identified, and iCRT3, -5, and -14 are shown to potently inhibit CRT while displaying minimal or less prominent effect on noncanonical Wnt signaling and other pathways, such as Hh, JAK/STAT, and Notch signaling.

How do these compounds work? Using biochemical, biophysical, and in silico methods, Gonsalves et al. (7) elegantly show that at least one of the mechanisms by which iCRT3, -5, and -14 act is to disrupt the interaction between β-catenin and TCF4, possibly by direct binding to β-catenin. Interestingly, the compounds display no significant effect on β-catenin interaction with E-caderin or α-catenin. Given that TCF and E-cadherin bind to overlapping interfaces on β-catenin (6), the specificity revealed by this work and another study (13) is significant and paves the way for future experiments to further dissect the underlying molecular/structural details, which will facilitate the rational



Compound	Target	References
iCRT-3,5,14, CPG049090, NC043 (I)	β-catenin-TCF interaction	7, 10, 13
ICG001 (II)	β-catenin-CBP interaction	14
XAV939, IWR (III)	Tankyrase/Axin	11, 12
Pyrvinium (IV)	Casein kinase 1α /Pygopus	9
CCT031374 (V)	β-catenin	15

Fig. 1. The Wnt/β-catenin signaling pathway and its small molecule inhibitors. Binding of a Wnt ligand to membrane receptor/coreceptor triggers an intracellular signaling cascade leading to the stabilization and nuclear translocation of β-catenin. β-catenin in the nucleus binds to T cell factor (TCF)/lymphoid enhancer factor (LEF) and a number of cofactors to regulate gene expression. Red and green arrows indicate events that negatively and positively, respectively, affect signaling output. Examples of recently discovered small molecule inhibitors are shown, with their sites of action detailed in the table below the diagram.

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design of next generation compounds. In general, a majority of clinically approved chemical inhibitors of signaling pathways targets enzymatic activities (16). Recent effort on high-throughput drug discovery has proven that protein–protein interaction sites can also be drug targets (16, 17). The disruption of β-catenin–TCF4 interaction by chemical blocking of a single interface, as suggested by correlative evidence in this study (7), implies an expanded repertoire of druggable targets in the Wnt/β-catenin pathway and lends confidence to future endeavors to target additional nuclear interactions of β-catenin (18).

Do iCRT compounds inhibit Wnt/CRT-dependent cellular processes? Gonsalves et al. (7) show that, at the concentrations (micromolar range) tested, iCRT3, -5, and -14 are effective at crippling Wnt/β-catenin target gene expression, Wnt-induced morphological transformation of mammary epithelial cells, β-catenin–dependent invasion of breast cancer cells, and proliferation of colon cancer cell lines containing β-catenin–activating mutations. Moreover, iCRT14 administration to mice housing xenografts of these colon cancer cells leads to compromised cell proliferation and a slight but consistent reduction in initial tumor growth. The successful use of *Drosophila* cells by Gonsalves et al. (7) to screen for Wnt inhibitors that affect mammalian cells has opened the door for similar undertakings to find inhibitors or activators of other conserved signaling pathways. Just as informative as the classic

iCRT3, -5, and -14 are shown to potently inhibit CRT while displaying minimal or less prominent effect on noncanonical Wnt signaling.

Drosophila genetics have been to our molecular understanding of mammalian biology, the small fruit flies continue to awe us, now in the modern realm of chemical genetics, with the power to uncover chemical regulators of fundamentally important developmental pathways.

The preliminary finding that iCRT3 is cytotoxic to three of six cultured primary human colon cancer samples suggests possible clinical value of the iCRT lead compounds. Regardless of clinical applicability, however, these iCRTs are a useful addition to our inhibitor toolbox for manipulating canonical Wnt signaling in cells and animals. A general concern regarding Wnt/β-catenin inhibitors as therapeutics stems from the fact that the pathway regulates a wide variety of cellular and developmental activities (3, 4, 19). Particularly prominent is its involvement in normal and cancer stem cells of various tissues (20). Although inhibitors of the pathway may help eradicate the so-called cancer stem cells, they could also affect normal stem cell self-renewal and tissue homeostasis, especially on long-term administration. Although it is encouraging that the iCRTs do not adversely affect the proliferation of Wnt-inactive normal cells, more vigorous tests are needed to assess their impact on primary tissue stem cells. Perhaps more practical is to consider the use of Wnt inhibitors in the context of combination chemotherapy with established anticancer agents or with drug delivery systems that specifically target cancer (stem) cells. Nevertheless, the discovery of specific inhibitors of the nuclear function of β-catenin promises contribution to both anticancer therapeutics and advancement of basic research into Wnt/β-catenin signaling.

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