Targeting Wnt Signaling: Can We Safely Eradicate Cancer Stem Cells?

Fumi Takahashi-Yanaga¹ and Michael Kahn²,³,⁴

Abstract

The Wnt signaling pathways have been conserved throughout evolution and regulate cell proliferation, morphology, motility, and fate during embryonic development. These pathways also play important roles throughout adult life to maintain homeostasis of tissues including skin, blood, intestine, and brain by regulating somatic stem cells and their niches. Aberrant regulation of the Wnt pathway leads to neoplastic proliferation in these same tissues. It has been suggested that Wnt signaling is also involved in the regulation of cancer stem cells (CSC), because there are many similarities in the signaling pathways that regulate normal adult stem cells and CSC. In this Perspective, we have focused on the Wnt/β-catenin signaling pathway, which is the most intensively studied and best characterized Wnt signaling pathway. We provide an overview on the function of the Wnt/β-catenin signaling pathway in CSC, and the possibility of the development of novel therapeutics to target this pathway.

Wnt/β-catenin Signaling Pathway (Canonical Pathway) and Cancer

Cell signaling cascades activated by Wnt proteins (collectively, the Wnt signaling pathways) have been well conserved throughout evolution. In addition to regulating cellular processes including proliferation, differentiation, motility, and survival and/or apoptosis, the Wnt signaling pathways play key roles in embryonic development and maintenance of homeostasis in mature tissues. Among the described Wnt signaling pathways, [the canonical pathway (Wnt/β-catenin pathway) and the noncanonical pathways (the planar cell polarity pathway, the Wnt/Ca²⁺ pathway, the protein kinase A pathway)], the Wnt/β-catenin signaling pathway is by far the best characterized (1–6).

The activity of the Wnt/β-catenin signaling pathway is dependent on the amount of β-catenin in the cytoplasm. Normally, cytoplasmic β-catenin is maintained at a low level through ubiquitin-proteasome-mediated degradation, which is regulated by a multiprotein “destruction” complex containing axin, adenomatous polyposis coli (APC), and glycogen synthase kinase-3β (GSK-3β). Upon binding of Wnt proteins to a receptor complex comprised of Frizzleds/low-density lipoprotein receptor-related protein (Fz/LRP), cytoplasmic dishevelled (Dvl), a protein downstream of the receptor complex is phosphorylated thereby inhibiting GSK-3β, resulting in the accumulation of nonphosphorylated β-catenin in the cytoplasm. Nonphosphorylated β-catenin avoids degradation and translocates into the nucleus. In the nucleus, β-catenin in the classical Wnt signaling cascade forms a complex with members of the T-cell transcription factor (TCF)/lymphoid enhancer-binding factor (LEF) family of transcription factors. To generate a transcriptionally active complex, β-catenin recruits the transcriptional coactivators, cAMP response element-binding protein (CREB)-binding protein (CBP) or its closely related homolog, p300 (7, 8), as well as other components of the basal transcription machinery, leading to the expression of a host of downstream target genes. Secreted Wnt inhibitor factor 1 (WIF1) and secreted Frizzled-related proteins (SFRP), soluble Wnt receptors and competitors of Fz, respectively, are Wnt target genes that function as endogenous inhibitors of the Wnt signaling pathway (Fig. 1; refs. 1–3).

The importance of aberrant Wnt signaling in some types of cancer (colorectal most definitively) is clear. However, aberrant Wnt signaling may play a role in many other types of malignancies even those in which the classical
mutations associated with the pathway (i.e., APC truncations, β-catenin mutations) are not present. For example, although our knowledge about the role of Wnt signaling in breast cancer is far from complete, its importance and significance has been the subject of numerous reports during the past 5 years (9, 10). In human breast cancer, there are many reports of inactivation of negative regulators of the Wnt signaling pathway. Similarly, there are numerous studies that have documented the amplification or overexpression of positive regulators of components of this pathway. Dvl is amplified and upregulated in 50% of ductal breast cancers (11). Frizzled-related protein 1 (FRP1/FRZB), a secreted Wnt inhibitor, located within chromosomal locus 8p11-21, is frequently deleted in human breast cancers. In approximately 80% of malignant breast carcinomas, Frp1 expression is either repressed, or absent, making it one of the most frequent alterations in breast cancers (12). Axin is downregulated in a small percentage of breast cancers (13). AXIN2, on chromosome 17q23-q24, exhibits frequent loss of heterozygosity in breast cancers (14). Both are negative regulators of the canonical Wnt signaling pathway. Collectively, loss of heterozygosity (23 to 40%), mutation (6 to 18%), and hypermethylation of the APC gene, have been shown to result in loss of expression in approximately 36 to 50% of breast tumors (15). In mice, it has long been known that misexpression of Wnt-1, -3, or -10 induces mammary adenocarcinomas (16). The APCMin mouse has also been shown to exhibit an enhanced incidence (~10%) of spontaneous mammary cancer and a greatly increased susceptibility (90%) to carcinogen-induced mammary cancer (17, 18).

**WNT Signaling in Stem Cells and Cancer Stem Cells**

Although most would agree that Wnt signaling is important in stem cell biology, there is no consensus on whether Wnt signaling is important for proliferation and maintenance of potency (pluripotency or multipotency, for example, see refs. 19–21) or differentiation of stem and/or progenitor cells (22, 23). Wnt/β-catenin signaling has been shown to maintain pluripotency in ES cells (20) and is critical for the expansion of neural progenitors, thereby increasing brain size (24). However, Wnt/β-catenin signaling is also required for neural differentiation of embryonic stem cells (25). Fate decision in neural crest stem cells (26), and Wnt3a has been reported to promote differentiation into the neuronal and astrocytic lineages by inhibiting neural stem cell maintenance (27). Clearly, Wnt/β-catenin signaling also plays a critical role in lineage decision and/or commitment. These dramatically different outcomes upon activation of the Wnt signaling cascade have fueled enormous controversy about the role of Wnt signaling in the maintenance of potency and induction of differentiation.

The similarities between normal adult stem cells and cancer stem cells (CSC; ref. 28), suggest that the signaling pathways (e.g., Wnt, Hedgehog, and Notch) involved in regulating somatic stem cell maintenance are also involved in the regulation of CSC (29, 30). Aberrant regulation of these same pathways leads to neoplastic proliferation in the same tissues (31, 32). Interestingly, progression of chronic myelogenous leukemia from chronic phase to blast crisis and imatinib resistance was correlated with increased nuclear β-catenin levels, a hallmark of increased Wnt/β-catenin transcription (33). Recent studies have revealed that multidrug resistance genes, including MDR-1, ABCG2, ABCA3, and BRCP1 are also intrinsically expressed in stem and/or progenitor cells from multiple adult tissues and that they may contribute to the side population (SP) phenotype of malignant cells (34–37). Wnt/β-catenin signaling seems to play an important role in ABCB1/MDR-1 transcription. This observation was initially based upon the increased expression of MDR-1 associated with intestinal crypt cells, which carry a defective APC tumor suppressor gene in both the Min mouse and FAP patients (38, 39). Putative TCF binding elements were also identified in the ABCB1 promoter (~1,813 to ~275 bp; ref. 38). Canonical Wnt signaling is believed to play an important role in the maintenance of hematopoietic progenitors and also in the lineage commitment of progenitors during hematopoiesis. Expression of survivin, which is a Wnt/CBP/β-catenin-regulated gene (40), is important during hematopoiesis (41), and it is prominently upregulated in CD34+ hematopoietic stem and/or progenitor cells upon differentiation and/or progenitor cells upon growth factor treatment. Survivin-deficient hematopoietic progenitors show defects in erythroid and megakaryocytic differentiation (42). Recently, continued expression of survivin upon differentiation has been associated with teratoma formation by hES cells (43). However, it is worth noting that β-catenin-deficient (44) and even β,γ-double-deficient (45) mice maintain apparently normal hematopoiesis through the Wnt signaling cascade (46), pointing to yet uncharacterized catenin-like molecule(s) that can compensate for the loss of both β and γ-catenin.

Interestingly, many of the cell surface markers (including LGR5/GPR49, CD44, CD24, and Epcam) that have been used to identify and isolate putative tumor stem cell populations in a variety of tissues are direct Wnt targets. The role of the Wnt signaling cascade, particularly in CSC in other malignancies, which do not carry classical activating mutations in the Wnt pathway, is becoming more apparent. For example, multiple myeloma is quite responsive to a wide array of therapeutic protocols including conventional cytotoxics, corticosteroids, radiation therapy, and an increasing number of targeted chemotherapeutic agents, e.g., the proteasome inhibitor bortezomib. Despite this, few if any patients are “cured” using these approaches and relapse remains a critical issue. The majority of multiple myeloma infiltrates phenotypically resemble normal terminally differentiated plasma cells with the ability to produce monoclonal immunoglobulin. That the majority of myeloma plasma cells are quiescent, particularly at diagnosis, led to the investigation of a restricted “stem cell” population critical for tumor growth. Thirty years ago, Salmon and Hamburger showed the ability of
∼90% of tumor samples from multiple myeloma patients to form colonies and that clonogenic growth occurred at a frequency of between 1 in 100 to 100,000 cells. Importantly, as with other CSC populations, multiple myeloma CSCs have been found to be relatively resistant to existing chemotherapies. Moreover, multiple myeloma stem cells display high expression of multidrug resistance transporters, intracellular detoxification enzymes, and relative quiescence, similar to both other CSC populations as well as normal stem cell populations.

In the Wnt signaling pathway, a change in coactivators (CBP versus p300) interacting with β-catenin (or catenin-like molecules in the absence of β-catenin; ref. 47), and more generally the basal transcriptional apparatus (48), may be very important for a cell deciding to either maintain its level of potency (be that embryonic or somatic stem cell), or to go on to differentiate and lose a level of potency. According to the model developed by Kahn and colleagues, CBP/β-catenin-mediated transcription is essential for stem and/or progenitor cell maintenance and proliferation, whereas a switch to p300/β-catenin-mediated transcription (e.g., increasing the expression of c-jun, fra-1, etc.) is the critical step to initiate differentiation and a decrease in cellular potency. Although a subset of the gene expression cassette that is regulated by the CBP/β-catenin arm is critical for the maintenance of potency and proliferation (e.g., Oct4, survivin, etc.), other genes that are regulated in this manner (e.g., hNkd and axin2) are in fact negative regulators of the CBP/β-catenin arm of the cascade (Fig. 2; refs. 49, 50). Assuming potency and activation of the CBP/β-catenin arm is the default pathway, at some point, in order for development to proceed, one must stop proliferation, exit cell cycle, and initiate the process of differentiation (51, 52). This process is critical for both normal development and tissue homeostasis. Furthermore, the inability to properly initiate and complete differentiation of somatic stem and/or progenitor cells may be the underlying malfunction in essentially all cancers; this, in essence, is another way of restating the CSC hypothesis. Therefore, we would propose that a wide range of mutations (some of which are cell type or tissue specific; e.g., bcr/abl, K-Ras, Her2, etc.) can lead to aberrant regulation of the underlying equilibrium between catenin/CBP and catenin/p300; i.e., between proliferation and maintenance of potency and the initiation of differentiation (Fig. 3). Thereby aberrant increase of the CBP/catenin interaction at the expense of the p300/catenin interaction could increase the number of symmetric divisions at the expense of asymmetric divisions. Recent evidence suggests that asymmetric division may function as a tumor suppressive mechanism (53).
Wnt/β-catenin Signaling as a Therapeutic Target

Accumulating evidence suggests that the Wnt/β-catenin signaling pathway is often involved in oncogenesis and cancer development. Given the fact that multiple mutations can lead to the nuclear translocation of β-catenin, there is a clear need for drugs that attenuate the nuclear transcriptional functions of β-catenin (54, 55). Inhibitors of the Wnt/β-catenin signaling pathway can be grouped into two classes, i.e., small-molecule inhibitors and biologic inhibitors. Small-molecule inhibitors include existing drugs such as nonsteroidal anti-inflammatory drugs (NSAID) and molecular-targeted agents such as the CBP/β-catenin antagonist ICG-001. Biologic inhibitors include antibodies, RNA interference (RNAi), and recombinant proteins. The majority of these inhibitors are in the preclinical stage of development, although at least one has entered clinical trial (also please see Table 1). Below we will briefly summarize development of inhibitors to date.

Existing drugs and natural compounds

A number of existing drugs and natural compounds have been identified as inhibitors and/or modulators of Wnt/β-catenin signaling pathway (reviewed in ref. 56). We will briefly discuss them below.

Nonsteroidal anti-inflammatory drugs. NSAIDs, such as aspirin and sulindac, inhibit the activity of cyclooxygenase (COX), a key enzyme in the arachidonic acid cascade. A number of experimental and epidemiological studies in humans suggested that aspirin and other NSAIDs show chemopreventive effects mainly against colon cancer (57–63), and inhibition of the Wnt/β-catenin signaling pathway is one of their potential mechanisms of action (64, 65). For instance, increased COX-generated PGE2 suppresses β-catenin degradation, resulting in activation of Wnt/β-catenin signaling. Therefore, suppression of elevated COX activity in cancer cells is likely to be an important factor for the anticancer activity of NSAIDs. However, treatment of colon cancer cell lines with celecoxib, a COX-2 selective inhibitor, was shown to inhibit Wnt/β-catenin signaling by inducing the degradation of TCFs, and this effect was independent of COX-2 (66–69).

At present, celecoxib is the only NSAID approved by the US Food and Drug Administration (FDA) for the treatment of familial adenomatous polyposis.

Vitamins. Retinoids, which are synthesized from vitamin A in the body, are used in some forms of cancer therapy (notably acute promyelocytic leukemia) and also chemoprevention. An active form of vitamin D, 1α,25-dihydroxyvitamin D3, and its synthetic derivatives have shown chemopreventive effects in animal models of colorectal and breast cancers. Although the mechanism by which vitamins inhibit Wnt/β-catenin signaling pathway is not fully understood, it is suggested that activated nuclear receptors for vitamins interact with β-catenin and compete with TCFs (70, 71). Recently it has also been suggested that both vitamin A and D might induce Wnt/β-catenin inhibitory proteins, e.g., Disabled-2 (Dab2) by retinoic acid (72, 73).
acids and Dickkopf-1 and -4 (Dkk-1 and Dkk-4) by vitamin D (72, 73).

**Polyphenols.** Polyphenols are a group of chemicals found in plants, characterized by the presence of more than one phenol unit or building block per molecule. Several polyphenols, such as quercetin, epigallocatechin-3-gallate (EGCG), curcumin, and resveratrol have been implicated as inhibitors of Wnt/β-catenin signaling pathway, although the mechanisms of action of these agents are not clear due to their inherent lack of specificity and inhibitory effects on multiple pathways (74–78).

The differentiation-inducing factors (DIF), first identified in *Dictyostelium discoideum* as putative morphogens required for stalk cell differentiation (79), also have a phenol unit and strongly inhibit the proliferation of human cancer cells. It has been reported that DIF-1 and DIF-3 inhibit the Wnt/β-catenin signaling pathway through the activation of GSK-3β (80–83). Apart from β-catenin, GSK-3β has many target proteins such as glycogen synthase, Tau, CREB, and AP-1. Cyclin D1, a known oncogene, is also one of target molecules of GSK-3β, and phosphorylation by GSK-3β triggers cyclin D1 degradation (84, 85). Because activators of GSK-3β, such as DIFs, could reduce cyclin D1 mRNA and protein levels, they may be applicable for the treatment of cancer and other proliferative disorders (86).

**Small-molecule inhibitors identified via high-throughput screen**

The molecularly targeted agents reported to date can be classified into four groups, i.e., β-catenin/TCF-antagonists, transcriptional co-activator modulators, PDZ domain of Dvl binders, and other mechanism-based inhibitors.

**β-catenin/TCF interaction antagonists.** A high-throughput screen of 6,000 natural and 45,000 synthetic compounds identified 8 natural product low molecular-weight antagonists of the interaction between β-catenin and TCF4 (87). Unfortunately these compounds are not highly selective for disrupting the β-catenin/TCF complex as they also interact with APC. Trosset and colleagues identified the synthetic compound PNU 74654 by structure-based virtual ligand (in silico) screening as a β-catenin/TCF antagonist (88). However, to our knowledge, the biological activity of PNU 74654 has not been reported. Recently, 2,4-diamino-quinazoline was identified by screening a large compound library and found to inhibit the β-catenin/TCF4 pathway. It is currently in the lead optimization stage of development (89).

**Transcriptional co-activator antagonists.** Several co-activators for Wnt/β-catenin transcription, including CBP, p300, B-cell lymphoma 9 (BCL9), and pygopus have been identified (7, 90, 91). Using a cell-based reporter screen and a small molecule secondary structure-templated chemical library, we identified the lead compound ICG-001, which selectively bound to CBP and prevented its interaction with β-catenin, resulting in the suppression of a subset of Wnt/β-catenin-driven gene expression. ICG-001 is highly selective and does not interact with the highly homologous co-activator p300. As described above, the switch from β-catenin/CBP to β-catenin/p300 controls fundamental stem and/or progenitor cell switching points, i.e., the initiation of a differentiative
program with a more limited proliferative capacity (92, 93).

**Targeting the PDZ domain of Dvl.** Dvl is an essential protein in the Wnt signaling pathway that transduces extracellular Wnt signals to downstream components. Dvl uses its PDZ domain, a common protein-protein interaction motif that recognizes short peptide motifs, to bind to the COOH-terminal region of the Wnt receptor Fz. Three compounds (NSC668036, FJ9, and 3289-8625), which have the ability to block Wnt signaling in vivo, were identified through in silico screening and nuclear magnetic resonance spectroscopy (94–96). These studies showed that this method can provide a useful tool for developing small-molecule inhibitors of the Wnt pathway.

**Alternative mechanism-based inhibitors.** In 2009, two additional reports describing Wnt/β-catenin inhibitors were published. Chen and colleagues described several small molecule inhibitors of Wnt response (IWR), which stabilize the protein Axin, and another group termed Wnt production inhibitors (IWP), which inhibit Porcupine, an essential protein for Wnt secretion (97). A recent report described the Wnt inhibitor XAV939, which induces the stabilization of Axin by inhibiting the poly-ADP-ribosylating enzymes Tankyrase 1 and 2 (98). These inhibitors successfully inhibited Wnt-mediated cellular responses, indicating that stabilization of Axin could be a new target for the inhibition of the Wnt/β-catenin cascade.

**Biologic inhibitors**

Therapeutic monoclonal antibodies against Wnt-1 and Wnt-2 have been developed and shown to inhibit Wnt signaling and suppress tumor growth in vivo (99–101). Similarly, small interfering RNA (siRNA) against Wnt-1 and/or Wnt-2 had potential therapeutic utility in cancer cell lines. Furthermore, therapeutic proteins, i.e., WIF1 and SFRPs, are presently being developed and tested in preclinical tumor models (102, 103).

**Perspectives on Therapeutic Intervention**

Drugs that target aberrant activation of the Wnt signaling cascade have enormous potential as novel cancer therapeutics. Furthermore, because of the importance of Wnt signaling in stem and/or progenitor populations, they may offer the ability to eliminate normally drug resistant CSCs, which are thought to be associated with relapse and metastasis. However, this enthusiasm needs to be tempered by the stark reality that the Wnt signaling cascade is also

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**Table 1. Summary of inhibitors against Wnt signaling pathway**

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<thead>
<tr>
<th>Inhibitors</th>
<th>Subcategory</th>
<th>Therapeutic</th>
<th>Pathway target</th>
<th>Development stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Small molecules</td>
<td>NSAIDs</td>
<td>Aspirin</td>
<td>β-catenin</td>
<td>Clinical</td>
</tr>
<tr>
<td>Existing drugs and natural compounds</td>
<td>Sulindac</td>
<td>β-catenin</td>
<td>Clinical</td>
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<td></td>
<td>Celecoxib</td>
<td>TCF</td>
<td>Clinical</td>
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<td></td>
<td>Etc. retinoids</td>
<td>β-catenin</td>
<td>Clinical</td>
<td></td>
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<tr>
<td>Vitamins</td>
<td>1α,25-dihydroxy-Vitamin D3</td>
<td>β-catenin</td>
<td>Clinical</td>
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<td>Polyphenols</td>
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<td>Unknown</td>
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<td>EGCG</td>
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<td>Resveratrol</td>
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<td>DIF</td>
<td>GSK-3β</td>
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<td></td>
<td>Etc.</td>
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<tr>
<td>Molecular targeted drugs</td>
<td>PNU 74654</td>
<td>β-catenin/TCF</td>
<td>Discovery</td>
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<td></td>
<td>2,4-diamino-quinazoline</td>
<td>β-catenin/TCF</td>
<td>Preclinical</td>
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<td>ICG-001-related analogs</td>
<td>CBP</td>
<td>Discovery</td>
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<td>NSC668036</td>
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<td>3289-8625</td>
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<td>Axin</td>
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<td>XAV939</td>
<td>Tankyrase 1 &amp; 2</td>
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<td>Biologics</td>
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<td>Recombinant proteins</td>
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<td>RNA interference</td>
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critical in normal somatic stem cell homeostasis and tissue maintenance. As described above, there are a number of drugs and natural compounds that have already been identified to have therapeutic value against cancers associated with aberrant Wnt signaling. However, often because of a lack of specificity, knowledge of their precise target molecule(s) and/or mechanisms by which they inhibit Wnt signaling have not been clarified to date. Identification of these target molecules and determination of the precise mechanism of action of these agents may provide novel targets for the expansion of our anticancer armamentarium. Unfortunately, there is limited evidence that these Wnt inhibitory compounds can target and eliminate the drug-resistant CSC population. Quite recently however, we have been able to show that CBP/catenin antagonists are able to target and eliminate drug-resistant leukemic stem cells both in vitro (Fig. 4) and in vivo (data are not shown).5

Elegant structural studies on the interactions between β-catenin and TCF (104–106), offered a priori an attractive mode for inhibition of the Wnt pathway. This high affinity protein-protein interaction has been successfully targeted via a small molecule high-throughput screen, to provide a series of inhibitors of TCF/β-catenin-mediated transcription (88). However, concerns arise about the development of specific inhibitors of this interaction due to the diverse partners besides TCF (e.g., APC and E-cadherin), which also bind to the central Arm repeats of β-catenin (107). Small molecule high-throughput screens have also provided interesting lead compounds (Disheveled PDZ domain and tankyrase inhibitors, etc.) for further development and may provide additional drug leads and novel targets in the future.

The functions of the coactivators CBP and p300 have been described as redundant in several studies (108), and their expression pattern during mouse development is almost identical (109). However, it is becoming increasingly clear that these highly homologous co-activators are not redundant under physiological conditions, and are responsible for distinct transcriptional programs (110–113). The selectivity of ICG-001 is at first quite surprising, given the fact that it interacts with CBP, a co-activator protein used by an extremely wide array of transcription factors (108).

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5 Y. Zhao and M. Kahn, manuscript in preparation.
However, ICG-001 (MW 548) blocks only a very small percentage of the CBP surface and the compound binds only to CBP, but not the highly homologous co-activator p300.

The critical region of interaction of ICG-001 was mapped to the NH2-terminus of CBP (amino acids 1 to 111). This region of CBP also interacts with the C-terminal transactivation domain of β-catenin residue (93). Interestingly, this region of CBP also contains binding sites for the retinoic acid (RA) receptor, RXR/RAR, and Vitamin D receptor VDR (108). Therefore, the mechanism of ICG-001, retinoids, and vitamin D may coincide in this regard as they all can antagonize the CBP/β-catenin interaction.

A more potent, specific CBP/β-catenin antagonist, PRI-724, is in development and set to enter phase I clinical trials in 2010. These clinical investigations should allow us to begin to address the question of whether we can safely target CSCs by modulating Wnt signaling.

In regard to recent publications about an increase in Wnt signaling with aging (114, 115) and the importance of stem cell homeostasis in aging and disease, it is interesting to speculate about whether a progressive imbalance in this co-activator equilibrium is associated with the aging process more generally. This observation would coincide with the fact that with many types of cancer, risk increases substantially with aging.

Finally, recent evidence correlated the loss of the β-catenin/E-cadherin interaction in immortalized breast epithelium with both the epithelial-mesenchymal transition and a CSC-like phenotype (116). Although activation of the Wnt signaling cascade was not formally shown, the dramatic increase in the CD44hi, CD24lo population is arguably a mark of activated Wnt signaling. Therefore, it is interesting to speculate that loss of the cytoskeletal-adherens junction role of β-catenin with a concomitant increase in the transcriptional role of β-catenin, may be a hallmark of the epithelial-mesenchymal transition, CSC-enhanced drug resistance, and metastatic capacity in a wide array of malignancies.

**Disclosure of Potential Conflicts of Interest**

M. Kahn, consultant, commercial research grant, ownership interest (including patents), board member and scientific advisory board member, Prism Biolab; ownership interest (including patents), University of Southern California. F. Takahashi-Yanaga disclosed no potential conflicts of interest.

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**References**

27. Muroya Y, Kondoh H, Takada S. Wnt proteins promote neuronal...
73. Pendás-Franco N, Aguilera O, Pereira F, González-Sancho JM,


99. Wei W, Chua MS, Grepper S, So SK. Blockade of Wnt-1 signaling leads to anti-tumor effects in hepatocellular carcinoma cells. Mol Cancer 2009;8:76.


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