Targeting Wnt Pathways in Disease

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Wnt-mediated signal transduction pathways have long been recognized for their roles in regulating embryonic development, and have more recently been linked to cancer, neurologic diseases, inflammatory diseases, and disorders of endocrine function and bone metabolism in adults. Although therapies targeting Wnt signaling are attractive in theory, in practice it has been difficult to obtain specific therapeutics because many components of Wnt signaling pathways are also involved in other cellular processes, thereby reducing the specificity of candidate therapeutics. New technologies, and advances in understanding the mechanisms of Wnt signaling, have improved our understanding of the nuances of Wnt signaling and are leading to promising new strategies to target Wnt signaling pathways.

As covered by other Wnt signaling investigators, the Wnt family of secreted glycoproteins act as ligands to activate multiple signal transduction pathways. The best understood is the Wnt/ β -catenin pathway, which activates the function of β -catenin in the nucleus to regulate expression of genes by binding to T-cell factor/lymphoid enhancer factor (TCF/LEF) transcription factors, in addition to its role in regulating cadherin-dependent cell adhesion at the plasma membrane. The Wnt/ β -catenin pathway acts in a context-dependent manner to regulate cell proliferation, migration, differentiation, and survival in both embryonic development and in adults.

Perturbations in the levels of Wnt/ β -catenin signaling are linked to many disease processes. Elevation of Wnt/ β -catenin signaling has been linked to cancer, whereas conversely attenuation of Wnt/ β -catenin signaling has been linked to a distinct set of diseases including Alzheimer's disease, familial exudative vitreoretinopathy (FEVR), and disorders of bone formation (Robitaille et al. 2002; reviewed in De Ferrari and Moon 2006; Hoepfner et al. 2009).

Reports have even shown that Wnt/ β -catenin signaling can regulate aspects of human immunodeficiency virus (HIV) including gene expression and replication, further highlighting the ubiquitous function of this pathway in

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almost all cell types (Wortman et al. 2002; Carroll-Anzinger et al. 2007; Kumar et al. 2008; Kameoka et al. 2009). As the diverse roles for Wnt/ β -catenin signaling in tissue homeostasis and disease continue to be elucidated, interest in therapeutic targeting of this pathway has expanded enormously.

In addition to the Wnt/ β -catenin pathway, β -catenin-independent pathways such as the planar cell polarity (PCP) pathway and Wnt/ Ca^{2+} pathway have been described. These pathways are less well understood in part owing to the paucity of established reporter assays. In addition, β -catenin-independent Wnt signaling often inhibits the Wnt/ β -catenin pathway, making the relative contribution of pathway activation versus inhibition to an observed phenotype difficult to distinguish. Studies in a variety of organisms have established that β -catenin-independent Wnt signaling is involved in regulating cell polarity during gastrulation in embryos, and in the polarized orientation of hair cells in the inner ear, as well as mesenchymal stem cell maintenance and renal development (reviewed in Sugimura and Li 2010). The β -catenin-independent Wnt pathway(s) are also linked to disease processes, notably cancer.

From a therapeutic point of view, the Wnt signaling pathways present several challenges to the development of a targeted drug, so it is not surprising that drug strategies specifically directed at this pathway are in a state of relative infancy. In addition to the existence of 19 Wnt ligands and 10 FZD receptor isoforms, specificity of targeting is further complicated by the convergence of downstream Wnt signaling events on promiscuous enzymes like glycogen synthase kinase 3 (GSK3) and on proteins that are central to fundamental and ubiquitous cellular structures such as the cytoskeleton and cell–cell junctions that are critical to all cells. Predictability of drug effects can be problematic with a pathway as ubiquitous as Wnt, particularly because it is quite likely that the majority of both somatic cells and stem cell niches in the body will show some effect with either Wnt activation or inhibition. Nevertheless, many studies in manipulation of Wnt signaling pathways have shown promise

that we will examine in more detail in this review.

WNT/ β -CATENIN SIGNALING IN CANCER

The Wnt/ β -catenin pathway has been associated with cancer ever since the gene *int-1* was identified as a mammary oncogene in mice (Nusse and Varmus 1982; Rijsewijk 1987). This relationship was solidified with the discovery that the adenomatous polyposis coli (APC) gene associated with familial adenomatous polyposis (FAP) is inactivated in $\sim 85\%$ of colorectal carcinomas leading to constitutive nuclear translocation of β -catenin (Kinzler et al. 1991; Nishisho et al. 1991; Su et al. 1993). As a result, the therapeutic targeting of Wnt/ β -catenin signaling has received considerable interest in the context of cancer.

Dysregulated Wnt/ β -catenin signaling occurs in many solid tumors and hematologic malignancies even in the absence of documented mutations. Frequently, altered levels of expression of Wnt/ β -catenin pathway regulators have been observed without mutations in the coding regions of the respective genes. For example, epigenetic silencing of genes that encode putative extracellular Wnt antagonists such as the secreted frizzled-related proteins (SFRPs) has been described in colon, breast, prostate, lung, and other cancers (Caldwell et al. 2004; Lee et al. 2004a; Suzuki et al. 2004; Fukui et al. 2005; Zou et al. 2005). Increased expression of other Wnt/ β -catenin pathway members such as Wnt ligands (Rhee et al. 2002; Wong et al. 2002; Milovanovic et al. 2004) or dishevelled (DVL) have also been described (Okino et al. 2003; Uematsu et al. 2003a,b).

CONTEXT-DEPENDENT CORRELATIONS BETWEEN β -CATENIN AND PATIENT SURVIVAL

The role of Wnt/ β -catenin activation via APC mutations in colorectal cancer progression as well as the initial discovery of *int-1* in a mammary tumor screen has led to the dogmatic view that this pathway is oncogenic. Indeed, studies correlating increased Wnt/ β -catenin signaling

in tumors to worse prognosis for patients with colorectal cancer have helped solidify this viewpoint (Cheah et al. 2002; Miyamoto et al. 2004; Lugli et al. 2007). However, it is important to emphasize that elevated Wnt signaling does not correlate with reduced patient survival in all types of cancer. For example, in melanoma, active Wnt/ β -catenin signaling as shown by nuclear β -catenin in tumors is associated with a lower proliferative index and correlates with a more favorable prognosis in melanoma patients (Kageshita et al. 2001; Maelandsmo et al. 2003; Bachmann et al. 2005; Chien et al. 2009b). Consistent with this observation, overexpression of Wnt3a in a murine melanoma model results in a less aggressive disease phenotype as well as an increase in melanocyte differentiation markers, which are often lost during melanoma progression (Chien et al. 2009b). Mechanistically, the effects of elevated β -catenin in melanoma differ from colorectal cancer because in the former the β -catenin promotes expression of the transcription factor *MITF*, which drives differentiation toward a melanocytelike cell fate and reduces cell movements (Dorsky et al. 2000; Takeda et al. 2000; Hornyak et al. 2001).

In some contexts, active Wnt signaling is correlated with less aggressive disease. Recently, discrete molecular subtypes of medulloblastoma were identified that included a subgroup characterized by constitutive Wnt/ β -catenin pathway activation. The tumors in this subgroup universally harbor activating mutations in β -catenin (*CTNNB1*), and appear to derive from a distinct anatomic region within the brain. These Wnt/ β -catenin-driven tumors are associated with a much better prognosis compared with those without active Wnt/ β -catenin signaling (Gibson et al. 2010). Similarly, Wnt activation appears to function as a tumor suppressor in some sarcomas, as inhibition of Wnt in human mesenchymal stem cells leads to high-grade sarcoma formation in nude mice (Matushansky et al. 2007). In mouse models of pancreatic ductal adenocarcinoma (PDAC), the forced expression of a nondegradable constitutively active β -catenin can arrest Kras-driven transformation, resulting in a different tumor type that is much more benign compared with

PDAC (Heiser et al. 2008; Morris et al. 2010). Other studies in prostate, ovarian, and even in colorectal cancer have also found that increased Wnt signaling in patient tumors is associated with a better prognosis in at least some stages of disease (Gamallo et al. 1999; Horvath et al. 2005; Elzagheid et al. 2008).

These findings suggest that the effects of Wnt/ β -catenin signaling differ widely depending on the stage of disease progression, cell of origin, or context in which the signaling occurs. In our current model, dynamic regulation of Wnt signaling is tightly regulated in cells, resulting in an ongoing modulation of cellular Wnt activation levels by feedback mechanisms, which involve other diverse signaling pathways. The identification of putative Wnt inhibitors including AXIN2, DKK1, and TCF7L2 as direct transcriptional targets of β -catenin signaling supports the model of a tightly regulated pathway. To develop and successfully implement Wnt-directed therapies, several contextual considerations are essential. For one, understanding the mechanism of Wnt dysregulation, whether it be through ligand overexpression or mutational activation, will be central to developing appropriate strategies for targeting the pathway in different diseases. Second, the idea that Wnt activation may be an important part of maintaining homeostasis in normal, noncancerous cells must be considered in the bigger picture. Finally, the effects of targeting this pathway will undoubtedly vary across different cell types, which have broad implications for both predicting the efficacy of treatment as well as for predicting potential side effects in the context of an entire organism.

NONCANONICAL β -CATENIN-INDEPENDENT WNT SIGNALING IN CANCER

Although most studies have focused on the well-described Wnt/ β -catenin pathway, Wnts can also activate pathways that act independently of β -catenin. These pathways, often referred to as “noncanonical” Wnt pathways, are currently known collectively as β -catenin-independent Wnt signaling. In both developmental models and in cultured mammalian cells, β -catenin-

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independent Wnt signaling is a key modulator of cellular adhesion and motility, and several studies have shown the involvement of this pathway in tumor cell migration and metastasis. Perhaps the best-studied activator of β -catenin-independent Wnt signaling is WNT5A, which has been shown to act both as a tumor suppressor as well as a promoter of tumor invasion depending on the model (Weeraratna et al. 2002; Kurayoshi et al. 2006; Pukrop 2006). In melanoma, WNT5A mediates a polarized redistribution of proteins such as myosin IIB to facilitate directional movement along a chemokine gradient (Witze et al. 2008). Other components of β -catenin-independent Wnt signaling such as VANGL1 and VANGL2 have also been implicated in tumor cell migration. These genes are mammalian homologs of the *Drosophila* PCP gene *van gogh/strabismus* and represent putative tetra-membrane-spanning proteins widely expressed in human cancers (reviewed in Katoh 2002). VANGL2 has recently been shown to form a Wnt-induced receptor complex with the receptor ROR2, which acts to sense Wnt dosage gradients in developing limb buds (Shafer et al. 2011). VANGL2 expression also appears to promote tumor cell migration and matrix metalloproteinase-dependent invasion in a human colon cancer line (Cantrell and Jessen 2010). VANGL1 also promotes metastases in murine models of colorectal and squamous cell carcinoma (Lee et al. 2004b, 2009; Kho et al. 2009). Interestingly, other roles for β -catenin-independent Wnt signaling besides migration are emerging. For example, WNT5A was found to potentiate androgen-mediated signaling through the androgen receptor in prostate cancer cells, thereby promoting tumor formation in a murine prostate cancer model (Takahashi et al. 2011).

Currently, the development of drugs targeting β -catenin-independent Wnt signaling has been limited by several key factors. First, assays for measuring activation of β -catenin-independent Wnt signaling are diverse, likely context dependent, and technically involved, thereby limiting the ability to perform large-scale exploratory studies such as high-throughput signaling. Second, our understanding of the mech-

anisms involved in β -catenin-independent Wnt signaling remains limited, even with regard to basic events such as the identification of receptors involved in ligand binding. Third, β -catenin-independent Wnt signaling can antagonize Wnt/ β -catenin signaling, so even specific targeting of ligands such as Wnt5A could conceivably have implications for the broad effects of Wnt/ β -catenin signaling in both cells and organisms. Nevertheless, there have been some efforts to target this pathway (Figs. 1 and 2; Tables 1 and 2) that have shown promise in early studies.

WNT PATHWAYS IN NEUROLOGIC DISEASE

Wnt-mediated pathways have a well-established role of the development of the central nervous system in embryonic development (reviewed in Salinas and Zou 2008). Therefore, it is not surprising that aberrations in Wnt signaling are common in neurologic diseases in the adult. One such disease that has received considerable attention is schizophrenia, in which genetic perturbations predicted to impact Wnt regulation have been recently identified. For example, a Scottish family with a high incidence of schizophrenia, depression, and bipolar disorder was found to carry a balanced chromosomal translocation involving disrupted in schizophrenia 1 (*DISC1*), encoding a protein subsequently found to have an important role in brain development and neural progenitor proliferation (Blackwood et al. 2001; Mao et al. 2009). *DISC1* directly interacts with and inhibits GSK3 β activity, resulting in enhanced β -catenin-mediated signaling (Mao et al. 2009). WNT1 was also observed at higher levels in the hippocampal region of brains of schizophrenic patients compared with controls on postmortem analysis (Miyaoka et al. 1999). Genetic linkage studies have also shown an association with schizophrenia and the Wnt receptor *FZD3* in a subset of schizophrenic Chinese patients (Yang et al. 2003a).

Other neurologic diseases have been linked to aberrant Wnt signaling as well. For example, the presenilin proteins, which have been associated with early-onset Alzheimer's disease, are

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Figure 1. Pharmacologic inhibitors of the Wnt/β-catenin signaling pathway and known interactions with pathway components (see Table 1 for references). This figure depicts the Wnt/β-catenin pathway in its active state, with cytoplasmic accumulation and nuclear translocation of β-catenin, along with the transactivation of target genes in the nucleus mediated by interaction between β-catenin and members of the TCF family of transcription factors. Note that in contexts in which downstream mutations in APC or β-catenin result in constitutive pathway activation, the use of upstream inhibitors that target ligand secretion, ligand binding, and even the APC-AXIN-GSK3β complex may not be sufficient to inhibit signaling. Also, targeting of β-catenin interactors can involve either the disruption of protein interactions or the direct inhibition of transcriptional activity (see also Table 1 for additional details and references).

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Figure 2. Pharmacologic activators and enhancers of the Wnt/β-catenin signaling pathway and known interactions with pathway components (see Table 2 for references). This figure depicts the pathway in the inactive state, in which constitutive phosphorylation of β-catenin by GSK3β targets the protein for ubiquitylation and subsequent proteasomal degradation. In the absence of nuclear β-catenin, TCF-family transcription factors are bound by members of the Groucho family of transcriptional repressors, thus inhibiting target gene expression.

Table 1. Drug candidates that inhibit Wnt signaling and diseases/disease models in which they have been studied

Drug candidate	Target	Diseases or disease model(s)	References
Biologics			
Wnt1 antibody	Wnt1	NSCLC, head and neck cancer, sarcoma, colon cancer, breast cancer	He et al. 2004, 2005a,b; Mikami et al. 2005; Wei et al. 2009
Wnt2 antibody	Wnt2	Melanoma, mesothelioma, nonsmall cell lung cancer	You et al. 2004a,b; Mazieres et al. 2005b
Wnt5a antibody	Wnt5a	Rheumatoid arthritis	Sen et al. 2001
Wnt16 antibody	Wnt16	Acute lymphoblastic leukemia	Mazieres et al. 2005a
Fzd5 antibody	Fzd5	Melanoma, rheumatoid arthritis	Sen et al. 2001; Weeraratna et al. 2002
Soluble Fzd7	Fzd7	Hepatocellular carcinoma	Wei et al. 2011
Fzd10 antibody (conjugated with Y90)	Fzd10	Synovial sarcoma	Fukukawa et al. 2008
Fzd7 ectodomain	Fzd7	Colon cancer	Vincan et al. 2005
Soluble Fzd8 CRD	Wnt binding	Teratocarcinoma	DeAlmeida et al. 2007
V3Nter	Wnt binding	Colon cancer	Lavergne et al. 2011
sFRP4	Wnt binding	Renal injury	Surendran et al. 2005
Small molecules			
Glucocorticoids	β -catenin	Osteosarcoma	Takayama et al. 2006
Retinoids	β -catenin	Colon cancer	Xiao et al. 2003
Vitamin D(3)	β -catenin	Colon cancer	Pálmer et al. 2001
IWP compounds	MBOAT family of acyltransferases	Inhibition of Wnt-dependent colon/prostate cancer proliferation	Chen et al. 2009
IWR compounds	Axin stability	Inhibition of Wnt-dependent colon/prostate cancer proliferation	Chen et al. 2009
XAV939	TNKS1, TNKS2	Colon cancer, cardiomyogenesis	Huang et al. 2009; Wang et al. 2011a
PKF115-584 PKF118-310 CGP049090 PKF118-744 PKF222-815	TCF4/ β -catenin	Colon cancer proliferation	Lepourcelet et al. 2004
ICG-001	CBP	Pulmonary fibrosis, colon cancer	Emami et al. 2004; Henderson et al. 2010
NC043	TCF4/ β -catenin	Colon cancer	Wang et al. 2011b
iCRT3,-5,-14	TCF/ β -catenin	Breast cancer, colon cancer	Gonsalves et al. 2011
IC261	CKI ϵ	Breast cancer	Kim et al. 2010
Fumagillin, TNP-470	MetAP-2	Angiogenesis	Zhang et al. 2006; Cirone et al. 2008
Phenylmethimazole	Wnt5a expression	Melanoma	Schwartz et al. 2009
Pyrrinium	CK1 α	Cardiac repair	Saraswati et al. 2010; Thorne et al. 2010

NSCLC, non-small-cell lung cancer; CRD, cysteine-rich domain; CBP, CREB-binding protein; CKI ϵ , casein kinase- ϵ .

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Table 2. Drug candidates that enhance Wnt signaling and diseases/disease models in which they have been studied

Drug candidate	Target or class	Diseases or disease model(s)	References
Biologics			
Purified Wnts	LPR5/6/ Fzd	Fracture repair, ES/iPS cell differentiation	Reya et al. 2003; Willert et al. 2003; Nostro et al. 2008; Lengerke et al. 2009; Vijayaragavan et al. 2009; Minear et al. 2010
DKK1 antibody (BHQ880)	DKK1	Osteoporosis, osteoarthritis, fracture healing, multiple myeloma	Wang et al. 2008; Fulciniti et al. 2009; Glantschnig et al. 2010; Komatsu et al. 2010
DKK1 antisense oligonucleotides	DKK1	Brain injury, osteoporosis	Cappuccio et al. 2005; Wang et al. 2007
DKK1 siRNA	DKK1	Alzheimer's disease, brain injury	Caricasole et al. 2004; Cappuccio et al. 2005
Sclerostin/SOST antibodies	Sclerostin	Osteoporosis, fracture healing	Li et al. 2009; Agholme et al. 2010; Ominsky et al. 2010; Padhi et al. 2011
Foxy-5	Wnt5a mimetic	Breast cancer	Säfhholm et al. 2006; Ford et al. 2009
Small molecules			
Lithium	GSK3 β	Bone formation, ES differentiation, Alzheimer's disease	de Boer et al. 2004; Zamani et al. 2009; Toledo and Inestrosa 2010
GSK3 β inhibitors	GSK3 β	Pancreatic transplant, bone formation, schizophrenia, multiple myeloma	Kulkarni et al. 2006; Roh et al. 2007; Freyberg et al. 2010; Graham et al. 2010; Gunn et al. 2010
WAY-316606	sFRP1	Bone formation	Moore et al. 2009
Isoquercitrin and isorhamnetin	Unknown	Adipocyte inhibition	Lee et al. 2011
Riluzole	GRM1	Melanoma, bipolar disorder	Yip et al. 2009; Biechele et al. 2010; Le et al. 2010

ES, embryonic stem; iPS, induced pluripotent stem.

negative regulators of canonical Wnt signaling (Kang et al. 2002). Additionally, variant alleles in the Wnt receptor LPR6 have been associated with Alzheimer's disease in population-based linkage analyses. Functional analysis of one of these common alleles revealed decreased β -catenin signaling when this variant was expressed in HEK293T cells (De Ferrari et al. 2007). Wnt may also have a role in the metabolism of the amyloid precursor protein (APP) and inhibit phosphorylation of the tau protein, two events thought to be critical in the pathophysiology of the disease (Mudher et al. 2001). Wnt signaling may also participate in neuroprotection after brain injury. Enhanced Wnt/ β -catenin was shown in newly

derived glial progenitors and astrocytes in a model of traumatic brain injury (White et al. 2010). These observations establish the potential rationale for exploring the use of Wnt-directed therapeutics in these disease conditions.

WNT SIGNALING IN AUTOIMMUNE DISEASE AND INFLAMMATION

Wnt signaling has long been implicated in development of the immune system (reviewed in Yu et al. 2010) but the effects of Wnt signaling in adult immunity are only beginning to be appreciated. Studies of adult T cells have shown that the Wnt effector TCF1 appears to be required

for the functional generation of memory CD8⁺ T cells (Gattinoni et al. 2009; Jeannot et al. 2010; Zhao et al. 2010; Zhou et al. 2010). Wnt signaling can also affect the differentiation of naïve CD4⁺ T lymphocytes. Blockade of Wnt by small interfering RNA (siRNA)-mediated knock-down of β -catenin or by treatment of CD4⁺ T cells with DKK1 inhibited the expression of Th2 cytokines (Notani et al. 2010). Wnt/ β -catenin has also been shown to affect immunosuppressive T regulatory (Treg) cells that have been implicated in the pathogenesis of autoimmune disease, cancer, and infectious diseases (Ding et al. 2008). Enhanced Wnt signaling in gut-associated dendritic cells also led to immune suppression and tolerance in a model of inflammatory bowel disease, the effects of which were partially mediated through the induction of Tregs (Manicassamy et al. 2010).

Aberrant Wnt signaling has also been observed in autoimmune disorders. In rheumatoid arthritis (RA) patients, Wnt and *FZD* gene expression is higher in synovial membranes of affected joints compared with controls without RA (Sen et al. 2002). Increased expression of *WNT7B* has also been shown in RA patients and exogenous expression of *WNT7B* in human synovial cells induced inflammatory cytokines such as interleukin 1 β , interleukin-6, and TNF α (Nakamura et al. 2005). β -catenin-independent Wnt signaling has also been implicated in the development of RA. *WNT5A* expression was noted to be higher in cultured synoviocytes from patients with RA compared with controls (Sen et al. 2000). The autoimmune disease systemic lupus erythematosus (SLE) has been associated with aberrant Wnt gene expression as well (Sheng et al. 2011). In a murine model of SLE, increased canonical Wnt pathway activity was shown in kidneys affected by lupus nephritis, suggesting a role for Wnt activity in mediating end organ damage associated with the disease (Tveita and Rekvig 2011).

In summary, manipulation of Wnt signaling may provide a novel means of shaping the immune response in autoimmune disease, tumor immunotherapy, transplantation, and vaccine development. Once again, the in-depth involvement of Wnt signaling pathways in im-

munological function highlights both the power of these pathways as regulators as well as the potential difficulties involved in the directed targeting of Wnt signaling in disease.

WNT SIGNALING IN TISSUE HOMEOSTASIS AND REGENERATIVE MEDICINE

Given the importance of Wnt pathways in development and stem cell biology, it should not be surprising that manipulating Wnt signaling has proven useful in the burgeoning field of regenerative medicine. One of the central problems that must be addressed in applying stem cell biology to regenerative medicine is how to specifically control stem cell renewal and differentiation to direct stem cells toward specific fates. There are numerous studies implicating Wnts as important components of the stem cell signaling network that can be used to direct specific cell fates (Nusse 2008). For example, Wnt signaling has been implicated in the self-renewal and maintenance of hematopoietic stem cells (Reya et al. 2003; Willert et al. 2003; Wang and Nakayama 2009). Also, canonical Wnt signaling appears to be required for differentiation of embryonic stem (ES) cells toward hematopoietic stem cells and primitive hematopoietic progenitors (Lengerke et al. 2008; Nostro et al. 2008; Wang and Nakayama 2009). β -Catenin-independent Wnt signaling has also been implicated in driving mesoderm specification and exit from the pluripotent state from ES cells (Vijayaragavan et al. 2009).

Wnt is important for the development and maintenance of other tissue types as well. For example, the maintenance of intestinal crypt stem cells requires the Wnt pathway transcription factor TCF7L2/TCF4 (Korinek et al. 1998). Accordingly, Wnt3a is one of a host of factors that can be used to derive human intestinal cells from induced pluripotent stem (iPS) cells (Spence et al. 2011). Wnt/ β -catenin signaling has been implicated in cardiomyogenesis from human ES cells in a biphasic manner, promoting cardiomyocyte differentiation when triggered at early stages of differentiation, while inhibiting cardiogenesis at later stages (Paige et al. 2010; Yamauchi et al. 2010).

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One of the most active fields in Wnt biology and regenerative medicine is in the field of bone metabolism. The initial discovery that Wnt pathways were related to bone formation in the adult was made when mutations in the Wnt coreceptor LRP5 were linked to the autosomal recessive disorder osteoporosis-pseudoglioma syndrome (OPPG) (Gong et al. 2001). Among other features of the syndrome, individuals with OPPG suffered with low bone mass and a predisposition to skeletal fractures. Carriers of the LRP5 mutation also had lower bone mass compared with age-matched controls. A different mutation in LRP5, G171V has been associated with increased muscle mass and skeletal strength in humans (Boyden et al. 2002). An indirect role for Wnt signaling in osteoblast biology has been suggested by the observation that *Lrp5* deficiency in the duodenum has an endocrine effect on osteoblasts via gut-derived serotonin binding the receptor hydroxytryptamine (Htr1b) expressed on osteoblasts (Yadav et al. 2008), but a more recent study refutes this view and indicates a direct role of LRP5 in osteoblasts (Cui et al. 2011). Several other studies have also shown an important direct role for Wnt/ β -catenin signaling in osteoblasts at multiple stages, including promoting osteoblast differentiation from progenitors as well as promoting osteoblast and osteocyte survival in vitro (Day et al. 2005; Rodda and McMahon 2006). In addition, β -catenin was found to regulate osteoprotegerin (OPG/TNFRSF11B) production in mature osteoblasts. Because OPG is an inhibitor of osteoclast differentiation, Wnt/ β -catenin signaling in osteoblasts appears to inhibit bone resorption as well (Glass et al. 2005; Holmen et al. 2005).

As new models for regeneration are developed, the role of Wnt/ β -catenin signaling as a therapeutic target will be further clarified. Because regeneration is a long-term process, the question of how persistent therapeutic activation or inactivation of Wnt signaling will affect an organism becomes more relevant. However, it should be noted that in developmental models, including embryonic development, the effects of Wnt/ β -catenin signaling can be elicited through rather short exposure to Wnt ligand.

During embryonic development, Wnt signaling over short time periods may completely alter the specification of cell fate, suggesting that prolonged treatment courses with activators or inhibitors may not be needed (Bakre et al. 2007; Ueno et al. 2007; Trowbridge et al. 2010). In zebrafish models, pulsed exposure to Wnt signaling can result in measurable improvements in fin regeneration, further supporting the idea that long-term, continuous activation or inhibition of the pathway may not be absolutely required to achieve the desired end result (Stoick-Cooper et al. 2007).

DRUGS THAT INHIBIT WNT SIGNALING

Given the aberrant Wnt pathway activation in cancer, it is not surprising that inhibition of Wnt signaling has been an active area of investigation in both academia and the biotechnology sector. One past limitation in identifying clinically useful drugs has been the lack of an obvious enzyme target to inhibit in the pathway. Another has been the complexity of Wnt pathway regulation. At least 19 Wnt isoforms have been identified in humans (Chien et al. 2009a), all of which share significant sequence homology. The mechanisms that determine the specificity of a particular Wnt isoform remain unresolved, even with regard to whether isoforms exclusively activate Wnt/ β -catenin signaling or β -catenin-independent Wnt signaling.

Existing Patient-Experienced Drugs with Wnt Inhibitory Activity

Despite the inherent difficulties in developing novel Wnt inhibitors, many existing FDA-approved drugs inhibit Wnt pathways (Table 1). Nonsteroidal anti-inflammatory drugs (NSAIDs) were initially found to inhibit polyp formation both in patients with FAP as well as in the corresponding murine model, APC^{min} (Labayle et al. 1991; Giardiello et al. 1993; Mahmoud et al. 1998; Yang et al. 2003b; Boon et al. 2004). In FAP patients, reduced levels of β -catenin were shown in polyps of patients treated with the NSAID sulindac (Boon et al. 2004). The selective COX2 inhibitor celecoxib has

been FDA approved in patients with FAP as it has been shown to reduce polyp formation as well (Steinbach et al. 2000; Phillips et al. 2002). It has also been shown to reduce nuclear β -catenin in colorectal cancer cell lines similarly to other NSAIDs (Steinbach et al. 2000). The Renatiomer, the NSAID etodolac, whose structure is a nonsuperimposable mirror image to the parent drug but itself does not possess COX inhibitory activity, was found to inhibit Wnt/ β -catenin target genes in HEK293 cells and enhance the apoptosis of chronic lymphocytic leukemia (CLL) cells in vitro (Jensen et al. 2008).

Several nuclear receptors have also been found to act, at least partially, through effects on the Wnt/ β -catenin pathway. Retinoid-mediated signaling through retinoic acid receptors can compete with several TCF factors for β -catenin binding, thus inhibiting Wnt/ β -catenin target genes (Xiao et al. 2003). Vitamin D derivatives can also inhibit Wnt signaling in colorectal cancer. Studies in colorectal carcinoma cell lines have shown that analogs of the active form allows the vitamin D receptor to bind β -catenin and compete with TCF7L2 to reduce Wnt target gene expression in these cells (Palmer et al. 2001). Additionally, in a human osteosarcoma line that is sensitive to glucocorticoid-induced cell cycle arrest, glucocorticoid-mediated down-regulation of cyclin D1 was found to occur via interactions between the glucocorticoid receptor and the TCF/ β -catenin complex (Takayama et al. 2006).

Other classes of drugs have been identified that can inhibit Wnt signaling by screening libraries of FDA-approved drugs. For example, the anti-helminthic drug pyrvinium was identified as an agent that could potentiate the activity of CK1 α , thus leading to enhanced β -catenin and coactivator Pygo degradation and diminished signaling (Thorne et al. 2010). Pyrvinium has shown promise as a promoter of tissue repair in a mouse model of myocardial infarction, in which the inhibition of Wnt/ β -catenin signaling led to global improvements in cardiac remodeling (Saraswati et al. 2010).

In summary, although the clinical use of drugs for the primary purpose of inhibiting

Wnt signaling is still in early development, there are numerous drugs or compounds in current usage that affect Wnt/ β -catenin signaling. Whether the effects of these drugs on inhibiting Wnt/ β -catenin may contribute to some of the efficacy or side effects seen with the current use of these drugs will likely become more apparent with improvements in our understanding of Wnt signaling across various tissues and diseases.

Approaches to Identifying Novel Inhibitors of Wnt Signaling

Because Wnt pathways are subjected to extensive regulation at various levels, high-throughput screening technologies have greatly facilitated the identification of both biologics and small molecule pathway regulators. One promising approach has been the use of cell-based reporter assays to screen siRNA or chemical libraries. These cell-based reporters provide a rapid, reliable, and quantitative means of interrogating Wnt/ β -catenin signaling, and have been critical tools in the identification and subsequent characterization of every Wnt-modulating small molecule identified to date (Biechele and Moon 2008). Genome-wide siRNA and drug library screening approaches are powerful but have many difficulties such as uneven transfection efficiency, the knockdown efficacy of siRNA sequences, or off-target effects leading to false hits. Such screens also offer little in the way of mechanistic data. These complications have been mitigated somewhat by improvements in assay automation and rigorous validation. Another approach to minimize the false hit rate and provide insights into mechanism is to integrate data from different platforms. For example, an integrated analysis from a genome-wide siRNA screen of modulators of Wnt signaling with protein interaction networks derived from mass spectrometry of 23 bait proteins or derived from known protein interactions in the literature identified 38 novel regulators, several of which were involved in chromatin remodeling (Major et al. 2008). Another siRNA-based loss-of-function screen of β -catenin-mediated transcription was integrated with gene copy number

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analysis in human colon cancer cells and identified cyclin-dependent kinase 8 (CDK8) as a key regulator of Wnt target gene expression (Firestein et al. 2008).

Strategies for Inhibiting Proximal Wnt Signaling

One attractive approach for inhibiting Wnt signaling has been targeting either ligands or extracellular receptors that are readily accessible to antibodies or small molecules (Fig. 1; Table 1). This biologics approach may be particularly relevant for diseases that display aberrant Wnt signaling in the absence of known downstream pathway mutations. In these cases, dysregulation of Wnt signaling is instead attributed to the aberrant expression of Wnt ligands. For example, WNT1 is overexpressed in numerous human cancers and a WNT1-blocking antibody both inhibited proliferation and induced apoptosis in many cancer cell types (He et al. 2004, 2005a,b; Mikami et al. 2005; Wei et al. 2009).

The FZD or LRP coreceptors are also potential targets for therapeutic manipulation. For example, synovial sarcomas show significant overexpression of FZD10 compared with normal tissue. In a mouse synovial sarcoma xenograft model, FZD10 antibody was used to target delivery of the radioisotope Yttrium-90 to tumors resulting in inhibition of tumor growth (Fukukawa et al. 2008). Using the FZD ectodomain to competitively bind Wnt ligand is another strategy that has been used. Overexpression of the FZD7 ectodomain attenuated proliferation in a colon cancer cell line in vitro as well as a murine xenograft assay (Vincan et al., 2005). Additionally, a soluble Wnt receptor consisting of a FZD8 cysteine rich domain (CRD) fused to a human Fc domain showed activity against teratoma lines in vitro and in vivo (DeAlmeida et al. 2007).

Another approach to target proximal events in Wnt signal transduction is to use endogenous extracellular modulators of Wnt such as Dickkopf (DKK), Wnt inhibitory factor-1 (WIF1), or SFRPs. SFRPs contain a cysteine-rich domain (CRD) similar to the extracellular Wnt-binding domain of the frizzled receptors, and have been

postulated to act as a decoy receptor by directly binding Wnt ligands. WIF1 also directly binds Wnt ligands, whereas DKK binds to and inhibits transduction via the LRP coreceptor. Dysregulation of these endogenous inhibitors is common in many tumor types, typically by promoter hypermethylation (Suzuki et al. 2004, 2008; Fukui et al. 2005; Zou et al. 2005). Although SFRP is lost early in the progression of colon cancer, restoration of SFRP in colon cancer can attenuate Wnt signaling even in the presence of downstream activating mutations (Suzuki et al. 2004). In another study, a peptide containing a frizzled CRD derived from a cell-surface collagen VIII termed V3Nter was able to act in an SFRP-like fashion and inhibit canonical Wnt signaling in colorectal carcinoma lines as well as inhibit tumor growth in a xenograft model (Lavergne et al. 2011).

In addition to the use of Wnt inhibitors as soluble proteins, forced cellular overexpression has also been tested as a strategy. Forced DKK3 expression in renal cell carcinoma lines resulted in the induction of apoptosis, although this appeared to be mediated by alterations in the non-canonical JUN kinase (JNK) pathway rather than β -catenin inhibition (Ueno et al. 2011). Viral vector-mediated overexpression of DKK3 inhibits prostate and breast cancer growth in mice (Edamura et al. 2007; Kawasaki et al. 2009). Although the use of endogenous Wnt inhibitors seems to be a reasonable strategy, the finding that proteins like SFRPs can potentially enhance Wnt signaling in some contexts, perhaps by acting as chaperones, suggests that the therapeutic use of these particular proteins may be complicated (Uren et al. 2000; Chien et al. 2009a; von Marschall and Fisher 2010). Furthermore, DKK family members can themselves transduce receptor-mediated cellular signals that are not completely understood at this time (Wu et al. 2000; Mukhopadhyay et al. 2006; Yamamoto et al. 2008).

Another approach to inhibit upstream events in Wnt signaling is disruption of the interaction between the receptor FZD and DVL. For example, a structure-based virtual ligand screening identified a small molecule that is capable of binding to the PDZ domain of DVL required

for its interaction with FZD receptors. This small molecule is able to inhibit Wnt3A-mediated signaling (Shan et al. 2005). Another study found that peptide binding to the DVL PDZ domain is more permissive compared with PDZ domains in other proteins and used peptide-phage display to identify peptide ligands, which bound to the PDZ domain of human DVL2. These peptide ligands were then shown to disrupt Wnt/ β -catenin signaling in cell lines (Zhang et al. 2009).

Drugs that interfere with Wnt secretion have also been used as an approach to inhibit proximal signaling. For example, the drug IWP-2, identified in a screen of a synthetic chemical library, was found to inhibit the activity of Porcupine, a membrane-bound acyltransferase that modifies Wnt ligands with a palmitoyl group that is required for their secretion and signaling activity (Takada et al. 2006; Chen et al. 2009).

Drugs Inhibiting Downstream Wnt Signaling

Given the wide range of biologic effects of Wnt-mediated signaling in the adult, targeting more upstream signaling events may lead to unwanted side effects. Furthermore, in diseases such as colon cancer in which there frequently are mutations in *APC*, upstream inhibition at the level of the receptor may be ineffective for preventing the effects of a downstream activating mutation.

High-throughput screening approaches have been used to identify promising lead compounds that may one day lead to the development of bona fide clinical Wnt inhibitors (Fig. 1; Table 1). For example, HEK293 cells expressing the SuperTOPFLASH Wnt-responsive reporter were screened with a chemical library that led to the identification of XAV939 as a potent inhibitor of Wnt/ β -catenin signaling. Mechanistic studies of this drug revealed a novel regulatory pathway by which the stability of Axin, a critical negative regulator of the pathway, was regulated by PARsylation and proteasomal degradation by tankyrases, TNKS1 and TNKS2 (Huang et al. 2009). This drug was able to inhibit colony formation of colorectal cancer cell lines and has also been used to inhibit β -catenin in a manner that promotes cardiomyocyte differen-

tiation from embryonic stem cells (Huang et al. 2009; Wang et al. 2011a). Other substrates of tankyrases must be considered if tankyrase inhibitors are to be used clinically. For example, the dominantly inherited disorder cherubism is characterized by interosseous fibrocystic lesions resulting from hyperactive osteoclasts results from single missense mutations in a region of the adaptor protein 3BP2, which is part of a multiprotein signaling complex containing the SRC family kinases, SYK, and RHO family guanine nucleotide exchange factor VAV (Ueki et al. 2007; Guettler et al. 2011). These mutations have been found to disrupt the binding of tankyrase to 3BP2, inhibiting 3BP2 degradation and resulting in high levels of SRC, SYK, and VAV activity in osteoclasts (Guettler et al. 2011; Levaot et al. 2011). These observations may be relevant if tankyrase inhibitors are to be considered as cancer therapeutics, for instance, owing to not only potential side effects such as osteoporosis, but also concerns about potentiating the SRC pathway, which is already hyperactive in many human tumors.

As the β -catenin TCF/LEF complex is the ultimate downstream effector required for transcription of Wnt target genes, disruption of this complex has been studied as a therapeutic strategy. The ability to rationally design drugs to intervene in this interaction has been facilitated by characterization of the crystal structure of TCF/ β -catenin complexes (Graham et al. 2000). One class of small molecules able to disrupt this interaction was identified by a high-throughput screening approach utilizing an assay based on the binding affinity of purified β -catenin to a TCF4 (TCF7L2) fragment (Lepourcelet et al. 2004). These compounds were able to inhibit growth of colorectal carcinoma cells in vitro (Lepourcelet et al. 2004). Another study used a small molecule secondary structure template library, which identified a compound, ICG-001 that was able to down-modulate β -catenin/TCF signaling by binding to the CREB response element-binding protein (CBP) and specifically inhibiting the CBP/ β -catenin interaction (Emami et al. 2004). This compound was also shown to inhibit tumor formation in a mouse colon cancer xenotransplant model as

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well as attenuate pulmonary fibrosis induced by bleomycin, which is thought to be in part mediated by wnt/ β -catenin signaling (Emami et al. 2004; Henderson et al. 2010). Another small molecule screen yielded the drug NC043, a diterpine alkaloid that can interrupt the β -catenin/TCF4 interaction and inhibit colorectal cancer growth in vivo (Wang et al. 2011b). Recently, several compounds were identified that inhibited β -catenin/TCF4 interactions utilizing a chemical library screen of *Drosophila* cells to mitigate functional genetic regulatory redundancies in mammalian cells. These compounds were able to inhibit β -catenin-dependent invasion of breast cancer cells and proliferation of colon cancer cell lines (Gonsalves et al. 2011). One conceptual difficulty in utilizing such drugs as therapeutics is that the structural basis for the interaction between β catenin and TCF is almost identical to that with E-cadherin, although there is some evidence for binding specificity mediated by β -catenin modifications (Graham et al. 2000; Huber and Weis 2001; Gottardi and Gumbiner 2004). Consequently, drugs designed to interrupt the β -catenin/TCF interaction may also disrupt the cadherins junction leading to unpredicted and potentially untoward side effects.

Other regulators of Wnt/ β -catenin signaling have also emerged as potentially useful targets. For example, casein kinase-I ϵ (CKI ϵ) was identified based on siRNA screening of breast cancer cell lines as a positive regulator of β -catenin-dependent transcription. Pharmacologic inhibition of CKI ϵ with the drug IC261 inhibited breast cancer cell proliferation (Kim et al. 2010). Many other kinases that positively regulate Wnt have emerged as potential targets including phosphatidylinositol 4-kinase type IIa (PI4KIIa), phosphatidylinositol-4-phosphate 5-kinase type Ib (PIP5KIb), PAR-1, TNIK, and MAP3K1 (Sun et al. 2001; Pan et al. 2008; Mahmoudi et al. 2009; Sue Ng et al. 2010).

As the tools for interrogating Wnt signaling continue to evolve, novel pathways and promising compounds will continue to be identified as modulators of Wnt/ β -catenin signaling. Because the effects of most of these compounds will be cell type/context-dependent, the chal-

lenge will be to understand the role of Wnt/ β -catenin signaling across various tissues in such a way as to develop a practical therapeutic strategy.

STRATEGIES TO ACTIVATE WNT SIGNALING

Although much work has been performed identifying antagonists of Wnt signaling, particularly with regard to cancer therapy, drugs that activate Wnt pathways may be beneficial in many diseases including osteoporosis and certain cancers (Fig. 2; Table 2). Several drugs exist that act, at least partially, by enhancing Wnt pathways. For example, lithium has long been known as a powerful morphogen in embryonic development. Research into the mechanism for this property identified it as an inhibitor of GSK3 β , which ultimately results in constitutive Wnt pathway activation (Klein and Melton 1996). Lithium is a well-known psychoactive drug and it has been postulated that its effects in the central nervous system (CNS) may be partially attributable to Wnt enhancement. Interestingly, many anti-psychotic drugs may act, at least to some degree, through interaction with Wnt pathway components. For example, the atypical anti-psychotic drug clozapine has been shown to increase levels of Dvl and phospho-Dvl in the rat brain, thereby enhancing β -catenin-mediated signaling (Roh et al. 2007).

Several studies have linked the use of lithium to increased Wnt/ β -catenin signaling. For example, treatment of Lrp5-deficient mice with lithium significantly increased trabecular bone mass. Lithium also increased bone mass in animals with age-related osteoporosis and oophorectomy-induced osteoporosis (Clément-Lacroix et al. 2005). Furthermore, a retrospective clinical study comparing patients receiving lithium to age-matched controls noted increased bone density, lower serum ALP, and osteocalcin (markers of bone turnover) in patients who received lithium (Zamani et al. 2009). The directed clinical use of lithium specifically as a Wnt modulator is complicated by the substantial toxicities in vivo that accompany the very high doses needed to achieve Wnt activation in different contexts. More potent GSK3 β inhibitors

are available and may also be clinically useful, although these agents are still considered experimental at this time. In vivo effects on bone metabolism comparable to lithium have been seen with an orally available potent dual inhibitor of GSK3 α/β (Engler et al. 2004). Pharmacologic inhibition of GSK3 β has been shown to potentiate regulatory T-cell activity in vitro and extend allograft survival in a mouse model of pancreatic islet cell transplantation (Graham et al. 2010). Unfortunately, inhibition of an enzyme as ubiquitous as GSK3 β has multiple consequences that are independent of the Wnt pathway, leading to significant side effects that have complicated the use of these otherwise potent compounds in human patients.

Other indirect means of enhancing Wnt signaling have been explored. For instance, knock-down of *DKK1* by RNA interference (RNAi) significantly inhibited bone loss in a rat model of estrogen depletion as well as in a model of glucocorticoid-mediated bone loss (Wang et al. 2007, 2008). Investigators have also used anti-*DKK1* monoclonal antibodies as a means to increase bone mass and mineralization in several small animal and nonhuman primate models (Glantschnig et al. 2010). *DKK1* antibody has also been shown to inhibit degenerative osteoarthritis in the setting of inflammatory arthritis and to facilitate fracture healing (Diarra et al. 2007; Komatsu et al. 2010). Additionally, in a small animal model of ischemic brain injury, expression of *DKK-1* was found to be strongly induced during injury. Treatment with antisense oligonucleotides against *DKK-1* significantly inhibited neurotoxicity (Cappuccio et al. 2005). Interest in inhibiting the function of *DKK* has also emerged because it has been appreciated that tumor-derived *DKK* may inhibit Wnt-induced osteoblastic differentiation, thus facilitating osteolytic bone metastases in diseases such as breast cancer and multiple myeloma (Tian et al. 2003; Bu et al. 2008; Qiang et al. 2008). An anti-*DKK1* antibody, BHQ880 was shown to increase osteoblasts and bone density in a (SCID)-hu murine model of human multiple myeloma as well as inhibit the growth of multiple myeloma cells on bone marrow stroma in vitro (Fulciniti et al. 2009).

Another extracellular regulator of Wnt signaling has been targeted as an approach to enhance bone formation. In *Xenopus*, a secreted cysteine-knot protein, Wise, was identified that acted as a context-dependent activator or inhibitor of Wnt signaling by interacting with LPR6 (Itasaki et al. 2003). Deficiency of a related mammalian gene product, sclerostin (SOST), is associated with the inherited disorder sclerosteosis, which results in hyperactive osteoblasts (Brunkow et al. 2001). SOST-deficient mice also showed markedly increased bone formation and bone strength (Li et al. 2008). Sclerostin was confirmed to bind LPR5/6 and inhibit Wnt/ β -catenin signaling (Li et al. 2005; Semënov et al. 2005). Several anti-sclerostin antibodies have been developed that have shown significant activity in enhancing bone mass in small animal and nonhuman primate models (Li et al. 2009; Agholme et al. 2010; Ominsky et al. 2010). A single dose, placebo-controlled phase I clinical trial with a humanized sclerostin antibody, AMG 785, has been recently reported. In this study, patients receiving single doses of AMG 785 had increased markers of bone turnover and higher bone mineral density compared with placebo, and treatment was generally well tolerated (Padhi et al. 2011). A phase II study of this agent is currently ongoing.

Another putative secreted inhibitor of Wnt signaling, SFRP1 is also an attractive target to enhance Wnt signaling therapeutically. *Sfrp1*-deficient mice have increased trabecular bone density and mineralization, consistent with the observation that deletion of *Sfrp1* inhibited age-associated bone loss in mice (Bodine et al. 2004). In one study, a small molecule inhibitor of SFRP1 was identified via a fluorescent polarization binding assay using purified human SFRP1 protein and a cell-based Wnt reporter assay. This small molecule was capable of stimulating ex vivo murine calvarial bone formation (Moore et al. 2009).

Although there has clearly been more work on identifying inhibitors, small molecule compounds that potentiate Wnt/ β -catenin signaling have also been described. One study identified flavonoid components of the *Persicaria hydropiper* plant as enhancers of Wnt/ β -catenin

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signaling that can block the differentiation of adipocytes from 3T3-L1 cells (Lee et al. 2011). In another study, a cell-based screen utilizing the BAR reporter of a chemical library of 1857 human-experienced drugs identified 44 compounds that enhanced Wnt3a-mediated stimulation (Pollock et al. 2003; Namkoong et al. 2007; Biechele et al. 2010). One of these activators, riluzole, is an FDA-approved drug for treating amyotrophic lateral sclerosis (ALS). Riluzole is thought to inhibit the metabotropic glutamate receptor GRM1 and is currently in clinical trials for melanoma based on its activity in melanoma cell lines, xenograft models, and a phase 0 patient trial (Pollock et al. 2003; Namkoong et al. 2007; Yip et al. 2009; Le et al. 2010). As Wnt/ β -catenin signaling is associated with an improved prognosis in melanoma, it is intriguing to speculate that the mechanism of action of this drug may be at least partially owing to its effect on enhancing Wnt/ β -catenin signaling (Chien et al. 2009b).

STRATEGIES FOR TARGETING β -CATENIN-INDEPENDENT WNT SIGNALING

Despite the mounting evidence for involvement of β -catenin-independent Wnt signaling in the pathogenesis of several disorders, approaches to target noncanonical Wnt signaling have been disappointingly limited. WNT5A antisense, dominant-negative WNT5A, and addition of a WNT5A antibody in vitro were shown to inhibit the production of proinflammatory cytokines such as IL-6, IL-15, and RANKL in cultured synoviocytes from RA patients (Sen et al. 2001). Small molecules represent another viable approach to targeting β -catenin-independent Wnt pathways. For example, the drug fumagillin, originally isolated from the fungus *Aspergillus fumigatus*, was initially identified as a compound that bound the protein methionine aminopeptidase 2 (MetAP-2) (Zhang et al. 2006). Fumagillin was subsequently found to disrupt noncanonical Wnt signaling in zebrafish embryos and to inhibit β -catenin-independent Wnt-mediated endothelial cell migration and angiogenesis (Zhang et al. 2006; Cirone et al. 2008). In another study, phenylmethima-

zole, an inhibitor of Toll-like receptor-3 (TLR3) was found to inhibit WNT5A expression in melanoma and pancreatic cell lines, thus inhibiting their growth and migration in vitro and in a murine xenograft model (Schwartz et al. 2009). A formylated hexapeptide derived from the primary WNT5A peptide sequence mimicked the ability of WNT5A to inhibit breast cancer cell migration, suggesting that peptide-based approaches may also be viable tools for modulating Wnt pathways (Säfhholm et al. 2006). As more studies elucidate the specific roles of β -catenin-independent Wnt signaling in disease, more therapeutic targets and strategies are likely to emerge.

CONCLUSION

As research tools to understand the molecular mechanisms of human diseases continue to develop, so does our understanding of the prominent role that aberrant Wnt-mediated signaling plays in an ever-expanding number of human diseases. Apart from the examples described here, a role for Wnt is emerging in a multitude of other disease processes for which pharmacologic manipulation could prove clinically valuable. To fully realize the potential of Wnt manipulation, more must be understood about the complex regulation of both canonical and noncanonical pathways. As research tools to understand the molecular mechanisms of human disease continue to develop, so does our understanding of the nuances of Wnt regulation in disease as well as in the context of normal tissue homeostasis. Our ability to target these pathways for therapeutic benefit is only in its infancy. However, promising new technologies are paving the way to drive more effective therapeutic targeting of Wnt pathways in the future.

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