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New avenues to target Wnt/β-catenin signaling

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Wnt/β-catenin signaling is an evolutionarily conserved signaling cascade with imperative roles during development and in adult stem cell maintenance. Hyperactivation of Wnt/β-catenin drives various cancers, whereas hypoactivation underlies bone malformations and neurodegenerative disorders. Although several small molecule modulators of Wnt/β-catenin signaling have been identified, none have progressed into clinical trials yet. Recent studies employing genomics and proteomics approaches have yielded more druggable targets, such as kinases and seven-transmembrane receptors. In addition, new assay methods enable a more targeted approach for high-throughput screening of this pathway and are expected to deliver clinical candidates in the coming decade.

Wnt proteins (Wnts) are a family of nineteen glycosylated and lipidated proteins that regulate cell growth and differentiation [1,2]. Wnts are the GPS system of the embryo: they determine body axes and drive organs to grow in the right direction. In the adult mammal, Wnts control the maintenance of self-renewing tissues such as bone marrow, gut and skin [3,4]. Aberrant Wnt signaling results in developmental malformations and underlies diverse human pathologies, such as colorectal cancer, osteoporosis and neurodegenerative disorders [3,4]. They elicit their effects through the activation of different intracellular signal transduction pathways. The precise signaling output of Wnts seems to depend on the repertoire of cell surface receptors present on recipient cells. For instance, binding of Wnt-5a to a member of the Frizzled family of seven-transmembrane receptors (7TMs) and a representative of the low-density lipoprotein receptor-related protein family (LRP5/6) stimulates the stabilization of β-catenin, which acts in concert with T-cell factor (TCF) to modulate the transcription of Wnt-responsive genes [5,6]. However, Wnt-5a can also inhibit this β-catenin-dependent pathway when it binds to the receptor tyrosine kinase ROR2 [6,7]. Thus, receptor levels and relative affinities of Wnt ligands to their receptors determine signaling output [8–10].

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Hyperactivation of β-catenin signaling has been implicated as a driver of various cancers, in particular colon cancer, whereas reduced signaling underlies aberrations in bone formation and neurodegenerative disease [3,11]. Consequently, there is a great interest in inhibitors or activators of this pathway for different indications. Small-molecule drug discovery on Wnt/β-catenin signaling has long been based on pathway screens employing TCF-dependent transcriptional reporter gene assays. Although these screens have yielded several small-molecule modulators of Wnt/β-catenin signaling [12–15], none have progressed into the clinical phase of drug discovery yet. In this respect, drug discovery on Wnt/β-catenin signaling is trailing behind research on other developmental pathways implicated in human disease, such as Notch and Hedgehog signaling [16]. In part, this has been because of the lack of druggable protein targets – like γ-secretase in Notch signaling, for example – in the Wnt/β-catenin pathway [17]. Recent RNAi screens and proteomics have yielded several enzymes, however, including protein and lipid kinases, ADP ribosylases and an acyltransferase, both as crucial regulators of Wnt/β-catenin signaling and as potential drug targets. The orphan G-protein-coupled receptor (GPCR) LGR5 was identified as a Wnt-regulated gene and stem cell marker, and new assay methods for the high-throughput screening (HTS) of β-catenin signaling have become available. These recent developments provide new avenues to target Wnt/β-catenin signaling that

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are discussed in this review. First, the role of Wnt/β-catenin signaling in health and disease is described below.

The Wnt/β-catenin signaling pathway

Wnt/β-catenin signaling is regulated through the controlled degradation of β-catenin in the cytoplasm. In quiescent cells, the cytoplasmic and nuclear abundance of β-catenin is kept at low levels by the action of a large protein assembly called the 'destruction complex'. The destruction complex consists of several scaffolding proteins, including Axin1/Axin2 and adenomatous polyposis coli (APC), the protein product of a tumor suppressor gene. In addition, it contains the Ser/Thr kinases glycogen synthase kinase 3 (GSK3α/GSK3β) and casein kinase Iα (CKIα) [1,2]. *De novo*-synthesized β-catenin is recruited to the destruction complex, where it is sequentially phosphorylated by CKIα and GSK3 at several N-terminal serine and threonine residues. These phosphorylations mark β-catenin for ubiquitination and subsequent degradation by the proteasome. In the absence of β-catenin, proteins of the TCF family repress Wnt-specific gene transcription [1,2].

Wnt/β-catenin signaling is initiated by binding of Wnts to a member of the Frizzled family and LRP5 or LRP6. The resulting ternary complex recruits several components of the destruction complex to the membrane. The function of the destruction complex is inhibited, and unphosphorylated β-catenin accumulates in the cytoplasm and eventually translocates to the nucleus. There, it associates with TCF proteins, converting TCF from a repressor into an activator of Wnt-responsive gene transcription [1,2].

Wnt/β-catenin signaling in cancer

In the early 1990s, interest in Wnt/β-catenin signaling was sparked by the finding that β-catenin binds to the tumor suppressor APC [18]. By then, deletions within a chromosomal region containing the APC gene had already been linked to a hereditary disease termed familial adenomatous polyposis, which gives rise to numerous intestinal polyps and, eventually, tumors. Loss-of-function mutations in APC turned out to be present not only in the large majority of familial adenomatous polyposis patients but also in sporadic colorectal carcinomas. These mutations typically resulted in truncated proteins that were incapable of binding β-catenin, Axin1 or Axin2 [3,19]. Since then, mutations in other core components of the destruction complex have been reported to be present in a wide variety of cancers. In a smaller set of colon carcinomas and in melanomas, mutations in the β-catenin gene have been observed [3,19]. These mutations are either truncations that lead to deletion of part of the N terminus of β-catenin or point mutations that affect the serine and threonine residues that are targeted by GSK3α/β or CKIα. These mutant β-catenin proteins are refractory to phosphorylation and thus escape proteasomal degradation. Consequently, β-catenin accumulates within affected cells. Stabilized and nuclear-localized β-catenin is a hallmark of nearly all cases of colon cancer, and proliferative Wnt-regulated genes such as the oncogene *c-myc* and the cell cycle regulator *cyclin D1* contribute to tumor progression [3,19].

Toward target-based screening of Wnt/β-catenin signaling

The prominent role of Wnt/β-catenin signaling in colorectal tumorigenesis has attracted considerable interest in the drug discovery research community. Several academic institutes and pharmaceutical companies have performed screening campaigns to identify low molecular weight inhibitors of Wnt/β-catenin signaling [12–14,20]. The most applied assay technology for HTS on Wnt/β-catenin signaling has been the TOPflash reporter gene assay. The TOPflash reporter consists of a luciferase gene preceded by a minimal promoter and several TCF-binding sites [21]. TOPflash screens, performed with up to 400,000 compounds, have yielded several hits [12–14]. The targets of these Wnt/β-catenin inhibitors are generally protein–protein interactions [12,14,20] for which no or insufficient structural information is available and structure–activity-relationship studies are consequently problematic. In addition, because of the large interaction surface between proteins and the large heterogeneity in protein–protein interfaces, they are not suitable for modulation by small molecules [22]. Most of the inhibitors identified in screens for Wnt/β-catenin signaling have no more than micromolar potencies, even after extensive chemical exploration. In contrast to protein–protein interfaces, receptors and enzymes are good drug targets [23]. Efforts to identify and specifically target members of these protein classes will probably result in a more efficient lead optimization process. In the following paragraphs, receptors and enzymes – specifically, 7TMs and protein kinases – that have recently been implicated in Wnt/β-catenin-dependent pathologies are described.

Stem cell marker LGR5

An interesting 7TM target for colon cancer was identified by the group of Clevers at the Hubrecht laboratory (Utrecht, The Netherlands). There, a crucial role was established for Wnt signaling in the maintenance of a small number of intestinal and gastric stem cells that were characterized by the expression of the Wnt target gene LGR5 [24,25], a leucine-rich repeat-containing GPCR. LGR5 was initially identified as a Wnt target gene using microarrays on colon carcinoma cell lines and patient-derived colorectal cancer specimens [26]. Expression pattern analysis showed that LGR5 is expressed in a limited number of cells near the base of the intestinal crypts. Lineage tracing revealed that LGR5-positive cells gave rise to all the differentiated cell types of the intestinal epithelium. Remarkably, a single LGR5-positive cell was shown to be sufficient to reconstitute a complete crypt-villus structure, the principle building block of the intestine, *in vitro* [27]. Within colon carcinomas, small numbers of LGR5-positive cells were found to be largely responsible for the proliferation of the tumor [28], giving strength to the idea that tumors contain defined compartments of stem cells that are responsible for their growth, as well as underlining the central role of Wnt signaling during this process. The closest relative of LGR5 is LGR6, which was recently shown to mark stem cells in hair follicles [29]. Its expression in cancers has not yet been addressed.

Because LRG5-positive cells are cells-of-origin of the complete intestinal lining, LGR5/6 may be used as a marker to select stem cells for regenerative medicine [30]. It is too early to predict whether modifying the activity of LGR5/6 with small-molecule drugs would have therapeutic utility. Targeting of chemotherapeutic drugs to LGR5, however, might be a feasible strategy to specifically inhibit β-catenin-dependent cancer stem cells. A prerequisite for identifying small-molecule (ant)agonists of LGR5 is the availability of assays to measure its activity. LGR5 is still an

'orphan' (i.e. no natural ligands are known) and does not display constitutive activity in assays measuring G-protein signaling [31]; however, recruitment of the intracellular scaffolding protein β-arrestin is a virtually universal trait of activated GPCRs [32]. β-arrestin recruitment was also recently shown for 7TMs that do not couple to G proteins, such as the complement fragment decoy receptor C5L2 [33] and the chemokine receptor CXCR7 [34]. β-Arrestin recruitment can be measured with high-content imaging approaches, employing fluorescent proteins tagged to the GPCR or to β-arrestin, bioluminescence resonance energy transfer, β-galactosidase enzyme fragment complementation and protease-mediated transcriptional reporter gene technology [32]. Applying these technologies to LGR5 might help in the identification of both natural and surrogate ligands to further validate the targeting of LGR5-positive stem cells in drug discovery.

Frizzleds: new drug targets in cancer?

Other potential 7TM targets for colon cancer are Frizzleds. For years, a doctrine in Wnt-oriented colon cancer research has been that inhibition of Wnt signaling at the level of Wnt reception would have no therapeutical benefit because aberrant activation of β-catenin signaling in most cancers occurs at the level of the destruction complex [35]. This premise is likely to be false, at least in some cases. For instance, several Wnt genes have been shown to be ectopically or overtly expressed in colon cancer, and in some instances, autocrine Wnt signaling has been reported to promote tumor growth [35,36]. In addition, several soluble Wnt antagonists, such as Wnt inhibitory factor-1 [37], soluble Frizzled-related proteins [38] and Dickkopf proteins [39] are epigenetically silenced in colorectal carcinomas, and restoration of their expression inhibits tumor progression. Inhibition of Wnt signaling at the level of Wnt/Frizzled interactions by treatment with antibodies against Frizzled [40], Wnts [41,42] or soluble Wnt-binding domains of Frizzleds (cysteine-rich domains) [43], or by transfection with vectors coding for soluble Frizzled-related proteins [35], reduced tumor growth in several models. These results hold promise for Frizzleds as drug targets for the treatment of cancer.

Assays to measure Frizzled function

In view of the role of Frizzleds in adult stem cell maintenance, it is essential to identify which receptors are involved in the aberrant activation of Wnt/β-catenin signaling in colorectal cancer to enable specific targeting of Frizzled family members in cancer. Thus, assay development for Frizzleds would need to yield assays that can measure the activity of individual receptor family members. This has proved challenging for two reasons. First, virtually every cell line expresses at least some Frizzleds endogenously. Second, the only commercially available purified recombinant Wnt ligand that activates Wnt/β-catenin signaling, Wnt-3a (R&D Systems, Abingdon, UK), does so through multiple Frizzleds [44]. Thus, high background signaling through the activation of endogenous Frizzleds usually confounds the analysis of single Frizzled receptor family members, unless they are overexpressed in a cellular background with low endogenous Frizzled expression levels. Functional cell-based assays measuring the activity of individual Frizzleds have been developed by stable transfection of human *Frizzled 1* and *Frizzled 2* cDNA in a HEK293 cell line containing a TCF-dependent β-lactamase reporter gene [44]. A limitation of reporter gene assays, in particular when applied in antagonistic format, is the high false-positive hit rate in HTS caused by the interaction of compounds with signaling components downstream of the receptor [45]. Thus, assays that measure the direct consequences of Frizzled receptor activation are required. The recruitment of Wnt signaling components that are reported to bind to Frizzled receptors upon Wnt binding, such as LRP5/6 and the scaffolding protein Dishevelled (Dvl), may therefore be exploited to make HTS assays measuring the proximity of these proteins to Frizzleds, for example by β-galactosidase enzyme fragment complementation or bioluminescence resonance energy transfer. An important advantage of both technologies is that the assay signal is generated by the specific interaction of labeled proteins and, therefore, is insensitive to activation of endogenous Frizzleds or downstream activation of Wnt/β-catenin signaling.

Although Frizzleds are 7TMs and structurally related to GPCRs, coupling of Frizzleds to G proteins has not been unambiguously proven [44,46]. Thus, most of the assay systems developed for measuring 7TM receptor activity cannot be used to measure Frizzled receptor signaling. However, β-arrestin recruitment assays may be applicable to Frizzleds.

Studies investigating the role of β-arrestins in Wnt/Frizzled signaling were fueled by results found for the closest Frizzled family relative Smoothened (Smo). Smo is a 7TM that shares with Frizzled not only its 7TM topology but also its N-terminal cysteine-rich domain. Smo has not been reported to bind Wnts, however, and solely functions in Hedgehog signaling. Like GPCRs, Smo has been shown to become phosphorylated by GPCR kinase 2 upon pathway activation, leading to β-arrestin-mediated internalization. Whereas β-arrestin and GPCR kinase 2 are involved in the desensitization of GPCRs, they are required for the activation of the Hedgehog pathway [47]. The same probably holds for Wnt/Frizzled signaling. Studies using β-arrestin knockout cells indicate that β-arrestin is required for the activation of Wnt/β-catenin signaling [48,49]. Furthermore, Chen *et al.* [50] demonstrated an indirect interaction between human Frizzled-4 and β-arrestin-2 through mutual binding to Dvl2, a process that required both Wnt stimulation and protein kinase C activation. These results suggest that Frizzleds recruit β-arrestin in a ligand-dependent manner. Thus, β-arrestin technology could be used for drug discovery on Frizzled receptors.

Targeting kinases in Wnt/β-catenin-driven cancers

Protein kinases are an important drug target class for oncology, with ten new chemical entities approved in the past decade [23]. However, two kinases that have been most firmly associated with Wnt/β-catenin signaling, GSK3α/β and CKIα, negatively influence Wnt/β-catenin signaling and, therefore, are not targets for colon cancer. Recent attempts to identify kinases that positively influence Wnt/β-catenin signaling have yielded several new drug targets (Fig. 1). These include two lipid kinases, phosphatidylinositol 4-kinase type IIα (PI4KIIα) and phosphatidylinositol-4-phosphate 5-kinase type Iβ (PIP5KIβ) [51]. These lipid kinases were identified by assessing the accumulation of β-catenin in HEK293T cells transfected with short inhibitory RNAs (siRNAs) targeting human kinases [51]. Another group of kinases that can activate Wnt/β-catenin signaling are the three human homologs of *Drosophila*

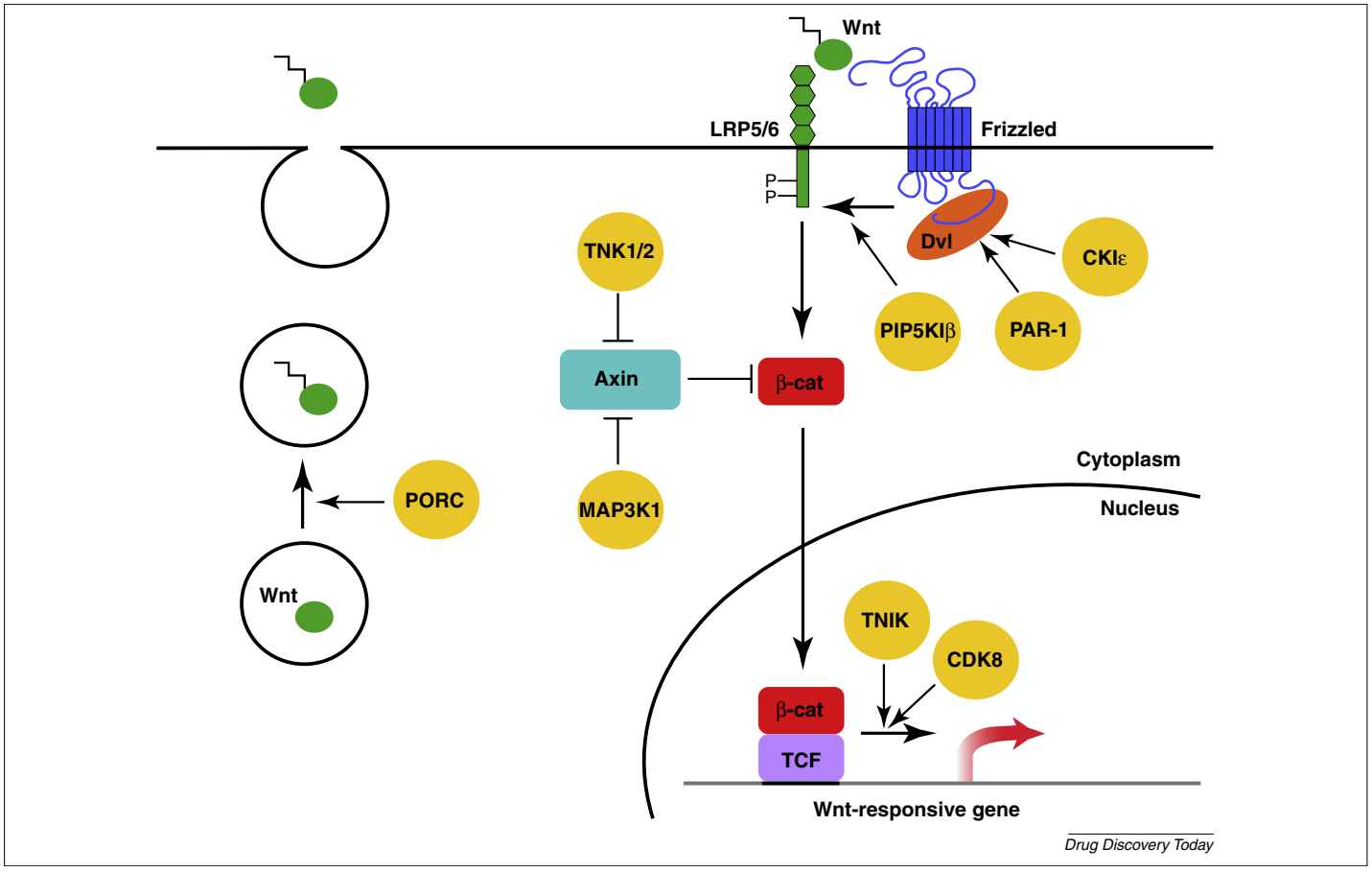

FIGURE 1

Novel enzyme targets in Wnt/β-catenin signaling. A schematic depiction of Wnt/β-catenin signaling. Left: Wnt protein is synthesized and is modified with lipid by the acyltransferase Porcupine (PORC). Right: After secretion, Wnts activate Frizzleds, leading to recruitment of Dishevelled (Dvl), phosphorylation of LRP5/6 at intracellular motifs, stabilization of β-catenin and transcription of β-catenin-dependent genes. PAR-1 associates with and phosphorylates Dvl [52]. Casein kinase I epsilon (CKIε) activates β-catenin signaling through phosphorylation of Dvl and, possibly, TCF. PIP5KIβ phosphorylates phosphatidylinositols, which is required for proper phosphorylation of LRP5/6 [51]. MAP3K1 binds to and inhibits Axin1 through its E3 ubiquitin ligase domain [54]. Tankyrases-1 and -2 (TNK1/2) ubiquitinate Axin, thereby causing its proteasomal destruction [61]. TNIK directly contacts both TCF and β-catenin and regulates β-catenin-induced gene transcription [53]. CDK8 activates Wnt/β-catenin-dependent gene transcription through an incompletely understood mechanism [58].

PAR-1, which were identified as Dvl-interacting kinases in immunoprecipitation experiments with lysates of *Drosophila* embryos [52]. Activation of PAR-1, PI4KIIα and PIP5KIβ during Wnt/β-catenin signaling has been demonstrated [51,52]. In addition, inhibition of these kinases, either through siRNA-mediated knockdown or through overexpression of kinase-dead mutants, interfered with Wnt/β-catenin signaling in several cell lines [51,52]. However, the involvement of these kinases in the constitutive activation of the Wnt/β-catenin pathway during cancer still needs to be addressed. Three other kinase targets, mitogen-activated kinase kinase kinase 1 (MAP3K1), Traf2-and-Nck-interacting kinase (TNIK) and casein kinase I epsilon (CKIε) have been shown to be important for the activation of Wnt/β-catenin signaling, not only in established cell lines but also in β-catenin-dependent cancer cell lines [53–55]. Inhibition of CKIε through pharmacological intervention or gene knockdown reduced the proliferation of β-catenin-dependent breast cancer cell lines [55]. How CKIε mediates the activation of Wnt/β-catenin signaling is not entirely clear but might involve phosphorylation of Dvl or TCF [56]. Mutations in CKIε have been identified in breast cancer, confirming a crucial role for this kinase in cancer [57]. These CKIε mutations suppressed Wnt/β-catenin signaling but promoted signaling through other Wnt pathways [57]. MAP3K1 was found to be present in Axin1 immunoprecipitates upon Wnt stimulation, and siRNAs against this kinase inhibited Wnt/β-catenin signaling in both HEK293T and colorectal cancer cell lines [54]. TNIK was identified as a TCF4-interacting protein in intestinal crypt cells and human colorectal carcinoma and was found to be essential for β-catenin-dependent transcriptional activation in colon cancer cell lines [53]. Whether the activity of MAP3K1 or TNIK is aberrantly altered in colorectal cancer remains to be determined.

The cyclin-dependent kinase CDK8 was identified in RNAi-based loss-of-function screens on a TOPFlash-harboring colon carcinoma cell line [58]. Knockdown of CDK8 inhibited Wnt/β-catenin-dependent reporter gene activity and proliferation of a panel of colon cancer-derived cell lines with activating mutations in Wnt/β-catenin pathway components. Conversely, overexpression of CDK8 stimulated tumor formation of mouse fibroblast cells injected into nude mice. Copy number gain of a region on chromosome 13, containing the CDK8 gene, was observed in nearly half of tumor specimens from human colorectal carcinomas, and elevated CDK8 protein expression was a hallmark of a subset of

colorectal cancers [58]. The molecular target of CDK8 within the Wnt/β-catenin pathway has not been defined, although evidence suggests that CDK8 activates β-catenin-dependent gene transcription through inhibition of the transcription factor E2F1 [59]. Its upregulation in colorectal cancer and its prominent role in Wnt/β-catenin-dependent cancer growth make CDK8 an interesting target for colon cancer. Of note, inhibitors of related CDKs are in clinical trials for other cancer types [60]. CDK8 inhibitor lead molecules may be identified by examining cross-reactivities of such inhibitors to CDK8.

Other enzymes in Wnt/β-catenin signaling

In addition to kinases, the repertoire of druggable targets within the Wnt/β-catenin pathway encompasses other enzymes. Recently, several enzymes – including tankyrases [13,61], a ubiquitin ligase [62,63] and an acyltransferase [13] – have been implicated in Wnt/β-catenin signaling (Fig. 1). Some of these enzymes have been shown to be amenable to modulation by small molecules. For instance, two low molecular weight tankyrase inhibitors (IWR-1 and XAV939), which antagonize Wnt/β-catenin signaling in HEK293 cells and in the cancer cell line DLD1 by causing stabilization of Axin1 levels, have been described [13,61]. Both compounds display nanomolar potency toward their target, and co-crystallization of XAV939 with the catalytic domain of tankyrase 2 has provided opportunities for even greater specificity and potency [64]. Tankyrase activity has been demonstrated to be important for zebrafish tailfin regeneration, intestinal homeostasis and murine kidney development, which are processes known to be regulated by Wnt/β-catenin signaling [13,65]; however, whether aberrant regulation of tankyrases plays a part in human disease is unknown.

An inhibitor of the acyltransferase Porcupine, which is essential for Wnt lipidation and secretion, inhibited Wnt/β-catenin signaling in mouse L cells with nanomolar potency [13]. Whether targeting of Porcupine is a viable approach for treating human disease is still uncertain, especially when considering that the human genome contains a single Porcupine gene that is predicted to enable maturation of a plethora of Wnts. However, the compounds support the rationale of targeting enzymes for drug discovery on Wnt/β-catenin signaling.

Activating Wnt/β-catenin signaling?

Whereas aberrantly activated Wnt/β-catenin signaling is linked to cancer, reduced β-catenin signaling has been implicated in osteoporosis and neurodegenerative disorders, such as Alzheimer's disease [11,66]. The role of Wnt/β-catenin signaling, and more specifically GSK3, in neurodegenerative disorders was suggested by the neuroprotective effect of lithium, which inhibits GSK3 isoforms and thereby causes activation of Wnt/β-catenin signaling [66]. This makes GSK3 an attractive target for which potent and selective inhibitors have been described [66]. Several other kinases have been shown to inhibit β-catenin activity, including (but not limited to) Nemo-like kinase [67] and Bruton's tyrosine kinase [68], but the role of these kinases in pathologies with diminished Wnt signaling has not been defined. Thus, there is ample opportunity to initiate target-based drug discovery to identify activators of β-catenin signaling.

Opportunities for specificity

A crucial issue that will need to be addressed is whether targeting of Wnt/β-catenin signaling can be sufficiently specific. A first concern is that many of the targets described here might perform vital roles in other signaling cascades. For instance, CDK8 may be pivotal for cell cycle regulation in non-cancerous cells, and whether global CDK8 inhibition is detrimental to normal cell homeostasis remains to be explored. Second, because Wnt/β-catenin signaling is essential for stem cell maintenance, the question is whether there can be a broad enough window between therapeutic efficacy and target-related toxicity. For instance, the intestine of mice deficient for TCF4 or overexpressing Dickkopf-1 display greatly diminished epithelial proliferation and altered ultrastructure [69,70]. Thus, global inhibition of Wnt/β-catenin signaling is predicted to cause intestinal toxicity; therefore, specificity will be an important determinant of the success of Wnt-inhibiting drugs. Of note, several siRNA approaches have identified a comparatively

TABLE 1

Druggable protein targets in Wnt/β-catenin signaling

Target name	Target class	Activates/inhibits β-catenin signaling	Molecular mode of action	Refs.
LGR5	7TM	N/A	β-Catenin target gene	[24,25,28]
LGR6	7TM	N/A	N/A	[29]
Frizzled	7TM	Activates	Wnt binding, LRP5/6 and Dvl recruitment	[37–39]
PIP5KIβ	Kinase	Activates	Phosphatidylinositol phosphorylation	[53]
PI4KIIα	Kinase	Activates	Phosphatidylinositol phosphorylation	[53]
PAR-1	Kinase	Activates	Dishevelled phosphorylation	[54]
MAP3K1	Kinase/ubiquitin ligase	Activates	Axin1 inhibition	[56]
TNIK	Kinase	Activates	Activation of β-catenin target gene transcription	[55]
CDK8	Kinase	Activates	Activation of β-catenin target gene transcription	[60]
CKIε	Kinase	Activates	Dishevelled phosphorylation, TCF phosphorylation	[57,59]
Porcupine	Acyltransferase	Activates	Wnt production	[13]
Tankyrase	Ubiquitin ligase	Activates	Axin1 ubiquitination/degradation	[13,63]
KLH12-Cullin3	Ubiquitin ligase	Inhibits	Dishevelled ubiquitination/degradation	[65]
Jade-1	Ubiquitin ligase	Inhibits	β-Catenin ubiquitination/degradation	[64]

small set of proteins that comprise the core Wnt/β-catenin signaling components [71]. Many proteins seem to be involved in Wnt/β-catenin signaling only in a given cell type or tissue [71]. Such context-specific pathway components provide opportunities for enhanced specificity.

Concluding remarks

Recent reports have implicated druggable proteins within the Wnt/β-catenin signaling pathway as targets in several pathologies (Table 1). This review discusses 7TMs and kinases with roles in β-catenin-mediated disease and suggests venues for assay development to identify small-molecule drugs modulating this clinically relevant signaling pathway. Such target-based approaches should outperform current drug development efforts targeting Wnt/β-catenin signaling and deliver clinical candidates in the coming years.

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