Review

Is Wilms Tumor a Candidate Neoplasia for Treatment with WNT/β-Catenin Pathway Modulators?—A Report from the Renal Tumors Biology-Driven Drug Development Workshop

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Abstract

The European Network for Cancer Research in Children and Adolescents consortium organized a workshop in Rome, in June 2012, on "Biology-Driven Drug Development Renal Tumors Workshop" to discuss the current knowledge in pediatric renal cancers and to recommend directions for further research. Wilms tumor is the most common renal tumor of childhood and represents a success of pediatric oncology, with cure rates of more than 85% of cases. However, a substantial minority (~25%) responds poorly to current therapies and requires "high-risk" treatment or relapse. Moreover, the successfully treated majority are vulnerable to the late effects of treatment, with nearly one quarter reporting severe chronic health conditions by 25 years of follow-up. Main purposes of this meeting were to advance our understanding on the molecular drivers in Wilms tumor, their heterogeneity and interdependencies; to provide updates on the clinical–pathologic associations with biomarkers; to identify eligible populations for targeted drugs; and to model opportunities to use preclinical model systems and prioritize targeted agents for early phase clinical trials. At least three different pathways are involved in Wilms tumor; this review represents the outcome of the workshop discussion on the WNT/β-catenin pathway in Wilms tumorigenesis. Mol Cancer Ther; 12(12); 2619–27. ©2013 AACR.

Introduction

The main purpose of the European Network for Cancer Research in Children and Adolescents (ENCCA) consortium, a European Union Framework 7–funded program, is to accelerate biology-based drug development, to facilitate access to novel therapies, and improve standards of care across Europe. The "Biology-Driven Drug Development Renal Tumors Workshop" (Rome, June 9–10, 2012) organized by ENCCA discussed these aspects in the context of Wilms tumor.

Wilms tumor, or nephroblastoma, the most frequent renal tumor of childhood, affects approximately 1 in 10,000 children before their 15th birthday, with a peak incidence between 2 and 3 years of age, and represents one of the successes of pediatric oncology, with an overall cure rate of more than 85%. The majority of patients with Wilms tumor in the Western world are treated within prospective, randomized multicenter trials conducted by either the International Society of Pediatric Oncology (SIOP, Europe) or the Children’s Oncology Group (COG, formerly NWTSG, North America; ref. 1).

At present, there is evidence of at least three pathways linked to the development of Wilms tumor that might be the cause of the heterogeneity in clinical phenotype and outcome observed in the tumors:

1. Wilms tumor with signs of activation of the WNT/β-catenin pathway (canonical WNT pathway), found...
in approximately 15%–20% of all anaplastic Wilms tumors.

2. Wilms tumor with activation of the IGF2 pathway, often with clear evidence of epigenetic aberrations, found in approximately two-thirds of all nonanaplastic Wilms tumors, with some overlap with WNT/β-catenin pathway deregulated group.

3. Wilms tumor with mutations in TP53, mainly found in anaplastic Wilms tumor, that comprises about 5%–10% of all Wilms tumors.

Here, we will review the current data on the heterogeneity in Wilms tumor, the importance of the WNT signaling pathway in a subset of tumors, and therapeutic opportunities. The other two groups will be discussed elsewhere.

**Wilms Tumor Heterogeneity**

Wilms tumor is an embryonic tumor of the kidney that is thought to arise from metanephric mesenchyme. Histologically, it resembles fetal kidney, with varying proportions of blastemal, epithelial, and stromal cells (which may include ectopic mesenchymal elements; ref. 2). About 40% of unilateral and nearly all bilateral Wilms tumors occur in association with nephrogenic rests, focal lesions reflecting developmental errors (3). Nephrogenic rests are apparently Wilms tumor precursor lesions, as tumors have been found developing within them. Nephrogenic rests are classified into intralobar nephrogenic rests (ILNR) and perilobar nephrogenic rests (PLNR) and are associated with different tumor histology and genetics (2).

Prevalent published model proposed that the clinical and histologic features of the Wilms tumors and associated nephrogenic rests are determined by the underlying molecular defects, and two genetic loci, WT1 gene at 11p13 and WT2 locus at 11p15.5, have been associated with Wilms tumor pathogenesis (reviewed in refs. 2, 4, 5). Further genes linked to Wilms tumor development include WTX on chromosome Xq11.2, CTNNB1 on 3p22.1, encoding β-catenin, and TP53 on 17p13.1 (reviewed in refs. 4, 5).

In this model, biallelic WT1 mutations lead to the development of ILNR, and following additional genetic changes, such as WNT/β-catenin pathway activation, most commonly due to CTNNB1-activating mutations, Wilms tumor develops. These Wilms tumors usually present a stromal-predominant histology with mesenchymal elements (2, 4–11). Alternatively, genetic or epigenetic changes deregulate the imprinting of IGF2-H19 locus at 11p15.5. This results in biallelic expression of IGF2, a gene normally expressed only from the paternally inherited allele, and in the development of PLNR, followed sometimes by additional genetic changes leading to Wilms tumor. These Wilms tumors usually have a blastemal- or epithelial-predominant histology (2, 4, 5, 12–14).

For the purposes of this review, we focus on the canonical WNT/β-catenin signaling pathway and its involvement in a subset of Wilms tumors.

**WNT/β-Catenin Signaling and Cancer**

The history of the WNT/β-catenin pathway had its very beginning in 1976 when wingless was identified as a gene involved in wing and haltere development in Drosophila (15). A few years later, it was identified as a segment polarity mutant in Drosophila (16). Independently, the Int1 gene was identified as a common integration site in mouse mammary tumors experimentally induced by the mouse mammary tumor virus (MMTV; ref. 17). In 1987, it was shown that the Drosophila homolog of Int1 was in fact wingless (18), formally linking the wingless pathway to cancer.

WNT proteins (WNT) are a family of secreted signaling proteins triggering cellular responses in a concentration-dependent manner. Lipid modification is required for efficient signaling and may be important for WNTs secretion. One of these lipids is palmitoleic acid, and porcine, a multipass transmembrane O-acyltransferase of the endoplasmic reticulum, is essential for WNTs palmitoylation and maturation (19).

The binding of WNT ligands to the transmembrane receptors Frizzled (FZ) and low-density lipoprotein receptor-related protein 6 (LRP6), or its close relative LRP5, initiates a signaling cascade that results in the activation of β-catenin–dependent transcription (19). At this level, the activity of WNTs is regulated by negative extracellular regulators: secreted frizzled-related proteins (SFRP), WNT inhibitory factor (WIF) proteins, and proteins of the Dickkopf (DKK) family (19). The WNT–FZ interaction is promiscuous, and the signaling includes a ligand-induced conformational change of the receptors, followed by FZ interaction with cytoplasmic Dishevelled 1 (DVL; ref. 19). A crucial step is the binding of AXIN to the cytoplasmic tail of LRP6. AXIN-LRP6 binding is regulated by phosphorylation of the LRP6 tail by at least two kinases, glycogen synthase kinase-3β (GSK3β) and casein kinase 1 (CK1), which require WNT-induced generation of phosphatidylinositols 4,5-bisphosphate [PtdIns(4,5)P2] at the plasma membrane (20). These events lead to the stabilization of β-catenin, which accumulates and travels to the nucleus to activate WNT target gene expression. β-Catenin acts as a transcriptional coactivator to stimulate target gene transcription by displacing the transcriptional repressor Groucho from T-cell factor/lymphoid enhancer factor (TCF/LEF) and recruiting an array of transcriptional coactivators and histone modifiers such as BRG1, CBP, BCL9, and pygopus (19).

In the absence of WNTs, cytoplasmic β-catenin protein is constantly degraded by the "destruction complex," which is composed of the scaffolding protein AXIN, the tumor suppressors antigen-presenting cell (APC), the signaling regulators WTX and DVL, and the kinases CK1 and GSK3β. Sequentially, CK1 and GSK3β phosphorylate the amino terminal region of β-catenin, resulting in β-catenin recognition by β-Trcp, an E3 ubiquitin ligase subunit, and subsequent β-catenin ubiquitination and...

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proteasomal degradation (Fig. 1). This continual elimination of β-catenin prevents β-catenin from reaching the nucleus, and WNT target genes are thereby repressed (19). Aberrant activation of WNT/β-catenin signaling is observed in many human cancers (reviewed in refs. 21, 22). Germline defects of the APC gene cause familial adenomatous polyposis, a condition predisposing to colorectal cancer, and APC mutations are also involved in the vast majority of sporadic colorectal cancers (21, 22). AXIN I and II are found mutated in sporadic malignancies, particularly hepatocellular and some colorectal cancers, as well as in some familial cancer syndromes (21, 22). Also, CTNNB1-activating mutations are observed in hepatocellular cancers, medulloblastoma, colorectal cancer, gastric cancer, ovarian cancer, and Wilms tumor (4, 5, 21, 22). The role of WTX mutations, almost exclusively described in Wilms tumors, is less established (23–25). Overexpression of WNT ligands or downregulation...
of WNT antagonists (Dkk, Sfrps, and Wif) has been reported in several human cancers (21, 22).

Activation of the Wnt/b-catenin pathway is one of the most important hallmarks of stemness of cancer stem cells (CSC; ref. 26). Intriguingly, many of the cell-surface markers, including Lgr5/Gpr49, C4D4, C2D4, and Epcam, that have been used to identify and isolate putative tumor stem cell populations, are directly regulated by the Wnt pathway, which also seems to play an important role in the transcription of Abcb1/mdr1, one of the multidrug resistance genes that may contribute to the side population phenotype of malignant cells (27).

Because the earliest indications of a role for Wnt signaling in cancer go back to the cloning of Int1 in MMTV-induced mouse mammary tumors, it is not surprising that mice have been a pivotal model organism. The role of Ctnnb1 has been studied in both loss-of-function and gain-of-function settings (28). The conventional knockout for Ctnnb1 is embryonic lethal at E6.5 due to failure of gastrulation and lack of A-P (anterior-posterior) axis formation (29). Conditional variants of these null mutations have further highlighted the role of endogenous b-catenin in a variety of processes (30, 31). An intrinsic problem in the interpretation of these mutations is the fact that b-catenin not only functions in the Wnt signaling pathway but also has additional roles in cell adhesion (32). It is, therefore, difficult to assign a knockout phenotype unequivocally to either of these roles. The generation of a mouse model where only the signaling function of b-catenin is removed, through combination of an N-terminal missense mutation (D164A) that disrupts the interaction with Bcl9 with a C-terminal truncation, has now started to answer this question in an elegant manner (33).

As the role of b-catenin in cancer is gain-of-function rather than loss-of-function, models that mimic this situation have been at least as informative as the models described above. As the four residues whose phosphorylation status controls b-catenin stability are all found in exon 3, Harada and colleagues generated a mouse model that carries a conditional exon 3 knockout allele (34). As removal of this exon leaves the remainder of the protein in the normal reading frame, this model allows for conditional activation of b-catenin mediated signaling. Just as in the conventional and conditional knockout models, this dominant stable allele has been used extensively in developmental and disease-related studies (28). Additional hypomorphic alleles, such as the model that mutates a phosphorylated tyrosine residue Y654E (35), leading to a more subtle increase in b-catenin signaling, are likely to provide further advances in our understanding of Ctnnb1 mutations in cancer.

Kidney Development and Wnt Signaling

Metanephric kidney development starts when the ureteric bud, an outgrowth of the Wolffian duct, invades the metanephric mesenchyme (reviewed in ref. 36). This event sets off a series of reciprocal inductions between these two cell populations, which result in the first branching of the ureteric bud and the formation of a condensate of mesenchymal cells, called the cap mesenchyme, around the tips of the ureteric bud. In the cap mesenchyme, cells positive for Six2 were found to be the progenitors of the complete nephron (37). Again, in response from signals from the tip, some of the cap cells undergo a mesenchymal-to-epithelial transition (MET) and form the renal vesicle. This epithelialized structure is patterned to form distinct proximal, medial, and distal domains and thus forms the complete functional nephron. Wnt signaling is essential in many of these processes. Canonical Wnt/b-catenin signaling is necessary for the initiation of nephrogenesis and the initiation of the MET that is required for metanephric mesenchyme to start to form epithelial tubules that contribute to the formation of the mature nephron. In fact, ectopic activation of b-catenin in kidney mesenchyme is sufficient to induce nephrogenesis, but is incompatible with subsequent epithelialization (38, 39). Activation of the noncanonical Wnt pathways calcium/nuclear factor of activated T-cells (NFAT) and planar cell polarity (PCP), together with attenuation of the canonical pathway, are required to complete the MET and renal tubule morphogenesis. Wnt9b, secreted by the ureteric bud, and signaling via b-catenin, determines which subset of Six2+ mesenchymal cells undergo MET and hence induces renal cell vesicle formation (40, 41). Wnt9b has a role upstream of Wnt4. This gene is expressed in the condensed mesenchyme containing the pretubular cells where it is necessary and sufficient for the nephron MET (42, 43). Wt1 regulates Wnt4 expression and coordinates the overall availability of the Wnt4 locus for interacting partners (44–46). Wnt4 in its turn signals, at least in the developing kidney, via the noncanonical calcium/Nfat Wnt signaling pathway (47, 48). Once the epithelialized nephron has formed, Wnt9b is needed for the control of polarity of cell division; in contrast to its role in the induction of the renal MET, this time its function is mediated by the noncanonical Wnt–PCP pathway (49). b-Catenin in its turn is essential for the development of the parietal epithelium (50). Disruption of this signalization might also be involved with Wilms tumor onset, as suggested by a gene-expression study (51). In all, it is clear that Wnt signaling, including the b-catenin mediated canonical pathway, plays a central role in kidney development.

Wilms Tumors and Wnt

As Wilms tumors show a direct relationship with abnormal development of the embryonic kidney, it is not surprising to find links between abnormal Wnt signaling and Wilms tumor formation. As anticipated, many lines of evidence point toward this being most important in the WT1/Ilnr-associated tumors. Wt1 and Ctnnb1 mutations occur in approximately 20% and 15% of Wilms tumors, respectively (4), with many studies describing an association between Wt1 and Ctnnb1 mutations in
Wilms tumors (4–7, 9, 10). Most mutations occurring in the CTNNB1 gene result in the loss of important regulatory phosphorylation sites, and are associated with constitutive activation of the WNT/β-catenin signaling pathway as well as with aberrant myogenesis (7–10). A recent review of the literature reported that of 154 CTNNB1 mutant tumors, 121 also had WT1 mutation with a surprising preference for CTNNB1 mutations affecting residue Ser45 (4). As loss of any of the four phosphorylatable residues in exon 3 of CTNNB1 is predicted to result in the same stabilization of the protein, the reason for this mutational preference is not clear.

More recently, WTX gene anomalies have been reported in approximately 20% of Wilms tumors (4, 23–25, 52–55). Although WTX anomalies were observed to be approximately equally frequent in tumors with and without WT1 mutations (4, 25, 53–55), when considering WTX and CTNNB1 mutations, it is difficult to draw any clear conclusion. Some studies reported that in WTX-mutated Wilms tumors, CTNNB1 mutations are more likely to occur in exons 7 and 8 (unlike exon 3 mutations, they do not affect phosphorylatable residues known to be involved in CTNNB1 stability; refs. 53, 54, 56). In contrast, a negative association with exon 3 CTNNB1 mutations has been speculated (reviewed in ref. 4). However, this was not confirmed in a study on more than 400 Wilms tumors that demonstrated a comparable frequency of exon 3 CTNNB1 mutations in tumors with and without WTX aberrations (25). Thus, although WTX was demonstrated to be part of the β-catenin destruction complex (57), and both CTNNB1-activating mutations and WTX-inactivating events would lead to the same endpoint, including inappropriate WNT/β-catenin pathway activation, mutations in these two genes may not represent mutually exclusive events. However, the published data regarding the co-occurrence of these mutations are somewhat discordant and the true nature of the association is yet to be established.

A recent study defined five subsets (S1–S5) of pathologic and clinical features of Wilms tumor based on global gene expression patterns combined with mutational status of WT1, CTNNB1, and WTX, and 11p15 copy number and methylation patterns (56). Among these, subset S2 tumors presented at an early median age, commonly arose within ILNRs, showed heterologous mesenchymal differentiation with expression profile enriched for genes expressed in the intermediate mesoderm and early metanephric mesenchyme, including low expression of WT1 and WNT/β-catenin pathway activation, and had a high frequency of WT1 and/or CTNNB1 exon 3 mutation (56).

The relationship between tumor lineages in Wilms tumor and WNT/β-catenin signaling pathway activation was further analyzed in a panel of tumors characterized for WT1, CTNNB1, and WTX status, as well as for nuclear accumulation of β-catenin. This showed that WNT/β-catenin pathway activation was restricted to Wilms tumors of mesenchymal lineage, which were associated with ILNRs, CTNNB1 mutation and/or β-catenin nuclear accumulation, and, occasionally, WT1 mutations (53). In a second study in which the epithelial and stromal components of β-catenin mutant tumors were microdissected, it was demonstrated that nuclear accumulation was confined to the mesenchymal cells, even though the epithelial component carried the same mutation (9). This implies that the signals that determine subcellular trafficking remain intact.

To improve the characterization of the molecular mechanisms and the involvement of the WNT/β-catenin pathway in Wilms tumors, both the genomic and expression status of WT1 and CTNNB1, and WTX mutations were characterized, as well as their gene-expression profiles, defining two major groups of tumors (52). Group A (WT-A), characterized by alterations in both CTNNB1 (mutations and/or nuclear accumulation) and WT1 (mutations and/or absence of expression), with stromal histology, and group B (WT-B), characterized by the absence of WT1 and CTNNB1 mutations, high WT1 transcription with nuclear WT1 protein, and no visible nuclear β-catenin and with blastemal or mixed histology. Comparison of gene-expression profiles of WT-A and WT-B showed that WTX/β-catenin was the most significant pathway identified (52).

Considering the role of WTX in the WNT/β-catenin pathway, it is intriguing to observe that in all these studies, WTX anomalies were found in Wilms tumors both with and without WNT pathway activation (52, 53, 56).

Gene-expression studies have the intrinsic caveat of assigning Wilms tumors to the WNT/β-catenin or non-WNT/β-catenin categories according to annotated lists of WNT target genes, whereas transcriptional targets can vary depending on the cellular context. Notwithstanding, it is clear that both genetic and gene-expression evidence indicates that deregulated WNT/β-catenin signaling is important in a substantial subset of Wilms tumors, and therefore, merits consideration as a potential therapeutic target. It should be noted that where it has been possible to study WNT disregulation in relation to risk of relapse in Wilms tumor, this does not seem to be an adverse risk factor, whether based on direct analysis of gene-expression patterns (56) or indirectly through association with WT1-mutated tumors (58). Thus, the potential therapeutic role of WNT-targeted agents may be to replace rather than intensify existing treatments.

An important question in the context of the aims of the ENCCA workshop is how far our understanding of the genetics of kidney development and its relation to malignant transformation provides clues for the therapeutic targeting of Wilms tumor. The focus of attention for this should be on the MET at the onset of nephron formation, as disruption of this is believed to be a key event in Wilms tumor formation. Both β-catenin and WT1 are involved in this process. Only Six2+ cells that respond to a WNT signal, likely activated by Wnt9b secreted by ureteric bud cells, will enter the differentiation pathway and go through the MET; nonresponding cells remain in the
WT1 is responsible for the execution of the MET through direct activation of Wnt4 expression, and loss of WT1 in the right cell type results in a developmental block at the MET stage (44, 45). Does this explain the early events in the WT1/β-catenin mutant/ILNR-associated Wilms tumor cases? It is important to realize that genetic loss of WT1 is the rate-limiting step, with activating mutations in CTNNB1 being a secondary event (6, 59). If there would be a direct link between WT1 and β-catenin that would explain their role in Wilms tumor and the observed Darwinian selection for activating mutations in CTNNB1 in WT1-mutant Wilms tumor, the function of CTNNB1 should be genetically downstream of WT1. A logical step would have been the signaling downstream of Wnt4 in the MET, if this would be mediated by β-catenin. This seems, however, not to be the case (47, 48). At present, there are no data on other roles for canonical WNT signaling post-MET that could explain this correlation from a cancer evolutionary point of view. An alternative scenario would be for WT1 loss and β-catenin activation to have independent roles in Wilms tumor formation without any selective pressure between them. More than one study has found nuclear staining for β-catenin to be mainly in the rhabdomyoblastic component of Wilms tumor samples (7, 9). If we follow the paradigm that WT1 loss (either through mutation or loss of expression) coincides with β-catenin pathway activation (52), this could suggest that loss of WT1 results in a block at the MET stage as observed (45), whereas β-catenin activation drives the ectopic differentiation, with other factors determining whether the mutant protein remains cytosolic or free to translocate to the nucleus (9). It is difficult to envision the pressure that would drive the selection for activated β-catenin in this scenario. A third possibility would be a role for β-catenin in the stemness of Wilms tumor CSCs. These CSCs have very recently been identified as a NCAM⁺ ALDH1⁺ population (60). The neural cell adhesion molecule (NCAM) is found in the cap mesenchyme. It is possible that the NCAM⁺ population shows an overlap with the Six2⁺ population, the known nephron progenitors (37), but this remains to be formally proven (61). If this is the case, however, it would take us back to the first, unsatisfactory, scenario.

Mouse models will be essential to understand the exact roles of different genes and types of mutations in Wilms tumor development. The first reproducible Wilms tumor mouse model has been generated through combined loss of WT1 and activation of Igf2 (62). These two events separately were not sufficient for Wilms tumor formation. This model corresponds to a subset of Wilms tumor cases (subset S3; ref. 56). So far, there have been no published reports on Wilms tumor mouse models based on activated β-catenin. Conditional loss of Apc in the developing kidney was found to result in renal carcinomas but not Wilms tumor (63). One reason to explain this would be that this model does not activate the canonical WNT signaling pathway in the correct developmental stage/cell type, but an alternative explanation could be that the level of β-catenin signaling resulting from this mutation is not compatible with Wilms tumor development. The wide variation in phenotypes found in different CTNNB1 and Apc mouse models suggests that some care should still be taken in interpreting the role of CTNNB1 mutations in human Wilms tumor onset. The selection for CTNNB1 Ser45 mutations in WT1-mutant Wilms tumor and exon 7/8 mutations in WTX-mutant cases might also be an important, yet underappreciated aspect of these tumors. Even more intriguing, a recent genome-wide association study aimed at identifying susceptibility loci for Wilms tumor, found the strongest evidence of association at the rs790356, which lies in a 68Kb-LD block at 11q14.1 containing the DLG2 gene, which codes for a member of the membrane-associated guanylate kinase proteins involved in the noncanonical WNT PCP pathway (64). This provides another twist to the role of WNT signaling in Wilms tumor. Despite this, the canonical WNT pathway provides at present the clearest link of a known oncogenic pathway, and a thorough exploration of its potential as therapeutic target is fully warranted.

Targeting the WNT/β-Catenin Pathway

The three major areas of targeting the WNT/β-catenin pathway are represented by the receptor/ligand interactions, cytosolic signaling components, and nuclear signaling components (Fig. 1). Although the number of WNT molecules and their functional redundancy argues against the utility of antibodies directed to any particular WNT, some studies demonstrated that certain tumor models rely heavily on specific WNTs (65). Tumors driven by multiple WNTs could be efficiently treated using a pan-WNT inhibitor, such as soluble ligand binding domain of FZ proteins, which would function like decoy receptors for WNT molecules (65). Therapeutic proteins such as WIF and SFRPs are presently being developed (27, 66). Also, the use of antibodies targeting the WNT receptors FZ, in their highly homologous extracellular cysteine-rich domain, responsible for the WNT–FZ interaction, or antibodies targeting LRP5/6, could be efficient in interfering with WNT signaling (65). A study showed that an antibody specific to Frizzled7 (FZD7) could induce apoptosis in primary tumor cells derived from a subset of Wilms tumors expressing FZD7 on their surface, thereby indicating dependency on the WNT pathway for their survival (67).

Because of the essential role of the DVL protein in the signaling cascade, different compounds targeting the PDZ domain of DVL, responsible for the DVL–FZ interaction, and thereby interfering with signal transduction, have been tested (27, 65, 66). Furthermore, a number of enzymes have been proposed as potential therapeutic targets (reviewed in ref. 68): the acyltransferase porcupine, essential for WNT lipidation and secretion, the
kinases PI4KIIα and PIP5Kβ, involved in phosphatidylinositol phosphorylation, which is required for LRP6 tail phosphorylation; PAR-1, which phosphorylates DVL; and CKIe, which activates the WNT signaling through phosphorylation of DVL and possibly TCF (68). Furthermore, potential druggable enzymes are those affecting the stability of cytoplasmic AXIN, such as Tankyrases 1 and 2 (TNK1/2), which promote the ubiquitination of AXIN, thereby causing its proteasomal degradation (22, 27, 65, 66, 68).

Ultimately, these myriad upstream signaling events converge on β-catenin, leading to its accumulation and nuclear translocation. Despite intense research efforts, progress has been slow in selectively and directly targeting β-catenin, which is likely to represent the ideal downstream target. Upon entering the nucleus, β-catenin displaces the corepressors from TCF/LEF transcription factors and recruits a variety of coactivators, which play critical roles in driving β-catenin-mediated transcription (19). The interactions between β-catenin and TCF and β-catenin/TCF complex and these coactivators are, therefore, potential targets useful to inhibit transactivation (22, 27, 65, 66). Furthermore, some protein kinases could represent additional potential targets for drug intervention. These include the Traf2- and Nck-interacting kinase and the cyclin-dependent kinase 8, which regulate WNT/β-catenin–dependent gene transcription (65, 68).

Further therapeutic opportunities are represented by an indirect targeting of the WNT/β-catenin signaling. Among drugs currently available, nonsteroidal anti-inflammatory drugs (NSAID), the tyrosine kinase inhibitor imatinib, and vitamins, have been demonstrated to modulate the WNT/β-catenin pathway (22, 27, 65, 69); a summary of WNT/β-catenin pathway inhibitors is displayed in Table 1.

### Table 1. Summary of inhibitors against the WNT signaling pathway

<table>
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<tr>
<th>Inhibitors</th>
<th>Therapeutic</th>
<th>Pathway target</th>
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<tr>
<td>Molecular targeted drugs</td>
<td>PNU74654</td>
<td>β-Catenin/TCF</td>
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<td></td>
<td>2,4-Diamino-quinazoline</td>
<td>β-Catenin/TCF</td>
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<td></td>
<td>ICG-001-related analogs</td>
<td>CBP</td>
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<td></td>
<td>IWP⁵</td>
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<td>XAV939</td>
<td>Tankyrase 1 and 2</td>
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<td>Recombinant proteins</td>
<td>WIF and SFRPs</td>
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<td></td>
<td>RNA interference</td>
<td>WNT proteins</td>
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<td>Existing drugs and natural compounds</td>
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<td>Celecoxib</td>
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<td>Vitamins</td>
<td>Retinoids</td>
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<td></td>
<td>1α,25-Dihydroxy-vitamin D₃</td>
<td>β-Catenin</td>
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NOTE: Data compiled from references 22, 27, 65, and 66.

⁴IWR inhibitor of WNT response.

⁵IWP inhibitor of WNT production NT.

By reviewing the current status of our knowledge on the WNT pathway in nephrogenesis and in Wilms tumorigenesis, it is apparent that many aspects remain unanswered. We highlight some of these questions, aiming to inspire future research.

1) What fraction of Wilms tumors depends on the WNT/β-catenin pathway? What surrogate biomarker can be used to identify them?
2) What is the relation between WT1 mutation and β-catenin activation?
3) How many different genetic events in Wilms tumor lead to WNT/β-catenin activation?
4) Why is β-catenin nuclear staining observed almost exclusively in the mesenchymal component of the tumor, although all the cells bear the mutation, as demonstrated by microdissection experiments (9)?
5) What is the importance of the noncanonical WNT pathways in Wilms tumor?
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Considering its deregulation in many human cancers, drugs targeting aberrant activation of the WNT/β-catenin signaling cascade have enormous potential. Furthermore, because of the importance of the WNT pathway in stem and/or progenitor populations, these drugs might be effective in eliminating normal drug resistant CSCs, which are thought to be associated with relapses and metastasis (22, 27, 65, 66, 69). However, we need to take extra care when thinking about drugs for use in pediatric patients. In fact, although the WNT pathway is critical in normal somatic stem cell homeostasis and tissue maintenance, we also have to be aware of its normal function during organogenesis and development because some WNT dependent processes, such as bone development, are still ongoing in the pediatric age group.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References


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