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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 (ENCCA) consortium organized a workshop in Rome, in June 2012, on “Renal Tumor Biology Driven Drug Development” to discuss the current knowledge in pediatric renal cancers and to recommend directions for further research. Wilms tumor (WT) is the most common renal tumor of childhood and represents a success of pediatric oncology, with cure rates of over 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 WT, their heterogeneity and interdependencies; to provide updates on the clinic-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 WT; this review represents the outcome of the workshop discussion on the WNT/β-catenin pathway in Wilms tumorigenesis.

Keywords

Wilms tumor; WNT/β-catenin pathway; WNT/β-catenin pathway modulators; therapeutic strategies

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

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improve standards of care across Europe. The 'Biology Driven Drug Development Renal Tumors Workshop' (Rome, 9-10 June 2012) organized by ENCCA discussed these aspects in the context of Wilms tumor (WT).

WT or nephroblastoma, the most frequent renal tumor of childhood affects ca 1 in 10,000 children before their 15th birthday, with a peak incidence between two and three years of age, and represents one of the successes of pediatric oncology, with an overall cure rate of over 85%. The majority of patients with WT 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) (1).

The ENCCA workshop discussed the following aspects of WT:

- What are the currently known genetic and epigenetic pathways operating in WT?
- How do they relate to the currently applied risk stratification used by the major clinical trial groups?
- How do they relate to the known biological risks of groups, subtypes and molecular biomarkers?
- Can we use knowledge of pathways in kidney development to better define molecular subtypes of WT?
- Can this knowledge help to identify the biological pathways to be targeted for drug development that should be prioritized for early-phase clinical trials in WT; and can it help identifying the patients most likely to respond?

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

1. WT with signs of activation of the WNT/β-catenin pathway (canonical WNT pathway), found in approximately 15-20% of all non-anaplastic WTs.
2. WT with activation of *IGF2* pathway, often with clear evidence of epigenetic aberrations, found in approximately two thirds of all non-anaplastic WTs, with some overlap with WNT/β-catenin pathway deregulated group.
3. WT with mutations in *TP53*, mainly found in anaplastic WT, that comprises about 5-10% of all WTs.

Here we will review the current data on the heterogeneity in WT, 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

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

Prevalent published model proposed that the clinical and histological features of the WTs and associated NRs 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 WT pathogenesis (reviewed in(2, 4, 5)). Further genes linked to WT development include *WTX* on chromosome Xq11.2, *CTNNB1* on 3p22.1, encoding β-catenin, and *TP53* on 17p13.1 (reviewed in (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, WT develops. These WTs 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 WT. These WTs 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 on its involvement in a subset of WTs.

WNT/ β-catenin signaling and cancer

The history of the WNT/β-catenin pathway has 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 MMTV virus (17). In 1987 it was shown that the Drosophila homologue of *Int1* was in fact *wingless* (18), formally linking the wingless pathway to cancer.

WNT proteins (WNTs) 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 Porcupine, 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) (Figure 1). At this level, the activity of WNTs is regulated by negative extracellular regulators: secreted frizzled-related proteins (SFRPs), WNT inhibitory (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) (19). A crucial step is 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 (GSK3 β) and casein kinase 1 (CK1), which require WNT-induced generation of phosphatidylinositol 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 co-activator to stimulate target gene transcription by displacing the transcriptional repressor Groucho from TCF/LEF and recruiting an array of transcriptional co-activators 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 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 proteasomal degradation (Figure 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 (21, 22)). Germline defects of the *APC* gene cause familial adenomatous polyposis (FAP), 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 WT (4, 5, 21, 22). The role of *WTX* mutations, almost exclusively described in WTs, is less established (23-25). Overexpression of WNT ligands, or down-regulation of WNT antagonists (DKK, SFRPs, WIF) have been reported in several human cancers (21, 22).

Activation of the WNT/β-catenin pathway is one of the most important hallmarks of stemness of cancer stem cells (CSCs) (26). Intriguingly, many of the cell surface markers, including LGR5/GPR49, CD44, CD24 and EPCAM, that have been used to identify and isolate putative tumor stem cell populations, 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).

Since 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 a 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 β-catenin in a variety of processes (30, 31). An intrinsic problem in the interpretation of these mutations is the fact that β-catenin not only functions in the WNT signaling pathway but 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 β-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 β-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 β-catenin stability are all found in exon 3, Harada et al 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 β-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 β-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 Wollfian duct, invades the MM (reviewed in (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/β-catenin signaling is necessary for the initiation of nephrogenesis and the initiation of the MET that is required for MM to start to form epithelial tubules that contribute to the formation of the mature nephron. In fact, ectopic activation of β-catenin in kidney mesenchyme is sufficient to induce nephrogenesis, but is incompatible with subsequent epithelialization (38, 39). Activation of the non-canonical WNT pathways calcium/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 β-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 pre-tubular 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 non-canonical 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 non-canonical WNT-PCP pathway (49). β-catenin in its turn is essential for the development of the parietal epithelium (50). Disruption of this signalization might also be involved with WT onset, as suggested by a gene expression study (51). In all, it is clear that WNT signaling, including the β-catenin mediated canonical pathway, plays a central role in kidney development.

Wilms tumors and WNT

As WTs show a direct relationship with abnormal development of the embryonic kidney, it is not surprising to find links between abnormal WNT signaling and WT formation. As anticipated, many lines of evidence point towards this being most important in the *WT1*/ILNR-associated tumors. *WT1* and β-catenin mutations occur in ca. 20% and 15% of WTs, respectively (4), with many studies describing an association between *WT1* and *CTNNB1* mutations in WTs (4-7, 9, 10). Most mutations occurring in the *CTNNB1* gene result in the loss of important regulatory phosphorylation sites, and are associated with the constitutive activation of 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 ca. 20% of WTs (4, 23-25, 52-55). While *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 reported that in *WTX*

mutated WTs, *CTNNB1* mutations are more likely to occur in exon 7, 8 (unlike exon 3 mutations, they do not affect phosphorylatable residues known to be involved in *CTNNB1* stability) (53, 54, 56). By contrast, a negative association with exon 3 *CTNNB1* mutations has been speculated (reviewed in(4)). However, this was not confirmed in a study on more than 400 WTs 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, consisting of 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 is somewhat discordant and the true nature of the association has yet to be established.

A recent study defined five subsets (S1 to S5) of pathologic and clinical features of WT 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 MM, 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 WT and WNT/β-catenin signaling pathway activation was further analyzed in a panel of tumors characterized for WT1, CTNNB1, WTX status, as well as for nuclear accumulation of β-catenin. This showed that WNT/β-catenin pathway activation was restricted to WTs 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 where 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 sub-cellular trafficking remain intact.

To improve the characterization of the molecular mechanisms and the involvement of the WNT/β-catenin pathway in WTs, both the genomic and expression status of *WT1* and β-catenin, 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 β-catenin (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 versus WT-B showed that WNT/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 WTs both with and without WNT pathway activation (52, 53, 56).

Gene expression studies have the intrinsic caveat of assigning WTs to the WNT/β-catenin or non-WNT/β-catenin categories according to annotated lists of WNT target genes, while 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 WTs, 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 WT, this does not appear 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 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 WT. 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 WT 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; non-responding cells remain in the stem/progenitor state (40, 41). *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 WT cases? It is important to realize that genetically 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 WT and the observed Darwinian selection for activating mutations in β-catenin in *WT1*-mutant WT, the function of β-catenin 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 appears 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 WT 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 WT 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) while 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 WT CSCs. These CSCs have very recently been identified as a $NCAM^+$ $ALDH1^+$ population (60). NCAM is found in the cap mesenchyme. It is possible that the $NCAM^+$ population shows overlap with the $SIX2^+$ population, the known nephron progenitors (37), but this remains to be formally proven (61). If this would be the case however, this 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 WT development. The first reproducible WT mouse model has been generated through combined loss of *WT1* and activation of *Igf2* (62). These two events separately were not sufficient for WT formation. This model corresponds to a subset of WT cases (Subset S3) (56). So far there have been no published reports on WT mouse models based on activated β-catenin. Conditional loss of *Apc* in the developing kidney was found to result in renal carcinomas but not WT (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 WT development. The wide variation in phenotypes found in different *CTNNB1* and *Apc* mouse models suggest some care should

still be taken in interpreting the role of *CTNNB1* mutations in human WT onset. The selection for *CTNNB1* Ser45 mutations in *WT1*-mutant WT 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 WT, 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 non-canonical WNT PCP pathway (64). This provides another twist to the role of WNT signaling in WT. 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 (Figure 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 extra-cellular 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 WTs expressing FZD7 on their surface, thereby indicating dependency on the WNT pathway for their survival (67).

Due to 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 (68): the acyltransferase Porcupine, essential for WNT lipidation and secretion, the kinases PI4KIIα and PIP5KIβ, involved in phosphatidylinositol phosphorylation, which is required for LRP6 tail phosphorylation; PAR-1 which phosphorylates DVL, and CKIε which activates the WNT signaling through phosphorylation of DVL and possibly TCF (68). Further 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 co-repressors from TCF/LEF transcription factors and recruits a variety of co-activators, 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 co-activators 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 (TNIK) and the cyclin dependent kinase 8 (CDK8), 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 (NSAIDs), the tyrosine kinase inhibitor Imatinib, and vitamins, have been demonstrated to modulate the WNT/β-catenin pathway (22, 27, 65, 69) summary of WNT/ β-catenin pathway inhibitors is displayed in Table 1.

Final remarks and remaining open questions

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 WTs 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 WT 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 non-canonical WNT pathways in WT?

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, while 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, since some WNT dependent processes, such as bone development, are still on-going in the pediatric age group.

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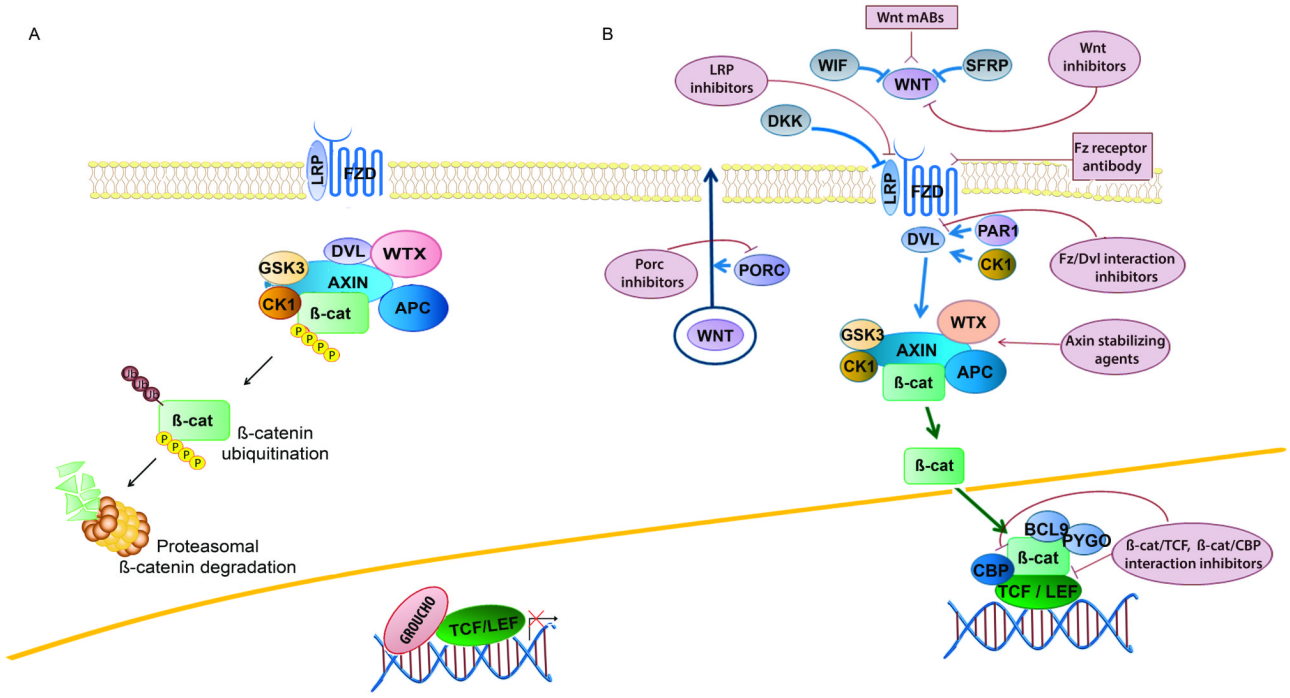

Figure 1.

Canonical WNT/β-catenin signaling and inhibitors. (A) WNT—off state: In the absence of WNT ligands, the destruction complex (containing Axin, APC, WTX, GSK3 and CKI) promotes N-terminal phosphorylation of β-catenin. This leads to ubiquitin-mediated proteasomal degradation of β-catenin and keeps intracellular levels low. Meanwhile, TCF/LEF type transcription factors recruit Groucho and histone deacetylases to repress WNT target genes. (B) WNT—on state: once WNT ligands bind to FZ/LRP6 co-receptors, the cytoplasmic tail of LRP6 is phosphorylated, the β-catenin destruction complex is inhibited through recruitment of its components to the FZ/LRP/DVL complex. Consequently, β-catenin accumulates intracellularly, translocates to the nucleus, and displaces Groucho from TCF/LEF. This interaction promotes the transcription of WNT target genes. Inhibitors of the WNT/β-catenin pathway (depicted in red) are shown and include: 1) agents targeting WNTs: antibodies against WNTs; soluble WNT receptors; proteins acting like WNT inhibitory proteins (WIF) and secreted frizzled related proteins (SFRPs); 2) FZ receptor antibody; 3) LRP inhibitors, acting like the members of the Dickkopf (DKK) family; 4) compounds targeting the PDZ domain of Dishevelled (DVL), responsible of DVL/FZ interaction and signal transduction; 5) molecules which stabilize the Axin protein; 6) molecules that inhibit Porcupine (Porc), an enzyme essential for WNT lipidation and secretion; 7) β-catenin/TCF and β-catenin/CPB interaction antagonists

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Table 1

Summary of inhibitors against WNT signaling pathway.

Inhibitors	Subcategory	Therapeutic	Pathway target
Molecular targeted drugs		PNU74654	β-catenin/TCF
		2,4-diamino-quinazoline	β-catenin/TCF
		ICG-001-related analogs	CBP
		NSC668036	Dvl
		FJ9	Dvl
		3289-8625	Dvl
		IWR*	Axin
		IWP**	Porcupine
		XAV939	Tankyrase 1 and 2
Biologics		Antibodies	WNT proteins
		Antibodies	FZ receptors
		Recombinant proteins	WIF and SFRPs
		RNA interference	WNT proteins
Existing drugs and natural compounds	NSAIDs	Aspirin	β-catenin
		Sulindac	β-catenin
		Celecoxib	TCF
	Vitamins	Retinoids	β-catenin
		1α,25-dihydroxy-vitamin D3	β-catenin

* IWR inhibitor of WNT response

** IWP inhibitor of WNT production NT

Data compiled from references (22, 27, 65, 66).