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Review article

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WNT SIGNALING PATHWAY IN DEVELOPMENT AND CANCER

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Wnt signaling pathway is one of the most important signaling pathways. The complexity of Wnt signals and their functional role is crucial in development and growth. It is the most active during embryogenesis facilitating new organism formation by cell differentiation, polarization and migration. Its activation is also common during development of many tumors and others diseases. In this review we shortly describe a role of Wnt pathway in development in order to better understand its role in cancer progression. We also describe current anti-cancer therapies targeting Wnt pathway.

Key words: *Wnt signaling pathway, cancer progression, β-catenin, epithelial-mesenchymal transition, planar cell polarity, tumor associated macrophages*

INTRODUCTION

Almost 50 years ago scientists discovered wingless gene in Drosophila melanogaster. Functional genetic studies revealed its role in developmental patterns (1). For example Wnt signaling pathway is associated with cell differentiation, polarization and migration during development. Therefore, its involvement in cancer biology was also expected.

The main component of the Wnt signaling pathway is the family of the Wnt proteins activating cell membrane receptors in paracrine and autocrine manner. Wnt proteins secreted by cells can induce cellular mechanisms by activation of Fzd (Frizzled) membrane proteins and through intracellular proteins and transcription factors regulate gene expression. The structure of Wnt pathway is based on three signaling pathways: canonical β-catenin dependent, and two β-catenin independent, non-canonical Wnt signaling pathways: PCP (planar cell polarity) signaling pathway and Ca^{2+} Wnt pathway.

Although Wnt signaling pathway disorders are best known in cancer progression, they can be a cause of other diseases development, for example Alzheimer's disease and bone abnormalities (2). In this review we describe the role of Wnt signaling pathway in development to understand its role in cancer. We also briefly point out anti-cancer therapies targeting Wnt proteins.

WNT/β-CATENIN SIGNALING

Structure

The structure of Wnt/β-catenin signaling also known as canonical Wnt pathway can be grouped as three main components, the membrane proteins, degradation complex and β-catenin protein (*Fig. 1*). In the cell membrane there are localized Frizzled (Fzd) receptors for Wnt proteins. Next to Fzd receptors are localized ligands belonging to low-density lipoprotein receptor-related protein group (LRP) which are encoded by the *lrp5* and *lrp6* genes (3, 4). The destruction complex is formed from adenomatous polyposis coli (APC), axin, glycogen synthase kinase 3 (Gsk3), casein kinase 1α (CK1α) and Dishevelled (Dvl) proteins (5). When Wnt/β-catenin pathway is inactive the destruction complex is formed around β-catenin protein to which ubiquitin particles are attached. In this process axin and APC are phosphorylated by CK1α and Gsk3 kinases. In the case when Wnt/β-catenin pathway is active CK1α and Gsk3 through Dishevelled are bounded to β-catenin enabling catalyzation of phosphorylation reaction (5).

Mechanism

Activation of trans-membrane complex composed of Fzd receptor and LRP5 or LRP6 co-receptor mediates β-catenin dependent canonical Wnt pathway. Stimulation of Fzd receptor *via* Wnt ligands for other co-receptors results in a non-canonical Wnt pathways activation (3, 4). The β-catenin is a protein involved in gene transcription. When canonical Wnt pathway is activated, β-catenin translocates into nucleus and acts as a transcription factor. However, when Wnt pathway is not active, β-catenin protein is actively degraded (*Fig. 1*). The intracellular level of β-catenin is regulated by canonical Wnt pathway. Stimulation of Fzd-LRP5/6 complex results in upregulation of the level of this protein. In case of Fzd-LRP ligands absence, the level of cytoplasmic β-catenin is low. Its degradation is ensured by the destruction complex (5). Activation of Fzd-Lrp5/6 complex by Wnt ligand, Dishevelled (Dvl) leads to inhibition of the degradation complex formation. The Dvl binding to Fzd results in exposure of Dvl's DIX domains, which are binding sites for axin. Besides, axin binds to intracellular tail of the LRP. Once bound to the receptor complex, axin is unable to bind β-catenin, furthermore - it inhibits Gsk3 activity. The effect of this

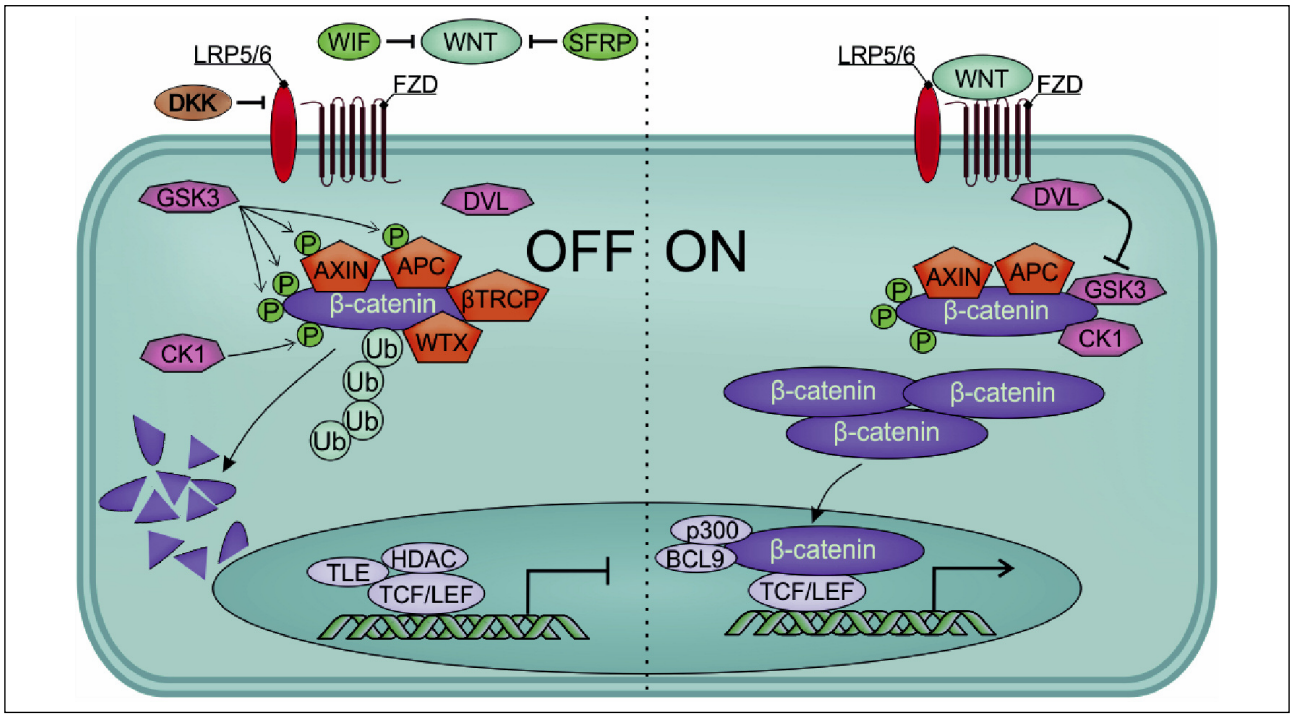

Fig. 1. Graphical illustration of mechanism of action of canonical Wnt pathway. Wnt pathway is inactive (OFF) when Wnt ligands are unbound to its receptor Frizzled (FZD). It can be inhibited by WIF, SFRP and DKK extracellular proteins. This situation results in β-catenin ubiquitination (Ub) and proteosomal degradation. Degradation is possible due to degradation complex formation (AXIN, APC, βTRCP, WTX) and functional phosphorylation of proteins building this complex by CK1 and GSK3 kinases. After Wnt proteins are bound to Frizzled (ON) and are in close neighborhood of co-receptor LRP, dishevelled (DVL) protein is bound resulting in failure of degradation of the complex formation. β-catenin is accumulated in cytoplasm and translocated to the nucleus to act as transcription factor and induce the gene expression.

cascade reaction prevents phosphorylation of β-catenin, its ubiquitination and proteasome degradation. Newly synthesized β-catenin accumulates in the cytoplasm eventually translocating into the nucleus. Nuclear β-catenin then replaces a repressor of T-cell factor/lymphoid enhancer factor (TCF/LEF) which are transcription factors activating Wnt-responsive genes. Besides, interaction of β-catenin and TCF/LEF recruits transcriptional co-activators and histone modifiers such as the ATP-dependent helicase Brahma-related gene 1 (BRG1, also known as SMARCA4), cyclic adenosine monophosphate response element, CREB-binding protein (CBP), p300, B-cell lymphoma 9 (BCL9) and pygopus (6) (*Fig. 1*).

Mode of action in development

Wnt/β-catenin signaling is an evolutionary conserved system that plays crucial role in embryogenesis, organogenesis and homeostasis. This pathway developed early in phylogenetics. It is common to all metazoan life forms and shares the same genes among such different species as human and *Drosophila* fly. The Wnt/β-catenin signaling is known as an important regulatory pathway that governs developmental process and the fate choices during tissue morphogenesis (7-9). The main role of canonical Wnt pathway is to provide symmetry-breaking signal common to all metazoan life forms by simultaneously regulating cell fate, whereas non-canonical signaling is responsible for cell polarity.

Canonical Wnt pathway plays a very important role starting from early embryogenesis. During gastrulation β-catenin promotes primitive streak formation in the posterior part of embryo. It may also function in a ligand-independent fashion to orient distal visceral endoderm anteriorly. Mutation of Wnt3 gene, which is a ligand of LRP5/6, disrupts gastrulation by blocking primitive streak and consequently mesoderm and definitive endoderm formation. Another evidence is that Lrp5/6 double mutants, as well as classical mouse mutant of the gene encoding Mesd (a specific chaperone for Lrp5 and 6) displays very similar patterning defects as the Wnt3 mutants. In summary - while early embryogenesis, Wnt/β-catenin signaling pathway regulates cell fate of embryonic endodermal cells that provides desired organogenesis (10).

Mode of action in cancer

Although canonical Wnt pathway plays an important role during development, it should be consequently downregulated in differentiated cells. Increased expression of β-catenin may be caused by the following factors: mutations in β-catenin gene, abnormalities in the β-catenin destruction complex, mutations in APC, overexpression of Wnt ligands, and loss of inhibition or decreased activity of regulatory pathways. The Wnt/β-catenin signaling is activated in many types of cancer.

Abnormal activation of the Wnt/β-catenin pathway has been reported as one of the predisposing factors in many cancer types. For example in cutaneus melanoma, nuclear β-catenin binds to TCF/LEF-type transcription factors (*Fig. 1*) and consequently stimulates expression of downstream genes, such as *cyclin D1* and *c-MYC*. Overexpression of mentioned genes alter cell cycle progression and contribute to tumorigenesis (11).

Epithelial-mesenchymal transition (EMT) is the process by which polarized epithelial cells acquire mesenchymal phenotype. This process can be induced by several signaling pathways including the Wnt/β-catenin. In this type of tumor, the upregulation of canonical Wnt pathway is caused by

overexpression of factor named Tiam1 - T lymphoma invasion and metastasis 1. Besides, increased Tiam1 expression is associated with metastasis in several cancers including colorectal, breast, prostate, lung cancer, renal cell carcinoma and hepatocellular carcinoma. Tiam1 acts on Wnt/β-catenin pathway indirectly: it activates Rac1 - a member of the Rho GTPase family, which subsequently modulates activity of the β-catenin/TCF complexes at Wnt-responsive promoters, enhancing target genes transcription (12). Korbut and co-workers paid attention on correlation between cancer stem cells, EMT process and Wnt/β-catenin signaling. It was proven that growth of primary tumors converting non-tumorigenic cells into cancer stem cells by processes related to the EMT. Numerous experiments confirmed that target genes of WNT signaling are implicated in cell-adhesion, which in consequence has an impact on EMT. This suggests a model that integrates a number of fundamental processes that underlie disease development and it should be put forward as an important target for novel therapies (13).

Downstream targets of β-catenin/TCF include genes expressing such factors as c-myc, cyclin D1, MMP-7, LGR5, uPAR, connexin 42, CD44, AF17, ENC1, laminin-5γa2, PPAR-δ, claudin-1, MT1-MMP. There have been detected 53 genes, whose expression was enhanced more than two-fold in response to the suppression of the β-catenin/TCF7L2 complex. Although genes upregulated by Wnt/β-catenin pathway have been intensively studied, the role of downregulated genes have not been fully understood. Many of those genes were found to be significantly associated with interferon signaling, suggesting that Wnt signaling might affect interferon-mediated immune responses. Among the genes, expression of IFIT2 - a gene encoding interferon-induced protein, was significantly lower in colonorectal cancer tissues than in normal tissues. Four members of IFIT family play a crucial role in the host antiviral defense. Interestingly, IFIT1 and IFIT2 in association with MITA (mediator of IRF3 activation) induce apoptosis *via* the mitochondrial pathway. Moreover, IFIT2 elicits apoptotic cell death independent of IFN stimulation. Since activation of the β-catenin/TCF7L2 complex is a common feature observed in colonorectal cancer cells, the expression levels of IFIT1 and IFIT2 were significantly downregulated compared to normal tissues. It has been proven, that β-catenin/TCF7L2 complex functions as a transcription downregulator of IFIT1 and IFIT2, however through unknown mechanism. In this way inhibition of apoptosis may confer pro-survival properties to cancer cells (14). Pajak *et al.* found positive correlation between β-catenin inhibition and decreased expression of secretory clusterin in human colon adenocarcinoma cells COLO 205. Epigallocatechin-3-gallate an important bioactive constituent of green tea extract which was widely believed to reduce proliferation of many cancer cell lines but dose-dependently increase COLO 205 cells viability and proliferation. Epigallocatechin-3-gallate stimulated secretory clusterin expression level, which underwent complex control through lipid rafts/PKC/Wnt/β-catenin pathway. Inhibition of β-catenin pathway significantly reduced secretory clusterin expression as well as cells viability and proliferation, reducing pro-tumour effect of epigallocatechin-3-gallate (15).

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide. It has been confirmed, that in highly malignant HCC there is upregulation of RPS15A - ribosomal protein s15a which plays a promotive role in the mRNA/ribosome interactions during early transcription. Recent studies indicated that RPS15A induced angiogenesis - tumor tissue with its overexpression demonstrated a higher microvascular density. The angiogenesis was associated with FGF signaling, especially FGF18, which secreted in the HCC microenvironment acts on FGFR3 receptor on the endothelial cell membrane. FGF18 activates downstream signaling phospho-AKT and phospho-ERK, increasing the angiogenic potency of a tumor. It has been proven, that Wnt/β-catenin pathway ligand - Wnt3a, increased level of RPS15A. To sum up: RPS15A mediates the nuclear transcription of FGF18 induced by the β-catenin activation (16). Another studies demonstrated that the Wnt/β-catenin pathway regulated protein expression of other angiogenic factors: MMP-2, MMP-9, VEGF-A, VEGF-C, bFGF in HCC cells (17). There is a study indicating that the inhibition of Wnt/β-catenin pathway could suppress migration and invasion ability of HCC cells, promote apoptosis and improve the efficacy of TACE - transcatheter arterial chemoembolization in rat model (18).

In breast cancer knockdown of ALX4 gene recovered cell proliferation, migration and invasion. Therefore, ALX4 is considered as an anti-tumor factor. It interrupts Wnt/β-catenin signaling. ALX reduces the protein level of β-catenin by promoting its phosphorylation *via* upregulating GSK3β, which subsequently results in its ubiquitination and proteasomal degradation. Lack of this mechanism causes expression of Wnt target genes (19).

In gliomas, PPAR gamma (peroxisome proliferator-activated receptor gamma) agonists inhibit cell proliferation by induction of cell-cycle arrest in G0/G1 phase. These two systems - Wnt/β-catenin and PPAR gamma act in an opposite manner. In several cellular systems β-catenin expression is inhibited by PPAR gamma agonists. This phenomenon may indicate, that decreased expression of PPAR gamma in glial tissue may directly lead to carcinogenesis, and indirectly - by weakening the inhibition of Wnt/β-catenin pathway (20).

WNT/PCP SIGNALING

Structure

The structure of Wnt planar cell polarity PCP signaling pathway is also based on transmembrane receptors Fzd and Wnt proteins. However, there is no involvement of the β-catenin (*Fig. 2*). The core PCP machinery consists of six proteins. Three out of six of so called ‘core module’ factors are transmembrane components: Frizzled (Fzd) (21), Vangl (also known as Strabismus (Stbm), Vang-like (Vangl) in vertebrates) (22) and Flamingo (Fmi, also known as Stan, Celsr in vertebrates) (21, 22). Recent studies, aimed to asses precise molecular structure of the Wnt/PCP pathway have shown, that Vangl side of the core complex consists of six Vangl molecules per each Fmi (25). The Wnt/PCP complex is composed of three cytoplasmic proteins: Dishevelled (Dsh; Dishevelled-like (Dvl) in vertebrates) (26), Prickle (Pk) (27), and Diego (Dgo; known as Inversin and Diversin in vertebrates) (28-30). The second group of Wnt/PCP factors consists of the Fat/Dachsous/four-jointed (Ft/Ds/Fj) group (also known as global module), which so far has not been as well studied as the core module (31, 32). All these molecules activate the final cytoplasmic effector molecules: small Rho GTPases (RhoA), c-Jun N-terminal kinase (JNK), and nemo-like kinase (NLK) (33-38).

Mechanism

Planar cell polarity (PCP) is a pathway driving tissue patterning organization and morphogenesis. This is an important feature of epithelia, which are defined by their apical-basal polarization and polarization within the plane of epithelium. The same pathway can also regulate polarity in nonepithelial cells during cell migration within tissues (39-41). A characteristic

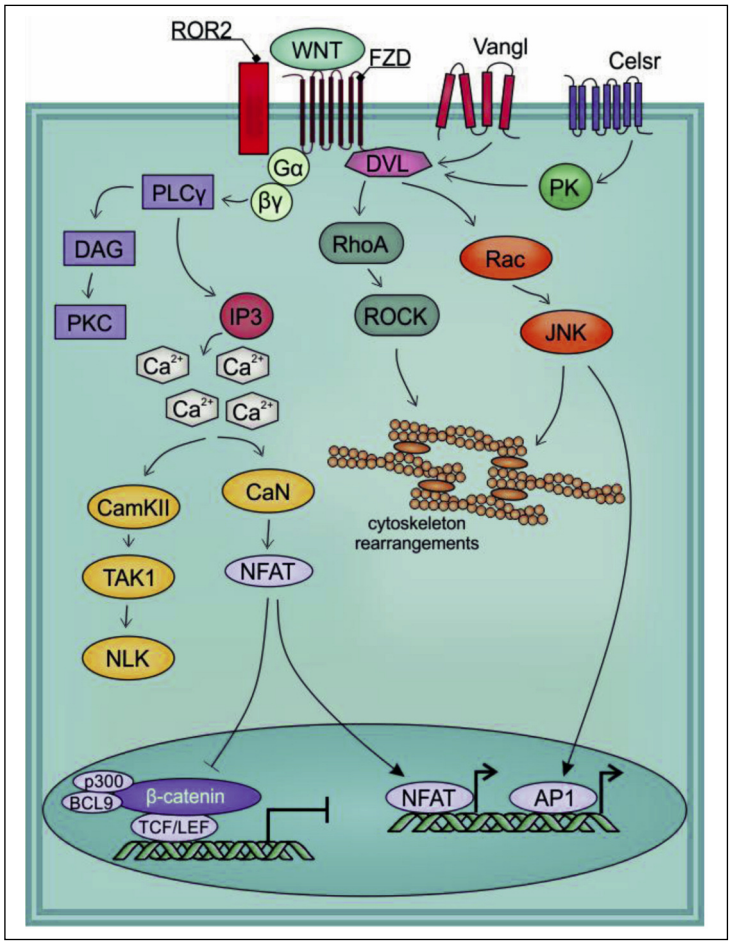

Fig. 2. Graphical illustration of mechanism of action of the non-canonical Wnt pathway. Both non-canonical Wnt pathways (PCP and calcium) are activated by Wnt proteins binding to Frizzled receptor and disheveled, however close neighborhood of ROR2 co-receptor, Vangl and Celsr is also crucial. The signal then is translocated by G proteins (Gα, Gβγ) to phospholipase C and finally to inositol trisphosphate (IP3) what results in calcium release. Increased Ca^{2+} level activates down-stream kinases and transcription factors such as NFAT which are positive regulators of non-canonical target gene expression and negative regulators of expression of β-catenin controlled genes. The PCP pathway after Wnt activation is also responsible for gene expression regulation but also for cell cytoskeleton remodeling together with ROCK and JNK kinases.

feature of Wnt/PCP is the asymmetric localization of its components that translate a global directional information into a polarized downstream result. Most of the molecular details of the pathway has been discovered and studied in *Drosophila*, but both the components and mechanisms of signaling are evolutionary conserved in vertebrates (42, 43). Wnt/PCP has important functions in a broad array of developmental and physiological cases in vertebrates, like: convergent extension during antero-posterior axis elongation, placement of motile and sensory cilia in cells, and polarization of skin and hair follicles (44-46).

Proteins belonging to core PCP machinery (Fzd, Vangl, Fmi, Dsh, Pk and Dgo) interact with each other in both inter- and intracellular ways. These interactions and the fact that these proteins are divided into two asymmetric PCP complexes in both adjacent cells, give the cell a planar orientation axis. Asymmetric localization of core components create two sub-complexes constituted of Fmi-Fzd-Dsh-Dgo and Fmi-Vangl-Pk located in opposite sides of the same cell. The core module creates intracellular connection through Fmi homophillic adhesions, which are aided by Fzd and Vangl proteins. This combination is required to spread polarity across the tissues (30, 47). Cytoplasmic proteins Dsh, Dgo and Pk take part both in positive and negative interactions, which help to stabilize the connections between cells. The second group of Wnt/PCP factors Ft/Ds/Fj provides additional information for the core Wnt/PCP to correctly orient local polarity to fit it into the tissue axes. Both Ds and Fj are expressed in oppositely oriented gradients in each of the studied tissue and body compartment (48). The expression gradients of Ds and Fj provide directional information, which is then translated into oriented cellular asymmetries that are at the end interpreted and used by downstream mechanisms. The final effectors of the global module, the protocadherins Ft and Ds form asymmetric structures that may orient in one of two directions at any apical cell-cell boundary. The transmembrane kinase Fj (48, 49) phosphorylates extracellular domains of Ds and Ft and modulates their interactions (50, 51).

Essentially, one of the most important features of Wnt/PCP is the asymmetric localization of components of both core module and global module, that translates a global directional cue into a polarized downstream output, which results in activation of small Rho GTPases (RhoA), c-Jun N-terminal kinase (JNK), and nemo-like kinase (NLK) (33-38).

Mode of action in development

As mentioned above the key aspect of the Wnt/PCP signaling is asymmetrical arrangement in the cells. The asymmetrical localization of receptors and others Wnt/PCP components is information for cytoskeleton rearrangement inside the cells and to follow this cell movement and directional growth (52). Proper function of complex organism is possible only when the polarity of each cell in a tissue is established, which allows settlement of proper tissue geometry during morphogenesis (53). In tissues during morphogenesis, signaling centers send out a polarizing signal. Cells respond to these signals and establish polarity through the Wnt/PCP core module (54) and Wnt protein gradients (33, 55, 56). If cells in the developing embryo were not polarized, they would develop into symmetric sphere made of different type of cells. Morphogenes, such as Wnts form a concentration gradient along cells in tissue. This gradient allows cells to differentiate according to local threshold concentrations of Wnt

proteins. Morphogen concentrations give quantitative information to generate a specific cell pattern (57).

Some examples of processes, which are regulated by Wnt/PCP signaling are: elongating anteroposterior body axis, orientations of Drosophila hair, fur in mice, sensory hair cells in inner ear and closing neural tubes. Defects in Wnt/PCP signaling have been associated with many developmental anomalies and diseases including open neural tube defects (58-60), polycystic kidneys (58, 61), heart defects (62), deafness (60), and *situs inversus*, also known as Kartagener's syndrome (63).

Table 1. Summary of drugs targeting Wnt pathway in clinical trials.

Drug	Mechanism of action	Condition or disease	Clinicaltrials.gov identifier	Phase	Clinical outcome
LGK974 (WNT974)	PORCN inhibitor	Solid malignancies	NCT01351103	Phase I: ongoing	Results from 68 patients showed manageable safety profile and potential for antitumor activity.
		Colorectal cancer	NCT02278133	Phase Ib/II: completed	Results not yet reported.
ETC-159 (ETC-1922159)	PORCN inhibitor	Solid malignancies	NCT02521844	Phase Ia/b: recruiting	Study open for recruitment.
Cirmtuzumab (UC-961)	Monoclonal antibody targeting ROR1	Chronic lymphotic leukemia	NCT02222688	Phase I: ongoing	Drug was well tolerated and had a long half-life. Patients had sustained stabilization of disease and a long progression-free survival.
		B-cell lymphoid malignancies	NCT03088878	Phase I: recruiting	Study open for recruitment.
		Breast cancer	NCT02776917	Phase I: not yet recruiting	Not yet recruiting.
OTSA 101-DTPA-90Y	Monoclonal antibody targeting FZD10	Synovial sarcoma	NCT01469975	Phase I: terminated (too slow accrual.)	Results from 16 patients: death occurred in 2/7 treated and in 6/7 untreated patients.
Vanituctumab (OMP-18R5)	Monoclonal antibody targeting frizzled receptors	Solid tumors	NCT01345201	Phase I: completed	Drug was well tolerated up to 2.5 mg/kg q3w and observed bone toxicity appeared manageable and reversible.
		Pancreatic cancer	NCT02005315	Phase I: completed	Drug was well tolerated in combination with nab-paclitaxel and gemcitabine (bone toxicity encountered).
		Breast cancer	NCT01973309	Phase I: completed	Drug was well tolerated in combination with paclitaxel (bone toxicity encountered).
		Non-small cell lung cancer	NCT01957007	Phase I: completed	Results not yet reported.
Ipafricept (OMP-54F28)	Fusion protein binding ligand of Fzd8 receptor	Hepatocellular cancer	NCT02069145	Phase I: completed	Results not yet reported.
		Ovarian cancer	NCT02092363	Phase I: completed	Results from 17 patients showed good safety profile in combination with paclitaxel and carboplatin (bone toxicity encountered).
		Pancreatic cancer	NCT02050178	Phase I: active, not recruiting	Study still ongoing.
		Solid tumors	NCT01608867	Phase I: completed	Drug was well tolerated and 20 mg/kg dose was established.
Niclosamide	Inhibitor of Wnt/β-catenin signalling	Colon cancer	NCT02687009	Phase I: recruiting	Study open for recruitment.
DKN-01	Monoclonal antibody targeting DKK1	Multiple myeloma	NCT01711671	Phase I: completed	Drug was well tolerated in 7/8 patients and the overall response rate was 57.1% (4/7).
		Non-small cell lung cancer	NCT01457417	Phase I: completed	DKN-01 was well tolerated and demonstrated clinical activity in non-small cell lung cancer.

		Esophagogastric malignancies	NCT02013154	Phase I: recruiting	Study open for recruitment.
		Advanced biliary cancer	NCT02375880	Phase I: active, not recruiting	Study still ongoing.
		Ovarian cancer	NCT03395080	Phase II: recruiting	Study open for recruitment.
BHQ880	Monoclonal antibody targeting DKK1	Multiple myeloma	NCT00741377	Phase Ib/II: completed	Drug was well tolerated and demonstrated potential clinical activity
		High risk smoldering multiple myeloma	NCT01302886	Phase II: completed	No significant anti-myeloma effect was observed; BHQ880 showed anabolic bone activity in 4/5 patients.
		Multiple myeloma	NCT01337752	Phase II: completed	Results not yet reported.

Mode of action in cancer

Correlation between up-regulation of PCP components along with several Wnt ligands and poor outcome has been shown in many different cancer types (64-66). On the other hand, tumor suppressive function of Wnt/PCP has also been described (67). It is presumed that Wnt ligands are able to activate different pathways depending on down-regulation of canonical signaling, context and interactions between different Wnt complexes. The Wnt/PCP influence on cancer progression is very complex and depends on cancer type and stage of its development (68).

The most important role of Wnt/PCP pathway in tumor development is its influence on cancer metastasis (69). In breast cancer fibroblast-derived exosomes can promote Wnt11/PCP signaling to increase invasive behavior (70). Asymmetry of PCP components is observed within cancer cells with Fzd complexes gathering in cell protrusions, and Vangl complexes localizing along the non-protrusive cell areas (70). On the other hand, Wnt/PCP plays different role in cancer cells than during morphogenesis. In tumors it gives no directional cues allowing cytoskeletal remodeling. Lately a new Prickle1-Rictor complex has been discovered which is responsible for regulation of focal adhesions and cancer cell migration. Disruption of this complex resulted in a heavy impairment of breast cancer cells dissemination and Prickle1-Rictor upregulation in basal breast cancers giving worse metastasis-free survival (69, 71). The Fzd-Dvl complex influences Prickle1-Rictor complex *via* cooperation with Smurf2, which can ubiquitinate and degrade Pk1. The Pk1 can also mediate down-regulation of RhoA activity, which influences protrusive activity of the cell, and therefore promotes cell migration (71). Also worth mentioning is the correlation of noncanonical Wnt pathway, pro-inflammatory cytokines and EMT process. Wnt pathway, especially noncanonical part is highly associated with epithelial-mesenchymal transition. The similar role was discovered for pro-inflammatory cytokine IL-8 which can trigger EMT process *via* several cellular pathways. One of them is Wnt signaling pathway which can be activated by overexpression of IL-8 (72). The positive correlation of high expression of IL-8 and subsequently Wnt pathway what lead to EMT process and finally to metastasis was proven in many cancer types (73).

WNT/CA^{2+} SIGNALING

Structure

Non-canonical calcium-dependent Wnt pathway is β-catenin independent, however Fzd and Wnt proteins are strongly involved in its signaling. The molecular significance is to control gene expression by intracellular deposit of calcium. In Wnt calcium signaling pathway, the Fzd protein is connected with trimeric G-protein that translocates signal to phospholipase C (PLC) and to main components of Wnt calcium pathway belonging to calcium related proteins: 1,2 diacylglycerol (DAG), inositol 1,4,5-triphosphate (IP3) and calcium calmodulin dependent protein kinase II (CaMKII). The final effectors of Wnt calcium pathway molecules are transcription factors, such as: nuclear factor associated with T cells (NFAT), NFκB and CREB (*Fig. 2*).

Mechanism

The molecular mechanism of calcium Wnt pathway is slightly different than PCP pathway because the key aspect is a regulation of intracellular level of calcium. After Wnt protein binding to Frizzled receptor the signal is translocated to Disheveled and G proteins. In this pathway transmembrane located co-receptor, ROR2 kinase inhibits canonical pathway as a consequence of Wnt5a binding (74). As it has been shown on *Fig. 2*, signal from G protein is translocated by phospholipase C leading to increase in concentration of signaling molecules: DAG, IP3 and Ca^{2+}. Calcium ions subsequently activate CaMKII, similarly as DAG activates protein kinase C (PKC) (75). CaMKII and PKC activate transcription factors NFκB and CREB. Calcium ions mobilized by IP3 can activate protein phosphatase calcinurin (CaN) that activates cytoplasmic protein NFAT . Activated NFAT may upregulate the expression of several genes in neurons, cardiac and skeletal muscle cells, and pro-inflammatory genes in lymphocytes (76, 77).

Mode of action in development

The biological role of Wnt/Ca^{2+} signaling is the least known among all Wnt signaling pathways family, but its impact on development is remarkable. When Wnt11 or Wnt5 levels are low, the intracellular calcium signaling also dramatically decreases (78, 79). Study conducted on *Xenopus* and *Zebrafish* embryos showed high impact of Wnt5 and Fzd5 on body axis formation, where both Wnt5 and Fzd5 are necessary for proper formation of secondary body axis (80). Expression of calmodulin dependent protein kinase II (CaMKII) in the blastula of *Xenopus* embryo is dorso-ventral dependent with higher expression on ventral side. It has been proven that when CaMKII is down-regulated on the ventral side, dorsal cell fate is prominent, so calcium Wnt signaling pathway promotes ventral cell fate during embryogenesis (81).

Mode of action in cancer

Depending on the cell type and current receptor availability, Wnt5a serves as a proto-oncogene or a tumor suppressor gene

(82). Abnormal expression of Wnt5a does not always directly lead to a loss of function, gain of function, gene amplification or rearrangement (83). The Wnt5a acts as a proto-oncogene in breast cancer (84), melanoma (66), prostate cancer (85) and pancreatic cancer (86). On the other hand tumor suppressing function of Wnt5a has been found in breast cancer (87), colon carcinoma (88), thyroid carcinoma (89), acute lymphoblastic lymphoma (90), acute myeloid lymphoma (91), esophageal squamous cell carcinoma (92) and neuroblastoma (93).

Wnt/β-catenin signaling pathway is up-regulated in many cancers. However, prognosis is often correlated with simultaneous down-regulation of Wnt/Ca^{2+} signaling (94).

One of the most interesting examples of tumor suppressing role of the Wnt5a gene was found during experiments on murine model of basal cell carcinoma (BCC) (95). The BCC develops in a knock-out mutant mouse with a persistently active hedgehog signaling pathway,. however activation of the Wnt5a signaling cascade could be therefore important for tumor regression, and could inhibit hedgehog signaling.

It was noted, that Wnt5a expression is required for proper embryonic development, serving as a proto-oncogene (96). In knock-out mouse deprived of Wnt5a gene function, multiple developmental abnormalities have been observed. However, in melanoma with upregulated Wnt5a signaling, invasiveness, increased cell motility and change in cell morphology were observed. These properties were mediated by the PCK showing its relation with calcium signaling (84, 97). In prostate cancer cell lines up-regulation of Wnt5a was caused due to hypomethylation of the Wnt5a promoter region (98). These modified cancer cells are more motile and invasive, they also have different morphology. Researchers found, that these changes are regulated by Ca^{2+}/CaMKII.

MODULATION OF WNT SIGNALING BY IMMUNE CELLS

Impaired Wnt pathway in immune and cancer cells mainly plays a pro-tumor role. In melanoma a correlation between high β-catenin activity and low anti-tumor respond of T-cells and decreased infiltration of dendritic cells have been reported (99). Moreover, increased IL-12 production due to β-catenin pathway activation in melanoma cells impairs dendritic cell maturation (100).

Tumor associated macrophages (TAMs) can influence cancer cells by modulating Wnt pathway. Macrophages in murine mammary cancer secrete Wnt-5a, which induces MMP-7 expression in cancer cells and facilitates invasion (84). Ojalvo *et al.* showed that TAMs enhances vasculogenesis by secreting Wnt-7b (101). Our previous microarray analyses showed increased *Wnt-5b*, *Wnt-7a*, and *Wnt-7b* expression in macrophages due to co-culture with cancer cells (102). We have also showed effect of TAMs on canine mammary cancer stem-like cells enhancing their pro-angiogenic properties and modulating Wnt/β-catenin signaling (103).

Canine mammary cancer study revealed that TAMs can regulate Wnt pathway in tumor cells. As mentioned before, canonical Wnt pathway is frequently up-regulated in cancer cells resulting in uncontrolled cells divisions, tumor growth and increased inflammation. Biological role of macrophages is to stop this process by secreting Wnt proteins to inhibit canonical pathway. Proteins upregulated in macrophages, such as Wnt-5a and Wnt-2a belong to canonical pathway inhibitors and non-canonical activators. Macrophages effectively limit divisions of cancer cells by inhibition of canonical Wnt pathway but they also increase activity of non-canonical Wnt pathway. Increased activity of β-catenin independent signals in cancer cells results in their differentiation, polarization and finally separation from tumor by epithelial-mesenchymal transition with subsequent metastasis (104).

Yeo and co-workers found the crucial role of Wnt7b in breast cancer angiogenic switch and metastasis. They found high expression of Wnt7b both in cancer cells TAM. After knock-down of Wnt7b in myeloid cells expression of Wnt/β-catenin pathway genes in tumor remained almost stable. But lack of Wnt7b expression in myeloid cells resulted in breakdown of angiogenic switch. They observed also decreased tumor lung metastasis mediated by TAMs. These results suggest high impact of Wnt pathway regulation mediated by myeloid cells on angiogenesis and metastasis of breast cancer cells (105).

Neutrophils and macrophages are known for their pro-metastasis role in tumors. *In vitro* study on cancer cells found that the DKK1, the main Wnt pathway inhibitor (*Fig. 1*), influenced secretion of chemoattractant for granulocytes and macrophages. When DKK1 expression is decreased, cancer cells secrete compounds increasing recruitment of macrophages and granulocytes. However, when DKK1 is overexpressed the result is opposite. Zhang *et al.* found this mechanism important for breast cancer metastasis (106).

TARGETING THE WNT SIGNALING PATHWAY IN THERAPY

Considering the role of the Wnt pathway in carcinogenesis, targeting Wnt signaling is a promising therapeutic approach. However, great complexity of the pathway and wide range of responses induced by Wnt signals are a major challenges that must be considered. Here we describe several examples of novel therapeutics targeting various components of the Wnt pathway in clinical trials for cancer treatment.

LGK974 (WNT974)

LGK974 is a PORCN inhibitor developed by Novartis. In preclinical studies, treatment with LGK974 caused regression of Wnt-related tumors in rats (107) and it was the first PORCN inhibitor approved for clinical trials. Phase I study which purpose is to find recommended dose of LGK974 in patients with solid malignancies is still ongoing (NCT01351103). Results from 68 enrolled patients have been reported. Measurement of AXIN2 which is β-catenin target gene showed Wnt pathway inhibition at a wide range of doses (108). Phase Ib/II clinical trial that assessed safety and anti-tumor activity of the triple combination of LGK974, LGX818 and cetuximab in patients with BRAFV600-mutant metastatic colorectal cancer with RNF43 mutations or RSPO fusions is completed, however results have not yet been reported (NCT02278133).

ETC-159 (ETC-1922159)

ETC-159 is another PORCN inhibitor that blocks secretion and activity of all Wnt proteins. Preclinical study showed, that ETC-159 was very effective in treating colorectal cancer (CRC) patient-derived xenografts with RSPO-translocation in mice (109). Phase Ia/b clinical trial that will evaluate safety and maximal/recommended doses of ETC-159 in patients with advanced or metastatic, or unresectable solid malignancies is now recruiting (NCT02521844).

Cirmtuzumab (UC-961)

UC-961 is a humanized antibody targeting receptor tyrosine kinase-like orphan receptor 1 (ROR1), a coreceptor in the Wnt signaling pathway. Phase I clinical trial evaluated

safety of Cirmtuzumab in patients with intractable chronic lymphocytic leukemia (NCT02222688). Drug was well tolerated and had a long half-life. Generally patients had sustained stabilization of disease after treatment with Cirmtuzumab and had a long progression-free survival (110). Another Phase 1b/2 clinical trial is open for recruitment and will evaluate safety and effectiveness of Cirmtuzumab in combination with Ibrutinib in patients with B-cell lymphoid malignancies (NCT03088878). Cirmtuzumab will also be studied in combination with paclitaxel in patients with metastatic or locally advanced, unresectable breast cancer (NCT02776917).

OTSA 101-DTPA-90Y

OTSA 101 was developed by OncoTherapy Science Inc. and it is a chimeric humanized monoclonal antibody targeting Frizzled 10 receptor. Non-radiolabeled OTSA 101 has weak antagonistic effect on synovial sarcoma cells, however Yttrium 90-radiolabeled anti-FZD10 antibody showed significant antitumor activity following single intravenous injection in mice with human xenografts (111). OTSA 101-DTPA-90Y was investigated in Phase I clinical trial (Clinicaltrials.gov NCT01469975), that evaluated biodistribution, tumor targeting, safety and optimal recommended dose of OTSA101-DTPA-90Y in the treatment of synovial sarcoma. In the report from Phase I, results of treatment of 16 patients with progressive metastatic synovial sarcoma resistant to doxorubicin were analyzed. Death occurred in 2/7 treated and in 6/7 untreated patients (112).

Vanituctumab (OMP-18R5)

OMP-18R5 is a human monoclonal antibody developed by OncoMed Pharmaceuticals initially identified as anti-Fz7 antibody, but later found to interact with five Fzd receptors (Fz1, Fz2, Fz5, Fz7, Fz8). It blocks canonical Wnt signaling (113). Four Phase I clinical trials have been completed. Results from study of Vantictumab in patients with solid tumors (NCT01345201) showed that drug was well tolerated up to 2.5 mg/kg q3w and observed bone toxicity appeared manageable and reversible (114). In other clinical trial, Vanituctumab was studied in combination with chemotherapy. Vanituctumab was shown to be safe in combination with nab-paclitaxel and gemcitabine in patients with stage IV pancreatic cancer (NCT02005315) (115) and in combination with paclitaxel in patients with 1st to 3rd-line metastatic HER2-negative breast cancer (NCT01973309) (116). Results from study of study of Vantictumab in combination with docetaxel in patients with previously treated non-small cell lung cancer have yet to be reported (NCT01957007).

Ipafricept (OMP-54F28)

OMP-54F28 is a fusion protein comprised of a cysteine-rich domain of Fzd8 receptor and the human immunoglobulin Fc domain. OMP-54F28 competes with the Fzd8 receptor for its ligand, thereby antagonizing Wnt signaling. In preclinical studies on a patient-derived hepatocellular carcinoma and ovarian cancer xenografts models, OMP-54F28 demonstrated anti-tumor activity (117). In a completed phase I clinical trial, OMP-54F28 was studied in patients with solid tumors. Drug was well tolerated and 20 mg/kg dose was established in this study (11). In other Phase I clinical trials OMP-54F28 was studied in combination with sorafemib in patients with hepatocellular cancer (NCT02069145) and in combination with paclitaxel and carboplatin in patients with ovarian cancer (NCT02092363). Drug was well tolerated in combination with paclitaxel and carboplatin (118). Phase I study of OMP-54F28 in combination with nab-paclitaxel and gemcitabine in patients with pancreatic cancer is still ongoing (NCT02050178).

Niclosamide

Niclosamide is an approved drug for parasitic infections. It has been shown that niclosamide promotes Frizzled1 endocytosis, downregulates Disheveled, induces LRP6 degradation and inhibits Wnt/β-catenin pathway (119-121). In mice implanted with human colorectal cancer xenografts, niclosamide administered orally led to tumor control and was well tolerated (120). Niclosamide is investigated in clinical trial (NCT02687009), that evaluates its safety in patients with colon cancer undergoing primary resection of their tumor. This is phase I study, and is now open for recruitement.

DKN-01

DKN-01 is a humanized monoclonal antibody against Wnt antagonist Dickkopf-related protein 1 (DKK1) developed by HealthCare Pharmaceuticals. DKK1 upregulation is related with canonical Wnt/β-catenin signaling inhibition (122). DKN-01 inhibits DKK1 and restores Wnt pathway through an increase of β-catenin signaling (123, 124). Clinical trials were carried out to evaluate safety of DKN-01 in monotherapy or in combination with other drugs in various tumor types.

Phase I study of DKN-01 in combination with lenalidomide and dexamethasone (NCT01711671) conducted in patients with relapsed or refractory multiple myeloma is complete. Drug was well tolerated in 7/8 patients and the overall response rate was 57.1% (4/7) (125). Another Phase I study evaluated DKN-01 in patients with multiple myeloma, advanced solid tumors and relapsed or refractory non-small cell lung cancer (NCT01457417). DKN-01 was well tolerated and demonstrated clinical activity in non-small cell lung cancer (126). Active Phase I study evaluates the safety and tolerability of DKN-01 in combination with paclitaxel or pembrolizumab in patients with relapsed or refractory esophagogastric malignancies is now open for recruitment (NCT02013154). Another active Phase I study evaluates anti-tumor activity of DKN-01 in combination with gemcitabine and cisplatin in patients with carcinoma primary to the intra- or exta-hepatic biliary system or gallbladder (NCT02375880). Phase II clinical trial will evaluate safety and efficacy of DKN-01 as a monotherapy or in combination with paclitaxel in patients with relapsed or refractory endometrioid endometrial or endometrioid ovarian cancer (NCT03395080).

BHQ880

BHQ880, developed by Novartis is another monoclonal antibody targeting DKK1 protein. Phase Ib study of BHQ880 in combination with zoledronic acid and anti-myeloma therapy in patients with relapsed or refractory multiple myeloma (NCT00741377) was well tolerated and demonstrated potential clinical activity (127). In monotherapy, BHQ880 was studied in Phase II clinical trial (NCT01302886) in patients with smoldering multiple myeloma at high risk of progression to active multiple myeloma. No significant anti-myeloma effect was observed, however single-agent BHQ880 showed anabolic bone activity in 4/5 patients (128). Considering that osteolytic bone disease affects more than 80% of patients with multiple myeloma and has negative impact on the overall survival, BHQ880 could be used in combination with other therapeutics (129). Other Phase II study of BHQ880 in patients with untreated multiple myeloma and renal insufficiency is completed with results yet to be published (NCT01337752).

CONCLUSIONS AND FUTURE DIRECTIONS

Although none of described therapies have been approved for clinical use, available data from clinical studies show that targeting Wnt signaling is a promising therapeutic approach. Even if targeting Wnt pathway alone may not be sufficient for cancer treatment, combination with chemotherapy may have synergistic effect. Particularly appealing strategy is a potential for Wnt inhibitors to eliminate cancer stem cells, which will sensitize resistant tumors to conventional therapies (130, 131). Major problem that has to be considered is a potential toxicity of Wnt alteration. For example, Wnt pathway plays fundamental role in bone metabolism, thus inhibition of Wnt modulators can increase risk of skeletal fractures (132). Despite the safety concerns, our knowledge of this pathway is rapidly increasing, which will hopefully led to precise therapeutic targeting of Wnt signaling pathway.

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REFERENCES

1. Sharma RP, Chopra VL. Effect of the wingless (wg1) mutation on wing and haltere development in Drosophila melanogaster. *Dev Biol* 1976; 48: 461-465.
2. Johnson ML, Rajamannan N. Diseases of Wnt signaling. *Rev Endocr Metab Disord* 2006; 7: 41-49.
3. Masuda T, Ishitani T. Context-dependent regulation of the β-catenin transcriptional complex supports diverse functions of Wnt/β-catenin signaling. *J Biochem* (*Tokyo*) 2017; 161: 9-17.
4. Karimaian A, Majidinia M, Bannazadeh Baghi H, Yousefi B. The crosstalk between Wnt/β-catenin signaling pathway with DNA damage response and oxidative stress: Implications in cancer therapy. *DNA Repair* (*Amst*) 2017; 51: 14-19.
5. van Kappel EC, Maurice MM. Molecular regulation and pharmacological targeting of the β-catenin destruction complex. *Br J Pharmacol* 2017; 174: 4575-4588.
6. Kretzschmar K, Clevers H. Wnt/β-catenin signaling in adult mammalian epithelial stem cells. *Dev Biol* 2017; 428: 273-282.
7. van Amerongen R, Nusse R. Towards an integrated view of Wnt signaling in development. *Dev Camb Engl* 2009; 136: 3205-3214.
8. Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell* 2006; 127: 469-480.
9. Holland JD, Klaus A, Garratt AN, Birchmeier W. Wnt signaling in stem and cancer stem cells. *Curr Opin Cell Biol* 2013; 25: 254-264.
10. Wang J, Wynshaw-Boris A. The canonical Wnt pathway in early mammalian embryogenesis and stem cell maintenance/differentiation. *Curr Opin Genet Dev* 2004; 14: 533-539.
11. Fan G, Ye D, Zhu S, *et al.* RTL1 promotes melanoma proliferation by regulating Wnt/β-catenin signalling. *Oncotarget* 2017; 8: 106026-106037.
12. Liu L, Wu B, Cai H, *et al.* Tiam1 promotes thyroid carcinoma metastasis by modulating EMT via Wnt/β-catenin signaling. *Exp Cell Res* 2018; 362: 532-540.
13. Korbut E, Ptak-Belowska A, Brzozowski T. Mechanisms promoting physiological cells progression into tumorigenesis. *J Physiol Pharmacol* 2012; 63: 565-570.
14. Ohsugi T, Yamaguchi K, Zhu C, Ikenoue T, Furukawa Y. Decreased expression of interferon-induced protein 2 (IFIT2) by Wnt/β-catenin signaling confers anti-apoptotic properties to colorectal cancer cells. *Oncotarget* 2017; 8: 100176-100186.
15. Pajak B, Kania E, Gajkowska B, Orzechowski A. Lipid rafts mediate epigallocatechin-3-gallate- and green tea extract-dependent viability of human colon adenocarcinoma COLO 205 cells; clusterin affects lipid rafts-associated signaling pathways. *J Physiol Pharmacol* 2011; 62: 449-459.
16. Guo P, Wang Y, Dai C, *et al.* Ribosomal protein S15a promotes tumor angiogenesis via enhancing Wnt/β-catenin-induced FGF18 expression in hepatocellular carcinoma. *Oncogene* 2018; 37: 1220-1236.
17. Qu B, Liu BR, Du YJ, *et al.* Wnt/β-catenin signaling pathway may regulate the expression of angiogenic growth factors in hepatocellular carcinoma. *Oncol Lett* 2014; 7: 1175-1178.
18. Wang BM, Li N. Effect of the Wnt/β-catenin signaling pathway on apoptosis, migration and invasion of transplanted hepatocellular carcinoma cells after transcatheter arterial chemoembolization in rats. *J Cell Biochem* 2018; 119: 4050-4060.
19. Yang J, Han F, Liu W, *et al.* ALX4, an epigenetically down regulated tumor suppressor, inhibits breast cancer progression by interfering Wnt/β-catenin pathway. *J Exp Clin Cancer Res* 2017; 36: 170. doi: 10.1186/s13046-017-0643-9
20. Vallee A, Lecarpentier Y, Guillevin R, Vallee J-N. Thermodynamics in gliomas: interactions between the canonical WNT/β-catenin pathway and PPARγ. *Front Physiol* 2017; 8: 352. doi: 10.3389/fphys.2017.00352
21. Adler PN, Krasnow RE, Liu J. Tissue polarity points from cells that have higher Frizzled levels towards cells that have lower Frizzled levels. *Curr Biol* 1997; 7: 940-949.
22. Wolff T, Rubin GM. Strabismus, a novel gene that regulates tissue polarity and cell fate decisions in Drosophila. *Development* 1998; 125: 1149-1159.
23. Chae J, Kim MJ, Goo JH, *et al.* The Drosophila tissue polarity gene starry night encodes a member of the protocadherin family. *Development* 1999; 126: 5421-5429.
24. Strutt H, Strutt D. Differential stability of flamingo protein complexes underlies the establishment of planar polarity. *Curr Biol* 2008; 18: 1555-1564.
25. Strutt H, Gamage J, Strutt D. Robust asymmetric localization of planar polarity proteins is associated with organization into signalosome-like domains of variable stoichiometry. *Cell Rep* 2016; 17: 2660-2671.
26. Theisen H, Purcell J, Bennett M, Kansagara D, Syed A, Marsh JL. Dishevelled is required during wingless signaling to establish both cell polarity and cell identity. *Development* 1994; 120: 347-360.
27. Gubb D, Green C, Huen D, *et al.* The balance between isoforms of the prickle LIM domain protein is critical for planar polarity in Drosophila imaginal discs. *Genes Dev* 1999; 13: 2315-2327.
28. Feiguin F, Hannus M, Mlodzik M, Eaton S. The ankyrin repeat protein Diego mediates Frizzled-dependent planar polarization. *Dev Cell* 2001; 13: 93-101.
29. Singh J, Mlodzik M. Planar cell polarity signaling: coordination of cellular orientation across tissues. *Wiley Interdiscip Rev Dev Biol* 2012; 1: 479-499.
30. Adler PN. The frizzled/stan pathway and planar cell polarity in the Drosophila wing. *Curr Top Dev Biol* 2012; 101: 1-31. doi: 10.1016/B978-0-12-394592-1.00001-6
31. Blair S, McNeill H. Big roles for fat cadherins. *Curr Opin Cell Biol* 2018; 51: 73-80.

32. Hulpiau P, van Roy F. Molecular evolution of the cadherin superfamily. *Int J Biochem Cell Biol* 2009; 41: 349-369.
33. Humphries AC, Mlodzik M. From instruction to output: Wnt/PCP signaling in development and cancer. *Curr Opin Cell Biol* 2017; 51: 110-116.
34. Minegishi K, Hashimoto M, Ajima R, *et al.* A Wnt5 activity asymmetry and intercellular signaling via PCP proteins polarize node cells for left-right symmetry breaking. *Dev Cell* 2017; 40: 439-452.e4.
35. Katoh M. WNT/PCP signaling pathway and human cancer (review). *Oncol Rep* 2005; 14: 1583-1588.
36. Marlow F, Topczewski J, Sepich D, Solnica-Krezel L. Zebrafish Rho kinase 2 acts downstream of Wnt11 to mediate cell polarity and effective convergence and extension movements. *Curr Biol* 2002; 12: 876-884.
37. Smit L, Baas A, Kuipers J, Korswagen H, van de Wetering M, Clevers H. Wnt activates the Tak1/Nemo-like kinase pathway. *J Biol Chem* 2004; 279: 17232-17240.
38. Hu D, Fang W, Han A, *et al.* C-Jun N-terminal kinase 1 interacts with and negatively regulates Wnt/beta-catenin signaling through GSK3beta pathway. *Carcinogenesis* 2008; 29: 2317-2324.
39. Zallen JA. Planar polarity and tissue morphogenesis. *Cell* 2007; 129: 1051-1063.
40. Overeem AW, Bryant DM, van Ijzendoorn SC. Mechanisms of apical-basal axis orientation and epithelial lumen positioning. *Trends Cell Biol* 2015; 25: 476-485.
41. Wang Y, Nathans J. Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development* 2007; 134: 647-658.
42. Croce JC, McClay DR. Evolution of the Wnt pathways. *Methods Mol Biol* 2008; 469: 3-18.
43. Schubert M, Holland LZ. The Wnt gene family and the evolutionary conservation of Wnt expression [internet]. *Landes Bioscience* 2013 [cited 2018 Jan 12]. Available from: https://www.ncbi.nlm.nih.gov/books/NBK6212/
44. Wu J, Mlodzik M. Wnt/PCP instructions for cilia in left-right asymmetry. *Dev Cell* 2017; 40: 423-424.
45. Macheda ML, Sun WW, Kugathasan K, *et al.* The Wnt receptor Ryk plays a role in mammalian planar cell polarity signaling. *J Biol Chem* 2012; 287: 29312-29323.
46. Yang Y, Mlodzik M. Wnt-Frizzled/planar cell polarity signaling: cellular orientation by facing the wind (Wnt). *Annu Rev Cell Dev Biol* 2015; 31: 623-646.
47. Usui T, Shima Y, Shimada Y, *et al.* Flamingo, a seven-pass transmembrane cadherin, regulates planar cell polarity under the control of Frizzled. *Cell* 1999; 98: 585-595.
48. Ma D, Yang C, McNeill H, Simon MA, Axelrod JD. Fidelity in planar cell polarity signalling. *Nature* 2003; 421: 543-547.
49. Yang C, Axelrod JD, Simon MA. Regulation of Frizzled by fat-like cadherins during planar polarity signaling in the Drosophila compound eye. *Cell* 2002; 108: 675-688.
50. Simon MA, Xu A, Ishikawa HO, Irvine KD. Modulation of fat: dachsous binding by the cadherin domain kinase four-jointed. *Curr Biol* 2010; 20: 811-817.
51. Thomas C, Strutt D. The roles of the cadherins Fat and Dachsous in planar polarity specification in Drosophila. *Dev Dyn* 2012; 241: 27-39.
52. Gelderblom M, Eminel S, Herdegen T, Waetzig V. C-Jun N-terminal kinases (JNKs) and the cytoskeleton - functions beyond neurodegeneration. *Int J Dev Neurosci* 2004; 22: 559-564.
53. Matis M, Axelrod JD. Regulation of PCP by the Fat signaling pathway. *Genes Dev* 2013; 27: 2207-2220.
54. Klein TJ, Mlodzik M. Planar cell polarization: an emerging model points in the right direction. *Annu Rev Cell Dev Biol* 2005; 21: 155-176.
55. Barrow JR. Wnt/PCP signaling: a veritable polar star in establishing patterns of polarity in embryonic tissues. *Semin Cell Dev Biol* 2006; 17: 185-193.
56. Sepich DS, Usmani M, Pawlicki S, Solnica-Krezel L. Wnt/PCP signaling controls intracellular position of MTOCs during gastrulation convergence and extension movements. *Development* 2011; 138: 543-552.
57. Yang Y. Wnt signaling in development and disease. *Cell Biosci* 2012; 2: 14. doi: 10.1186/2045-3701-2-14
58. Simons M, Mlodzik M. Planar cell polarity signaling: from fly development to human disease. *Annu Rev Genet* 2008; 42: 517-540.
59. Copp AJ, Greene ND. Genetics and development of neural tube defects. *J Pathol* 2010; 220: 217-230.
60. Curtin JA, Quint E, Tsipouri V, *et al.* Mutation of Celsr1 disrupts planar polarity of inner ear hair cells and causes severe neural tube defects in the mouse. *Curr Biol* 2003; 13: 1129-1133.
61. Simons M, Walz G. Polycystic kidney disease: cell division without a c(l)ue? *Kidney Int* 2006; 70: 854-864.
62. Garriock RJ, D'Agostino SL, Pilcher KC, Krieg PA. Wnt11-R, a protein closely related to mammalian Wnt11, is required for heart morphogenesis in Xenopus. *Dev Biol* 2005; 279: 179-192.
63. Pennekamp P, Menchen T, Dworniczak B, Hamada H. Situs inversus and ciliary abnormalities: 20 years later, what is the connection? *Cilia* 2015; 4(1): 1. doi: 10.1186/s13630-014-0010-9
64. Hong CF, Chen WY, Wu CW. Upregulation of Wnt signaling under hypoxia promotes lung cancer progression. *Oncol Rep* 2017; 38: 1706-1714.
65. Kurayoshi M, Oue N, Yamamoto H, *et al.* Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. *Cancer Res* 2006; 66: 10439-10448.
66. Weeraratna AT, Jiang Y, Hostetter G, *et al.* Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. *Cancer Cell* 2002; 1: 279-288.
67. Tell S, Yi H, Jockovich M-E, Murray TG, Hackam AS. The Wnt signaling pathway has tumor suppressor properties in retinoblastoma. *Biochem Biophys Res Commun* 2006; 349: 261-269.
68. Daulat AM, Borg JP. Wnt/planar cell polarity signaling: new opportunities for cancer treatment. *Trends Cancer* 2017; 3: 113-125.
69. Daulat AM, Bertucci F, Audebert S, *et al.* PRICKLE1 contributes to cancer cell dissemination through its interaction with mTORC2. *Dev Cell* 2016; 37: 311-325.
70. Luga V, Zhang L, Viloria-Petit AM, *et al.* Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell* 2012; 151: 1542-1556.
71. Zhang L, Luga V, Armitage SK, *et al.* A lateral signalling pathway coordinates shape volatility during cell migration. *Nat Commun* 2016; 7: 11714. doi: 10.1038/ncomms11714
72. Moon HG, Zheng Y, An CH, Kim YK, Jin Y. CCN1 secretion induced by cigarette smoking extracts augments IL-8 release from bronchial epithelial cells. *PloS One* 2013; 8: e68199. doi: 10.1371/journal.pone.0068199
73. Zhao Z, Wang S, Lin Y, *et al.* Epithelial-mesenchymal transition in cancer: role of the IL-8/IL-8R axis. *Oncol Lett* 2017; 13: 4577-4584.
74. Mikels A, Minami Y, Nusse R. Ror2 receptor requires tyrosine kinase activity to mediate Wnt5A signaling. *J Biol Chem* 2009; 284: 30167-30176.
75. Sheldahl LC, Park M, Malbon CC, Moon RT. Protein kinase C is differentially stimulated by Wnt and Frizzled homologs in a G-protein-dependent manner. *Curr Biol* 1999; 9: 695-698.

76. Hogan PG, Chen L, Nardone J, Rao A. Transcriptional regulation by calcium, calcineurin, and NFAT. *Genes Dev* 2003; 17: 2205-2232.
77. Feske S, Okamura H, Hogan PG, Rao A. Ca^{2+}/calcineurin signalling in cells of the immune system. *Biochem Biophys Res Commun* 2003; 311: 1117-1132.
78. Eisenberg LM, Eisenberg CA. Onset of a cardiac phenotype in the early embryo. In: Myofibrillogenesis (Cardiovascular Molecular Morphogenesis Series), Dube DK (ed). Boston, Birkhauser/Springer Verl. 2002, pp. 181-205.
79. Westfall TA, Brimeyer R, Twedt J, *et al.* Wnt-5/pipetail functions in vertebrate axis formation as a negative regulator of Wnt/β-catenin activity. *J Cell Biol* 2003; 162: 889-898.
80. He X, Saint-Jeannet JP, Wang Y, Nathans J, Dawid I, Varmus H. A member of the Frizzled protein family mediating axis induction by Wnt-5A. *Science* 1997; 275: 1652-1654.
81. Kuhl M, Sheldahl LC, Malbon CC, Moon RT. Ca^{2+}/calmodulin-dependent protein kinase II is stimulated by Wnt and Frizzled homologs and promotes ventral cell fates in Xenopus. *J Biol Chem* 2000; 275: 12701-12711.
82. McDonald SL, Silver A. The opposing roles of Wnt-5a in cancer. *Br J Cancer* 2009; 101: 209-214.
83. Sukarawan W, Simmons D, Suggs C, Long K, Wright JT. WNT5A expression in ameloblastoma and its roles in regulating enamel epithelium tumorigenic behaviors. *Am J Pathol* 2010; 176: 461-471.
84. Pukrop T, Klemm F, Hagemann T, *et al.* Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA* 2006; 103: 5454-5459.
85. Wang Q, Symes AJ, Kane CA, *et al.* A novel role for Wnt/Ca^{2+} signaling in actin cytoskeleton remodeling and cell motility in prostate cancer. *PloS One* 2010; 5: e10456. doi: 10.1371/journal.pone.0010456
86. Ripka S, Konig A, Buchholz M, *et al.* WNT5A - target of CUTL1 and potent modulator of tumor cell migration and invasion in pancreatic cancer. *Carcinogenesis* 2007; 28: 1178-1187.
87. Leris AC, Roberts TR, Jiang WG, Newbold RF, Mokbel K. WNT5A expression in human breast cancer. *Anticancer Res* 2005; 25: 731-734.
88. MacLeod RJ, Hayes M, Pacheco I. Wnt5a secretion stimulated by the extracellular calcium-sensing receptor inhibits defective Wnt signaling in colon cancer cells. *Am J Physiol-Gastrointest Liver Physiol* 2007; 293: G403-G411.
89. Kremenevskaja N, von Wasielewski R, Rao AS, Schofl C, Andersson T, Brabant G. Wnt-5a has tumor suppressor activity in thyroid carcinoma. *Oncogene* 2005; 24: 2144-2154.
90. Roman-Gomez J, Cordeu L, Agirre X, *et al.* Epigenetic regulation of Wnt-signaling pathway in acute lymphoblastic leukemia. *Blood* 2007; 109: 3462-3469.
91. Martin V, Valencia A, Agirre X, *et al.* Epigenetic regulation of the non-canonical Wnt pathway in acute myeloid leukemia. *Cancer Sci* 2010; 101: 425-432.
92. Wang X, Liu D, Zhou K, *et al.* Expression of Wnt11 and Rock2 in esophageal squamous cell carcinoma by activation of the WNT/PCP pathway and its clinical significance. *Pathol Res Pract* 2016; 212: 880-885.
93. Blanc E, Roux GL, Benard J, Raguenez G. Low expression of Wnt-5a gene is associated with high-risk neuroblastoma. *Oncogene* 2005; 24: 1277-1283.
94. De A. Wnt/Ca^{2+} signaling pathway: a brief overview. *Acta Biochim Biophys Sin* (*Shanghai*) 2011; 43: 745-756.
95. Nitzki F, Zibat A, Konig S, *et al.* Tumor stroma-derived Wnt5a induces differentiation of basal cell carcinoma of Ptch-mutant mice via CaMKII. *Cancer Res* 2010; 70: 2739-2748.
96. Yamaguchi TP, Bradley A, McMahon AP, Jones S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* 1999; 126: 1211-1223.
97. Dissanayake SK, Weeraratna AT. Detecting PKC phosphorylation as part of the Wnt/calcium pathway in cutaneous melanoma. *Methods Mol Biol* 2008; 468: 157-172.
98. Wang Q, Williamson M, Bott S, *et al.* Hypomethylation of WNT5A, CRIP1 and S100P in prostate cancer. *Oncogene* 2007; 26: 6560-6565.
99. Spranger S, Bao R, Gajewski TF. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* 2015; 523: 231-235.
100. Yaguchi T, Goto Y, Kido K, *et al.* Immune suppression and resistance mediated by constitutive activation of Wnt/β-catenin signaling in human melanoma cells. *J Immunol* (1950). 2012; 189: 2110-2117.
101. Ojalvo LS, Whittaker CA, Condeelis JS, Pollard JW. Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. *J Immunol* 2010;184: 702-712.
102. Krol M, Pawlowski KM, Majchrzak K, Gajewska M, Majewska A, Motyl T. Global gene expression profiles of canine macrophages and canine mammary cancer cells grown as a co-culture in vitro. *BMC Vet Res* 2012; 8: 16. doi: 10.1186/1746-6148-8-16
103. Rybicka A, Eyileten C, Taciak B, *et al.* Tumour-associated macrophages influence canine mammary cancer stem-like cells enhancing their pro-angiogenic properties. *J Physiol Pharmacol* 2016; 67: 491-500.
104. Krol M, Mucha J, Majchrzak K, *et al.* Macrophages mediate a switch between canonical and non-canonical Wnt pathways in canine mammary tumors. *PLoS One* 2014; 9: e83995. doi: 10.1371/journal.pone.0083995
105. Yeo EJ, Cassetta L, Qian BZ, *et al.* Myeloid WNT7b mediates the angiogenic switch and metastasis in breast cancer. *Cancer Res* 2014; 74: 2962-2973.
106. Zhuang X, Zhang H, Li X, *et al.* Differential effects on lung and bone metastasis of breast cancer by Wnt signalling inhibitor DKK1. *Nat Cell Biol* 2017; 19: 1274-1285.
107. Liu J, Pan S, Hsieh MH, *et al.* Targeting Wnt-driven cancer through the inhibition of Porcupine by LGK974. *Proc Natl Acad Sci USA* 2013; 110: 20224-20229.
108. Janku F, Connolly R, LoRusso P, *et al.* Abstract C45: Phase I study of WNT974, a first-in-class Porcupine inhibitor, in advanced solid tumors. *Mol Cancer Ther* 2015; 14 (12 Suppl. 2): C45-C45.
109. Jimeno A, Gordon M, Chugh R, *et al.* A first-in-human phase I study of the anticancer stem cell agent Ipafricept (OMP-54F28), a decoy receptor for Wnt ligands, in patients with advanced solid tumors. *Clin Cancer Res* 2017; 23: 7490-7497.
110. Choi MY, Widhopf GF, Yu J, *et al.* Immunotherapeutic targeting of ROR1-dependent, non-canonical Wnt5a-signaling by cirmtuzumab: a first-in-human phase I trial for patients with intractable chronic lymphocytic leukemia. [abstract]. *Blood* 2016; 128: 3224.
111. Fukukawa C, Hanaoka H, Nagayama S, *et al.* Radioimmunotherapy of human synovial sarcoma using a monoclonal antibody against FZD10. *Cancer Sci* 2008; 99: 432-440.
112. Giraudet AL, Badel JN, Cassier P, *et al.* SYNFRIZZ-A phase Ia/Ib of a radiolabelled monoclonal AB for the treatment of relapsing synovial sarcoma. [Abstract 223]. *J Nucl Med* 2014; 55 (Suppl. 1): 223.
113. Gurney A, Axelrod F, Bond CJ, *et al.* Wnt pathway inhibition via the targeting of Frizzled receptors results in

decreased growth and tumorigenicity of human tumors. *Proc Natl Acad Sci* 2012; 109: 11717-11722.
114. Smith DC, Rosen LS, Chugh R, *et al.* First-in-human evaluation of the human monoclonal antibody vantictumab (OMP-18R5; anti-Frizzled) targeting the WNT pathway in a phase I study for patients with advanced solid tumors. [Abstract]. *J Clin Oncol* 2013; 31 (Suppl. 15): 2540. doi: 10.1200/jco.2013.31.15_suppl.2540
115. Messersmith W, Cohen S, Shahda S, *et al.* Phase 1b study of WNT inhibitor vantictumab (VAN, human monoclonal antibody) with nab-paclitaxel (Nab-P) and gemcitabine (G) in patients (pts) with previously untreated stage IV pancreatic cancer (PC). [Abstract]. *Ann Oncol* 2016; 27 (Suppl. 6): doi: 0.1093/annonc/mdw371.69
116. Mita MM, Becerra C, Richards DA, *et al.* Phase 1b study of WNT inhibitor vantictumab (VAN, human monoclonal antibody) with paclitaxel (P) in patients (pts) with 1st-to 3rd-line metastatic HER2-negative breast cancer (BC). *J Clin Oncol* 2016; 34 (Suppl. 15: 2516. doi: 10.1200/JCO.2016.34.15_suppl.2516
117. Yeung P, Beviglia L, Cancilla B, *et al.* Wnt pathway antagonist OMP-54F28 (FZD8-Fc) inhibits tumor growth and reduces tumor-initiating cell frequency in patient-derived hepatocellular carcinoma and ovarian cancer xenograft models. [abstract 1907]. *Cancer Res* 2014; 74 (Suppl. 19): 1907. doi:10.1158/1538-7445.AM2014-1907
118. O'Cearbhaill RE, McMeekin DS, Mantia-Smaldone G, *et al.* Phase 1b of WNT inhibitor ipafricept (IPA, decoy receptor for WNT ligands) with carboplatin (C) and paclitaxel (P) in recurrent platinum-sensitive ovarian cancer (OC). *Ann Oncol* 2016; 27: 114-135.
119. Lu W, Lin C, Roberts MJ, Waud WR, Piazza GA, Li Y. Niclosamide suppresses cancer cell growth by inducing Wnt co-receptor LRP6 degradation and inhibiting the Wnt/β-catenin pathway. *PLoS One* 201; 6: e29290. doi: 10.1371/journal.pone.0029290
120. Osada T, Chen M, Yang XY, *et al.* Antihelminth compound niclosamide downregulates Wnt signaling and elicits antitumor responses in tumors with activating APC mutations. *Cancer Res* 2011; 71: 4172-4182.
121. Chen M, Wang J, Lu J, *et al.* The anti-helminthic niclosamide inhibits Wnt/Frizzled1 signaling. *Biochemistry* 2009; 48: 10267-10274.
122. Katoh M, Katoh M. Molecular genetics and targeted therapy of WNT-related human diseases. *Int J Mol Med* 2017; 40: 587-606.
123. D'Amico L, Mahajan S, Capietto AH, *et al.* Dickkopf-related protein 1 (Dkk1) regulates the accumulation and function of myeloid derived suppressor cells in cancer. *J Exp Med* 2016; 213: 827-840.
124. Malladi S, Macalinao DG, Jin X, *et al.* Metastatic latency and immune evasion through autocrine inhibition of WNT. *Cell* 2016; 165: 45-60.
125. White BD, Chien AJ, Dawson DW. Dysregulation of Wnt/β-catenin signaling in gastrointestinal cancers. *Gastroenterology* 2012; 142: 219-232.
126. Edenfield WJ, Richards DA, Vukelja SJ, *et al.* A phase 1 study evaluating the safety and efficacy of DKN-01, an investigational monoclonal antibody (Mab) in patients (pts) with advanced non-small cell lung cancer. *J Clin Oncol* 2014; 32 (Suppl. 15): 8068. doi: 10.1200/jco.2014.32.15_suppl.8068
127. Iyer SP, Beck JT, Stewart AK, *et al.* A phase IB multicentre dose-determination study of BHQ880 in combination with anti-myeloma therapy and zoledronic acid in patients with relapsed or refractory multiple myeloma and prior skeletal-related events. *Br J Haematol* 2014; 167: 366-375.
128. Munshi NC, Abonour R, Beck JT, *et al.* Early evidence of anabolic bone activity of BHQ880, a fully human anti-DKK1 neutralizing antibody: results of a phase 2 study in previously untreated patients with smoldering multiple myeloma at risk for progression. *Blood* 2012; 120 (54th ASH Annual Meeting Abstracts): 331.
129. Raje N, Roodman GD. Advances in the biology and treatment of bone disease in multiple myeloma. *Clin Cancer Res* 2011; 17: 1278-1286.
130. Catalano V, Dentice M, Ambrosio R, *et al.* Activated thyroid hormone promotes differentiation and chemotherapeutic sensitization of colorectal cancer stem cells by regulating Wnt and BMP4 signaling. *Cancer Res* 2016; 76: 1237-1244.
131. Cianfrocca R, Rosano L, Tocci P, *et al.* Blocking endothelin-1-receptor/β-catenin circuit sensitizes to chemotherapy in colorectal cancer. *Cell Death Differ* 2017; 24: 1811-1820.
132. Kahn M. Can we safely target the WNT pathway? *Nat Rev Drug Discov* 2014; 13: 513-532.

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