**Review**

**Wnt signaling in cancer**

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Wnt signaling is one of the key cascades regulating development and stemness, and has also been tightly associated with cancer. The role of Wnt signaling in carcinogenesis has most prominently been described for colorectal cancer, but aberrant Wnt signaling is observed in many more cancer entities. Here, we review current insights into novel components of Wnt pathways and describe their impact on cancer development. Furthermore, we highlight expanding functions of Wnt signaling for both solid and liquid tumors. We also describe current findings how Wnt signaling affects maintenance of cancer stem cells, metastasis and immune control. Finally, we provide an overview of current strategies to antagonize Wnt signaling in cancer and challenges that are associated with such approaches.

In this review we focus on novel insights into Wnt signaling in cancer, gained from studies published within the past 5 years. We describe recently discovered Wnt pathway components and novel functions of the Wnt pathway for cancer stemness, metastasis and immune surveillance. Furthermore, we review the current progress on targeting the Wnt pathway.

**INTRODUCTION**

More than 40 years ago, the wingless gene was discovered in a mutagenesis screen for visual phenotypes, affecting various developmental patterning processes in *Drosophila melanogaster*.\(^1\) Subsequently, further genetic screens identified many components of the Wnt family of signaling proteins as key mediators of patterning decisions during embryonic development.\(^2\) A connection of the Wnt pathway to cancer was implicated by the discovery that activation of int1 (Wnt1), which, either by proviral insertion into the Wnt1 locus or transgenic overexpression in mice, resulted in mammary hyperplasia and tumors.\(^3\)\(^–\)\(^5\) It was also shown that the *Drosophila* gene wingless and the murine proto-oncogene Wnt1 are orthologous.\(^6\) Furthermore, injection of murine Wnt1 mRNA into embryos of *Xenopus* could induce axis duplication.\(^7\) These observations suggested that genes involved in Wnt signaling are highly conserved through evolution. In 1991, mutations of the adenomatous polyposis coli (APC) gene were discovered as the underlying cause of the hereditary colon cancer syndrome termed familial adenomatous polyposis.\(^8\),\(^9\) The APC gene was found to interact with β-catenin\(^10\),\(^11\) and loss of function of APC resulted in overactive T-cell factor (TCF)/β-catenin signaling.\(^12\) These findings established a direct link between Wnt signaling and human colorectal cancer.

In the past years, many genetic and biochemical studies have sought to identify novel Wnt pathway components and their functions. Identified components and processes include the Wnt secretory machinery, Wnt co-receptors, components of the β-catenin destruction complex and nuclear co-factors. With the advance in sequencing technology and the comprehensive structural characterization of cancer genomes,\(^13\),\(^14\) it became evident that mutations in the Wnt pathway occur frequently in human cancers.\(^15\)–\(^18\) Despite the fact that major pathway components have been characterized, the function of Wnt signaling within the context of cancer biology is intriguingly complex and remains only partially understood.

In recent years, novel insights into multiple levels of canonical Wnt signaling were obtained, refining the model of how the pathway is regulated. Production of Wnt ligands in secreting cells is an important and surprisingly complex step in Wnt signaling. The ER resident acyl-transferase Porcupine is required for the attachment of palmitoleic acid to Wnt ligands.\(^19\) Thereafter, lipid-modified Wnt ligands bind to the transmembrane protein Evi/Wls and are shuttled to the plasma membrane via the Golgi apparatus.\(^20\)–\(^22\) The transport of Wnts from the ER to the Golgi is assisted by p24 proteins.\(^23\),\(^24\) After secretion of Wnt ligands, Evi/Wls is undergoing clathrin based endocytosis and is recycled to the Golgi apparatus by the retromer complex.\(^25\),\(^26\) Finally, Evi/Wls is transported back to the ER to re-engage in Wnt secretion.\(^22\) Wnt proteins can either be tethered to the plasma membrane or exit the cell via multiple routes, including direct release from the plasma membrane by solubilization,\(^27\) the formation of exosomes\(^28\) or on lipid protein particles.\(^29\) The variety of mechanisms by which Wnt ligands are released may correspond to their diverse roles during development and organismal maintenance. For example, although membrane-bound Wnt3 ligands retain a short range, but high level of Wnt signaling in intestinal organoids,\(^30\),\(^31\) exosome-bound Wnt2b in the

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epididymal lumen ensures long-range effects needed for sperm maturation.\(^{32}\) It is unclear which release mechanism of Wnt ligands is most prevalent in cancer. However, the presence of exosome-based Wnt signaling in the breast cancer microenvironment\(^{33}\) as well as short range Wnt signaling in RNF43/ZNRF3 double mutant intestinal organoids\(^{31}\) suggest that tissue-specific mechanisms exist.

Beyond secreted Wnts, members of the R-spondin ligand family were discovered as positive effectors of Wnt signaling.\(^{34,36}\) R-spondins bind to leucine-rich repeat containing G-protein-coupled receptors (Lgr) 4-6.\(^{3,7}\) In the absence of R-spondin binding, the two homologues E3 ubiquitin ligases ZNRF3/RNF43 target the Frizzled (Fzd) receptor for lysosomal degradation.\(^{3,7,8}\) Binding of R-spondins to Lgr4-6 inhibits the activity of ZNRF3/RNF43 and leads to the accumulation of Fzd receptors on the cell surface.\(^{3,7,8}\) Being transcriptional targets of Wnt signaling, ZNRF3 and RNF43 function as negative feedback regulators in Lgr5-positive cells.\(^{3,7,8}\) The interaction of ZNRF3 and RNF43 with the Fzd receptor was found to be dependent on Dishevelled (Dsh).\(^{40}\) The important role of the R-spondin/Lgr5/RNF43 module in cancer has been demonstrated in several tumor subtypes of colorectal cancer (CRC), pancreatic ductal adenocarcinoma (PDAC) and endometrial cancer which all harbor inactivating mutations of RNF43\(^{41,42}\) (Figure 2).

At the level of the destruction complex, YAP/TAZ, two transcriptional regulators of the Hippo pathway were recently identified as novel Wnt regulators.\(^{43}\) Complementing previous evidence for a negative effect of YAP/TAZ on Wnt signaling,\(^{44-46}\) Azzolin et al. proposed a double role for these factors: in the absence of Wnt ligands, YAP/TAZ are part of the destruction complex and recruit β-TrCP, thereby acting as negative regulators of Wnt signaling. However, in an activated pathway state, YAP/TAZ and bound β-TrCP are displaced from Axin1 by LRP during sequestration of the destruction complex. Free YAP/TAZ can subsequently act as positive transcriptional effectors of Wnt signaling.\(^{47,48}\) The dual role of YAP/TAZ in the Wnt pathway underlines the close connection of Wnt and Hippo signaling.

The transcriptional response to Wnt signaling activation is orchestrated by a complex network of β-catenin binding factors in the nucleus (reviewed in Lien and Fuchs\(^{49}\) and MacDonald et al.\(^{46}\)) of which novel cancer related components were recently identified. LATS2 kinase, a repressor of YAP/TAZ activity, was found to downregulate Wnt signaling by competing with BCL9\(^{50}\) a co-activator of the TCF/LEF transcriptional complex which is highly expressed in human tumors.\(^{51}\) Furthermore, the DNA-repair gene PAF (PCNA-associated factor) was found to be specifically overexpressed in colon cancer and intestinal stem cells.\(^{52}\) Overexpression of PAF induced intestinal neoplasia in a mouse model. Mechanistically, PAF enhances Wnt signaling by recruiting the...
histone methyltransferase EZH2 to the TCF transcriptional complex (Figure 1).

Beyond the transcriptional response of canonical Wnt signaling, Wnt-dependent stabilization of proteins (Wnt/STOP) has been introduced as a novel β-catenin-independent mechanism. Canonical Wnt signaling, which peaks during G2/M phase due to priming of LRP6 by Cyclin Y/CDK14, was shown to promote β-catenin-independent stabilization of proteins. Activation of this β-catenin-independent Wnt cascade leads to inhibition of GSK3β and the subsequent blockade of poly-phosphorylation and poly-ubiquitination of target proteins, which were predicted to comprise 20% of the proteome. These targets include prominent oncogenes such as c-Myc, which is degraded by the E3 ubiquitin ligase FBXW7 (Figure 1). Wnt/STOP has also been proposed to affect chromosomal stability, cell division and endolysosomal biogenesis. It remains to be further elucidated whether tissue- and time-dependent preferences for Wnt/STOP or transcriptional Wnt responses exist and can be exploited for cancer therapy.

Although the canonical pathway is comparatively well understood, the non-canonical Wnt pathways are more diverse and less well characterized (reviewed in Anastas and Moon). The Wnt ligands Wnt5a and Wnt11 can bind to a panel of different receptors to preferentially activate non-canonical Wnt signaling, including receptors of the Fzd family and other receptors such as ROR2, ROR1 or RYK (reviewed in Wang and Katoh). Binding of these non-canonical Wnt ligands can activate multiple intracellular pathways, of which the planar cell polarity (PCP) and calcium signaling pathways are most extensively studied (Figure 1b). The PCP pathway is implicated in cell orientation during development, but also has a role in metastasis formation, whereas the calcium signaling pathway controls intracellular influx of calcium which can activate various downstream kinases including PKC and CaM kinase II (reviewed in De and Yang and Mlodzik; see Figure 2c).

Figure 2. Mutation rates of Wnt pathway components in selected cancer entities. Percentage of cancer patients with mutations of selected canonical Wnt pathway related genes. Information was retrieved from the ICGC data portal (accessed 5/2016). The frequency of exonic mutations was determined based on cases with single nucleotide variant data in the MELA-AU, SKCA-BR, SKCM-US, PACA-US, PACA-CA, COAD-US, COCA-CN, READ-US, BRCA-UK and BRCA-US studies.
The most prominent ligand receptor pair in non-canonical Wnt signaling is the Wnt5a-ROR2 module. Binding of Wnt5a leads to the formation of the ROR2 receptor complex (Figure 1b). In addition to Wnt/PCP and Wnt/Ca²⁺signaling, further cascades can be triggered by ROR2. By binding of Filamin A to its prolin-rich domain (PRD), ROR2 can directly cause the formation of filopodia and subsequent cell migration. Of note, although related, ROR2 and ROR1 show a relatively low homology in their PRD, indicating differing functions of these receptors in cell migration. Although non-canonical Wnt signaling plays an undisputed—but understudied—role in most cancer types, our review will primarily focus on canonical Wnt signaling.

EMERGING ROLES OF WNT IN CANCER

Gastrointestinal cancers

The impact of Wnt signaling on carcinogenesis of colorectal cancer is well-studied (reviewed in Polakis67). Loss of APC is the main driver of Wnt signaling in colorectal cancer and its important role was further highlighted by several recent studies. By genome editing of APC using the CRISPR/Cas9 technology, the carcinogenesis of CRC could be modeled ex vivo in human intestinal organoids. Furthermore, studies of human CRC samples and tumors from mouse models revealed that different mutations of APC result in distinct levels of canonical Wnt pathway activity and are associated with characteristic tumor locations within the large intestine. Using a mouse model with reversible knockdown of APC via shRNA, it was demonstrated that adenomas could regress to normal tissue if APC function is restored, underlining the importance of continuous Wnt signaling for tumor maintenance.

Moreover, it was also shown that in spite of truncated APC, Wnt pathway activity can still be modulated by interference with Wnt secretion. Interestingly, a molecular classification of colorectal cancers based on expression and mutation data demonstrates that despite comparable frequencies of APC mutations between subtypes, Wnt target genes can be differentially expressed. Molecular subtypes with high expression levels of Wnt target genes were associated with better overall survival rate after relapse compared to subtypes with low expression levels of the respective genes.

Besides APC, mutations in the R-spondin/Lgr5/RNF43 module were implicated as drivers of Wnt-dependent tumor growth. Deleterious RNF43 mutations have been described in ~19% of CRC cases and are mutually exclusive to APC mutations. In addition, R-spondin3 mutations and fusion proteins expressed at a high level have been described in 10% of CRC cases. RNF43 mutant CRC are strongly dependent on Wnt secretion, rendering them highly susceptible to Wnt secretion targeted therapy.

Chromosomal instability (CIN) is frequently observed in CRC and associated with poor prognosis. Loss of function of Wnt pathway components, particularly APC, have been linked to CIN by multiple mechanisms. Although direct interactions of APC with the cytoskeleton and the transcriptional Wnt response via β-catenin are known routes to CIN, Wnt/β-catenin/Wnt/STOP were introduced as new mechanism: Erych et al. recently showed that loss of basal Wnt/STOP leads to increased microtubule assembly rates and subsequent CIN in HCT116 cells, while reconstitution of normal assembly rates could reverse the CIN phenotype.

Development of PDAC is mainly driven by oncogenic Ras signaling and the impact of Wnt signaling has not been fully understood. Unlike CRC, mutations of key Wnt pathway components are rare in PDAC (Figure 2), but nuclear localization of β-catenin is regularly found. Results from mouse models indicate that Wnt signaling can initiate tumor formation when activated at distinct tumor stages (reviewed in White et al.70). However, stabilized β-catenin can also inhibit reprogramming of acini into preneoplastic lesions in the presence of mutated KRAS,

indicating a more complex role of Wnt signaling during tumor development. Recent studies show that PDAC relies on Wnt ligand stimulation, as PDAC cell lines carrying a mutation in RNF43 are particularly sensitive towards treatment with the Porcupine inhibitor LGK974. Furthermore, induction of the Wnt antagonist DKK1 as well as treatment with the anti-Fzd antibody OMP18R5 delays PDAC formation.

Autocrine Wnt7b was found to increase Wnt signaling in pancreatic cell lines and to promote anchorage-independent cell growth.

Wnt signaling is also activated in cholangiocarcinoma, but genomic alternations of major Wnt pathway components are rare, with the exception of RNF43. Pharmacological inhibition of Wnt signaling, both at the level of β-catenin and Wnt secretion, reduces proliferation of cholangiocarcinoma cells in a mouse model. Interestingly, Wnt secreting inflammatory macrophages in the microenvironment are required to maintain high Wnt signaling in the tumor.

Furthermore, secreted inhibitors of Wnt signaling such as SFRP2 are frequently silenced by hypermethylation in cholangiocarcinoma.

Taken together, novel findings reinforce the view that dependence on Wnt signaling of gastrointestinal cancers can be mediated by different, tissue-specific routes.

Leukemia

In recent years, knowledge about the role of Wnt signaling in hematopoesis and leukemia has increased. Normal hematopoietic stem cells (HSC) depend on a finely controlled level of Wnt signaling for long-term maintenance, whereas Wnt activity is substantially increased in most leukemias. Acute myelogenous leukemia (AML) is the most common type of acute leukemia in adults and characterized by frequent chromosomal translocations. In MLL-fusion positive AML mouse models, leukemia initiating cells (LiCs) can arise from HSC as well as myeloid progenitor cells after progression through a pre-LIC state. β-catenin appears to be essential for the progression of pre-LiCs to the LIC state and for LIC self-renewal.

Frequent translocation products found in AML, such as AML1-ETO, MLL-AF9 and PML-RARα positively affect canonical Wnt signaling in patient samples and derived cell lines.

The most common leukemia in childhood is acute lymphoblastic leukemia (ALL). The majority of LiCs in T-cell ALL (T-ALL) harbor activating mutations of the Notch signal pathway (reviewed in Ferrando). However, canonical Wnt signaling in HSC and thymocytes synergizes with PTEN loss and c-Myc amplification to generate a β-catenin dependent and Notch independent T-ALL subset in mouse studies and human T-ALL patients. Besides the driving role of canonical Wnt signaling during tumorogenesis of specific T-ALL subsets, active β-catenin appears to play an important role during LIC self-renewal in a broader context. Giambra and colleagues showed that LiCs in bulk NOTCH1 driven T-ALL mouse models are marked by high Wnt activity. The characterization of β-catenin in these tumors eliminated LiCs without affecting the short-term viability of the bulk tumor.

Chronic lymphocytic leukemia (CLL) is the most prevalent form of adult leukemia in western countries. Canonical Wnt signaling is active in CLL cells and its inhibition increases apoptosis in vitro. Next to frequent silencing of Wnt inhibiting factors such as DKK1, somatic mutations in Wnt pathway related genes (for example, FZD5, BCL9) were found in 14% of studied cases. Knockdown of mutated Wnt pathway members reduced cell viability in CLL cells carrying the targeted Wnt pathway alteration, while those without Wnt pathway mutations remained unaffected. These findings demonstrate that a subset of CLL is dependent on active Wnt signaling for survival. In summary, canonical Wnt signaling is able to drive tumor development in major leukemia subtypes and is required for maintenance of leukemia initiating cells.
Melanoma

About 25% of melanomas arise from benign nevi (commonly known as moles) which typically consist of quiescent BRAFV600E or NRASQ61K mutant melanocytes that have undergone a process of oncogene induced senescence (reviewed in Jones and Cichowski). Canonical Wnt signaling has been found to delay the onset of oncogene induced senescence in both BRAFV600E or NRASQ61K expressing primary melanocytes and thereby increase the chance of tumor development.

Although canonical Wnt signaling appears to contribute to melanoma development, its role in disease progression is controversial. Several clinical and translational studies have shown an increased overall survival rate of patients carrying melanoma with elevated nuclear β-catenin levels. However, in a mouse model with a mutant PTEN and BRAF genotypen, activated β-catenin lead to accelerated melanoma development and promotion of metastasis. The impact of Wnt signaling on response towards BRAF inhibitors in melanoma is also unclear. Active Wnt signaling was shown to cooperate with BRAFV600E inhibition to induce apoptosis in melanoma cell lines. However, clinical studies demonstrated that patients with lower nuclear β-catenin levels had a better prognosis under BRAF inhibitor treatment.

The role of non-canonical Wnt signaling in melanoma progression has been investigated extensively. Activity of Wnt5a/ROR2 leads to increased cell motility as well as a pseudo-senescent phenotype, which is induced by external stresses. Interestingly, stimulation with IL-6 has been shown to induce the expression of Wnt5a in melanoma cells itself, thereby forming a positive feedback loop. Thus, Wnt5a mediated non-canonical Wnt signaling leads to self-promoting invasive and resistant phenotypes in melanomas. It is important to note that Wnt5a ligand binding to alternative co-receptors, such as LRP6, can result in a canonical Wnt signaling response in a subset of melanoma cells. Consequently, the distinction between canonical and non-canonical signaling effects is not dichotomous and might explain controversial findings.

The progression of melanoma has been described by a phenotype switching model with melanoma cells changing between proliferative and invasive states. These prevailing phenotypes are determined in part by the balance between canonical and non-canonical Wnt signaling. Recently, ROR1 and ROR2 co-receptor abundance was linked to the two different melanoma phenotypes. Wnt5a treatment of proliferative ROR1 positive melanoma cells led to ROR1 degradation, a high ROR2 expression and increased invasiveness of melanoma cells in vivo. Hypoxic culture conditions were identified as a trigger for changing the cellular Wnt signaling response eventually leading to phenotypic switching.

Breast cancer

Wnt signaling is activated in over 50% of breast cancer patients and linked to reduced overall survival. The role of canonical Wnt signaling in triple negative breast cancer development and progression has been studied intensively. However, high levels of nuclear β-catenin were also found in other breast cancer subtypes. Only a small fraction of tumors harbor somatic mutations of key pathway regulators such as β-catenin (Figure 2), but canonical Wnt ligands and receptors are often overexpressed in breast cancers whereas secreted antagonists are silenced. In mice, MMTV-Wnt induced tumors are dependent on continuous Wnt signaling which leads to progenitor-like signatures in tumor cells. Overexpression of R-spondin2 alone was shown to initiate mammary tumors in mouse models. Recently the model of canonical Wnt signaling in mammary tumors has been refined by analysis of clonal heterogeneity within the tumor. Cleary et al. identified two tumor cell-lineages of luminal and basal descent in a MMTV-Wnt model. The luminal subclone was characterized by secretion of canonical Wnt ligands, which was necessary for tumor growth of the basal-like recipient cells. These findings indicate that mammary tumors can consist of polyclonal cell populations that cooperate to generate distinct, subpopulation specific Wnt activity levels.

WNT SIGNALING AND CANCER STEM CELLS

The self-renewal potential of cancer cells is described by the cancer stemness model and has been used to explain many malignant phenotypes. Although the concept of cancer stemness is still controversially discussed, the vital role of the Wnt pathway for the function of normal and cancer stem cells is commonly accepted (reviewed in Rey and Clevers). One of the hallmarks of stem cells is their ability to maintain long telomeres by function of the TERT gene. TERT expression was found to be directly enhanced by binding of β-catenin to its promoter region and thereby links telomerase activity to Wnt signaling. The R-spondin receptor Lgr5 is a marker of intestinal stem cells and can fuel tumor growth when APC is deleted in these cells. Lineage tracing experiments demonstrate that single Lgr5-positive cells can give rise to additional Lgr5-positive cells and other cell types in colon adenoma, indicating that Lgr5 is a potential cancer stem cell marker. Myant et al. show that RAC1 is required for expansion of the Lgr5 population after APC loss. RAC1 activation drives ROS production and thereby activates NFκB signaling, which then enhances Wnt signaling. These findings are supported by another study showing that co-activation of the NFκB and Wnt pathway can induce dedifferentiation of normal intestinal cells into stem cells and thereby initiate tumor development in a mouse model. The important role of the tumor environment for maintenance of cancer stemness is highlighted by several studies. Hepatocyte growth factor secreted by myofibroblasts in the tumor microenvironment can increase Wnt activity and induce stemness features in colorectal cancer cells. Malanchi et al. demonstrated that breast tumor cells induce the stromal expression of the extracellular matrix protein peristin in order to form a metastatic niche. Peristin interacts with Wnt1 and Wnt3a, thereby inducing Wnt signaling and sustaining a CSC phenotype. In another study, MMP3 secreted by mammary epithelial cells was found to stimulate canonical Wnt signaling in mammary stem cells by sequestration of Wnt5b, thereby counteracting the inhibitory effect of the non-canonical Wnt pathway. CD44v6 was described as another CSC marker in colorectal cancer and its expression is promoted by canonical Wnt signaling and cytokines secreted from tumor-associated cells, resulting in increased metastatic capacity.

Recently, several studies uncovered potential links between non-coding RNAs and Wnt signaling in cancer stem cells. microRNA-146a was shown to stabilize β-catenin by repression of Numb, leading to maintenance of Wnt signaling and symmetric division of colorectal cancer stem cells. In mammary stem cells, miR-142 recruits the APC mRNA for degradation and thereby increases canonical Wnt signaling. The long non-coding RNA hsa-miR-142 recruits the APC mRNA for degradation and thereby increases canonical Wnt signaling. The long non-coding RNA LncTCE7, which is highly expressed in hepatocellular carcinoma and liver stem cells, was found to activate expression of TCF7 by recruitment of the SWI/SNF complex to the promoter of TCF7. This activation of canonical Wnt signaling is associated with an increased self-renewal capacity of liver CSC. In non-small cellular lung cancer, overexpressed miR-582-3p maintains stemness features by targeting negative the regulators of Wnt...
signaling Axin2, DKK3 and SRP1 for degradation, thereby increasing β-catenin mediated Wnt activity.Expression signatures of intestinal stemness in tumor samples were correlated with disease prognosis in colorectal cancer. Although both the intestinal stem cell and cancer stem cell signature could readily identify patients with poor prognosis, Melo et al. show that the expression of accepted Wnt target/stemness genes such as Axin2 and Lgr5 were lower in patients with poor prognosis, due to promoter methylation of those genes. No correlation was found between expression level of Wnt target/stemness genes and the number of CD133-positive stem cells or nuclear β-catenin levels. Thus, stem cell signatures likely reflect the general differentiation state of the tumor tissue rather than the number of Wnt driven cancer stem cells.

### WNT SIGNALING IN METASTASIS
Metastasis is a hallmark of late stage cancer and a major challenge to therapy. A main adaptive change of tumors during therapy is an epithelial to mesenchymal transition (EMT, reviewed in Scheel and Weinberg). EMT describes the process by which polarized epithelial cells transform into migratory mesenchymal cells with invasive properties. Transcriptional factors that are responsible for EMT include, among others, SNAI2. Cytoplasmic SNAI2 concentration is kept in check by GSK3β phosphorylation and subsequent ubiquitylation by β-TrCP. Activation of canonical Wnt signaling stabilizes SNAI2 by inhibiting GSK3β kinase activity and initiates EMT transcriptional programs in breast cancer cells. Another candidate gene that regulates EMT is ASPP2, a protein that binds to a β-catenin/E-cadherin complex and inhibits N-terminal phosphorylation of β-catenin, leading to its stabilization. Reduced expression of ASPP2 leads to EMT and is associated with poor survival in hepatocellular and breast cancer. In colon cancer cells with hyperactivated canonical Wnt signaling, pharmacological inhibition of the PI3K-Akt signaling leads to a nuclear accumulation of β-catenin and FOXO3a which results in increased cell scattering and metastasis. These results show that both active and non-active canonical Wnt signaling can enhance EMT, depending on the tissue type. An involvement of the non-canonical Wnt pathway in EMT was implied by high co-expression of Fzd2, its ligands Wnt5a/b and EMT markers. It was shown that Fzd2 expression enhances EMT and cell migration via Fyn and Stat3. Targeting of Fzd2 by a specific antibody reduces tumor growth and metastasis in a xenograft mouse model of colon cancer.

Recently, exosomes were found to be potential mechanism by which tumors prime their metastatic niche. Exosomes are small vesicles secreted by cells and function in intercellular communication. It was shown that they can be vehicles for the transport of active Wnt ligands or incorporate β-catenin. Exosomes secreted from fibroblast in the tumor microenvironment can enhance motility and proliferative activity of breast cancer cells via the Wnt/PCP pathway. Co-injection of breast cancer cells with fibroblast in orthotopic mouse models was shown to promote metastasis. Mechanistically, this results from a tethering of Wnt11 to fibroblast-derived exosomes. Another route by which distant metastasis is proposed to spread is via circulating tumor cells (CTCs). Single-cell RNA sequencing of CTCs was performed for prostate and pancreatic cancer and both studies identified a role for Wnt signaling. In CTCs of pancreatic cancer, Wnt2 expression increased anchorage-independent sphere formation and their metastatic propensity. In another study, the non-canonical Wnt signaling pathway was found to be upregulated in CTCs of prostate cancer cells that are resistant to androgen receptor inhibition. Taken together, there is increasing evidence that both canonical and non-canonical Wnt signaling can support tumor metastasis in a highly tissue-specific manner.

### WNT SIGNALING AND ANTI-TUMOR IMMUNITY
Overcoming immune evasion by cancer cells is a promising therapeutic approach and immune checkpoint blockade was shown to be highly effective in the treatment of melanoma and other tumor types. Wnt signaling controls proliferation, maturation and differentiation of T-cells and dendritic cells, but a role of tumor intrinsic Wnt signaling in immune evasion has only recently emerged. Spranger et al. show that a Wnt signature in cutaneous melanoma samples correlates with T-cell exclusion. Using a mouse model of melanoma with Braf/PTEN mutant background and constitutively high β-catenin activity, the authors show that T-cell priming against tumor antigens is failing due to defective recruitment of CD103+ dendritic cells. β-catenin signaling downregulates the chemokine CCL4, which negatively affects the recruitment of dendritic cells to the tumor. Restoration of intra-tumoral dendritic cells by injection could furthermore increase the efficiency of anti-CTLA4 and anti-PD-L1 therapy. Moreover, upregulation of IL-12 production in melanoma by increased β-catenin signaling can also lead to impaired dendritic cell maturation and induction of regulatory dendritic cells.

A different mechanism of immune evasion was recently demonstrated in lung and breast cancer. It was shown that latency competent cancers self-impose a slow-cycling state by autocrine inhibition of Wnt signaling by DKK1, thereby evading innate immune response. As therapy against immune checkpoint inhibitors are showing promising results also in other tumor entities such as colorectal cancer, further studies investigating the interplay between tumor intrinsic Wnt signaling and immune response are expected.

### PHARMACOLOGICAL INHIBITORS AND CLINICAL TRIALS
The concept that inhibition of Wnt signaling is an universal strategy for the treatment of cancer has been controversially discussed in the past. As clinical data suggests, elevated Wnt signaling is only linked to a worse outcome for a subset of human cancers. Therefore, current strategies aim at targeting Wnt signaling in distinct tumor subclasses or with specific mutational backgrounds. An overview of small molecular inhibitors and antibodies that are currently in clinical testing is presented in Figure 3 and Table 1.

In recent years, the knowledge on the role of Wnt secretion for carcinogenesis has advanced considerably and unveiled novel therapeutic targets. Small molecule inhibitors, including IWP-1 and LGK974, were shown to selectively inhibit the acyl-transferase Porcupine and thus Wnt secretion, leading to a size reduction of MMTV-Wnt1-driven tumors and head and neck cancer xenotransplants. Furthermore, Jiang et al. showed that mutations of RNF43 results in dependency of pancreatic adenocarcinomas on Wnt ligands. Mutations of RNF43 and R-spondin fusion proteins, which occur mutually exclusive with APC mutations in colorectal cancer, were subsequently presented as a predictors for an effective therapy targeting Wnt secretion. Based on these results, a phase I/II trial of LGK974 was initiated for patients with metastatic colorectal cancer harboring mutations of RNF43 or R-spondin fusions. In addition, a novel orally available Porcupine inhibitor ETC-159 was found to prevent growth of R-spondin-fusion positive CRC, thereby evading innate immune response. As therapy against immune checkpoint inhibition of Wnt signaling by DKK1, thereby evading innate immune response. As therapy against immune checkpoint inhibitors are showing promising results also in other tumor entities such as colorectal cancer, further studies investigating the interplay between tumor intrinsic Wnt signaling and immune response are expected.

Besides the intracellular perturbation of Wnt secretion, an array of drugs targeting extracellular Wnt ligands and their receptors are under development. OMP-54F28 is a fusion protein consisting of a Fzd8 and a human IgG1 Fc domain. This decoy receptor for Wnt ligands reduces the size of tumor xenografts and overall tumor initiating cell number in mouse models of hepatocellular...
carcinoma and ovarian cancer. Currently, the substance is undergoing three phase 1b trials in liver, ovarian and pancreatic cancer in combination with established therapeutics. Furthermore, a phase I clinical trial testing the safety of OMP131R10, a RSPO3-binding antibody, has been initiated in June 2015 for patients with solid tumors and metastasized colorectal cancer.

OMP18R5 is a monoclonal antibody targeting five of ten human Fzd receptors. It was shown to inhibit the growth of human tumor xenografts and to synergize with standard-of-care therapeutic agents, but first data from clinical studies suggested adverse effects on skeletal constitution. This finding was considered as an on-target effect of Wnt inhibition since Wnt signaling has a key role in bone development and disease. Currently, OMP18R5 is studied in phase I clinical trials alone or in combination with taxanes in breast and pancreatic cancer, as well as non-small cell lung cancer. Besides targeting key functional members of the Wnt signaling cascade, other pharmaceuticals exploit the specific expression patterns of Fzd receptors by cancer cells. For example, Fzd10 is almost exclusively overexpressed in a variety of defined cancer types. OTSA101 is a radioactive anti-Fzd10 antibody which is currently in a phase I trial for the treatment of advanced synovial sarcoma.

In addition to approaches targeting Wnt secretion and ligands, an inhibitor of the downstream Wnt pathway is currently undergoing clinical trials. PRI-724 and the closely related compound ICG-001 specifically target the complex formation of β-catenin and CBP while enhancing the formation of β-catenin/p300 complexes in ALL cells. Treatment with PRI-724 therefore inhibits the self-renewing downstream effects of β-catenin-CBP activity and leads to reduction of tumor burden. Following promising results from phase I trials, a new phase II trial of PRI-724 in combination with bevacizumab therapy in metastatic colorectal carcinoma patients is planned. Tankyrase inhibitors such as XAV939, which stabilize Axin by blocking its PARsylation, have shown promising results as Wnt inhibitors. Subsequently, additional compounds targeting this enzyme have been developed. However, no tankyrase inhibitor is currently undergoing clinical testing, which may be linked to their toxicity in preclinical models.

A better understanding of non-canonical Wnt signaling in tumor metastasis and growth has led to novel therapeutic approaches. Two Wnt5a analog small peptides, Foxy-5 and Box-5 have been developed to either activate or inhibit Wnt5a-dependent signaling, thereby reducing metastasis in selected tumors. First results from a phase I clinical trial of Foxy-5 suggest a good tolerability. The introduction of both Wnt5a agonists and antagonists, as well as the identification of anti-secretion therapy responsive tumor subsets further guide the clinical practice into the direction of personalized treatments. However, as data from selected clinical trials suggests, the possibility of serious on-target side effects across stem cell niches in the organism needs to be considered in future drug safety evaluations.

OUTLOOK

In recent years, a multitude of studies have contributed to a deeper understanding of canonical and non-canonical Wnt signaling in cancer. Schematic representation of the canonical Wnt signaling pathway with pharmaceutical modulators. All depicted drugs are currently undergoing testing in Phase 1/2 against various types of cancer (see also Table 1).
| Compound      | Mode of action               | Trial Phase | Tumor entities                                                                 | Originator                               | Preliminary clinical results                                                                 | Starting date     | Trial identifier   |
|---------------|-----------------------------|-------------|--------------------------------------------------------------------------------|------------------------------------------|---------------------------------------------------------------------------------------------|-------------------|-------------------|
| LGK974 (WNT974) | Inhibitor of Porcupine      | 1/2         | Metastatic colorectal cancer with Wnt pathway mutations; head and neck squamous cell carcinoma with Notch receptor mutations | Novartis                                 | None                                                                                        | October 2014      | NCT02278133       |
| ETC-159       | Inhibitor of Porcupine      | 1           | Solid tumors                                                                   | D3-Institute, experimental Therapeutics Centre (ETC), Duke-NUS | None                                                                                        | July 2015         | NCT02521844       |
| OMP-54F28 (Ipafricept) | Fzd8-Fc Decoy receptor     | 1           | Hepatocellular carcinoma; ovarian cancer; pancreatic ductal adenocarcinoma   | Bayer HealthCare Pharmaceutical; OncoMed Pharmaceuticals | None                                                                                        | February 2014     | NCT02092363       |
| OMP18R5 (Vantictumab) | Anti-Fzd7 antibody   | 1           | Non-small cell lung cancer; pancreatic ductal adenocarcinoma; metastatic breast cancer | Bayer HealthCare Pharmaceutical; OncoMed Pharmaceuticals | Well tolerated; Increased bone turnover; LEF1 is a potential biomarker for treatment response | September 2013    | NCT01957007       |
| OTSA101       | Yttrium90 radiolabeled Anti-Fzd10 antibody | 1           | Synovial sarcoma                                                              | OncoTherapy Science                      | Heterogeneous uptake; one case of thrombopenia complicated by hemoptysis with fatal outcome | November 2011     | NCT01469975       |
| OMP131R10     | Anti-R-spondin3 antibody    | 1           | RSPO3 biomarker-positive metastatic colorectal cancer                          | OncoMed Pharmaceutical; Celgene          | None                                                                                        | June 2015         | NCT02482441       |
| Foxy-5        | Wnt5a mimetic               | 1           | Breast cancer; colorectal cancer; prostate cancer                             | WntResearch                              | No dose-limiting toxicity identified; Phase 1b trial planned                               | January 2016      | NCT02655952       |
| PRI-724        | Inhibitor of TCF-CBP interaction | 1/2     | Acute and chronic myelogenous leukemia; colorectal adenocarcinoma, pancreatic adenocarcinoma | PRISM BioLab and University of Southern California | No dose-limiting toxicity in pancreatic cancer trial, some evidence of clinical activity | April 2015         | NCT01606579       |

Abbreviation: TCF, T-cell factor.
signaling on a mechanistic level. The effect of aberrant canonical Wnt signaling is not only restricted to cancer cells, but dynamically interacts with the microenvironment and immune system. It also became clear that the function of non-canonical Wnt signaling is similar in development and cancer. While non-canonical Wnt signaling regulates convergent extension and tissue mobility during development, it can also mediate motility of cancer cells during metastasis. Moreover, there is a better understanding of how canonical and non-canonical Wnt signaling interact. The balance between both pathways is maintained by mechanisms that are distinct for different tissue types and their corresponding tumors. This knowledge is currently translated into a refined approach of targeting the Wnt pathway in cancer, taking into account both the functional and mutational status of canonical and non-canonical Wnt pathways in different cancer types.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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