Cancer and Metastasis Reviews (2020) 39:625–645
https://doi.org/10.1007/s10555-020-09887-6

Wnts and the hallmarks of cancer

Zheng Zhong[1] · Jia Yu[1] · David M. Virshup[1,2] · Babita Madan[1]

Published online: 8 May 2020

Abstract
Since the discovery of the first mammalian Wnt proto-oncogene in virus-induced mouse mammary tumors almost four decades ago, Wnt signaling pathway and its involvement in cancers have been extensively investigated. Activation of this evolutionarily conserved pathway promotes cancer development *via* diverse mechanisms. Cancer is a complex disease and one outstanding conceptual framework for understanding its biology is the "Hallmarks of Cancer". In this review, we focus on the involvement of Wnt signaling in the ten hallmarks of human cancer. These widespread roles of Wnt signaling in human cancers highlight the importance and feasibility of targeting this signaling pathway for cancer treatment.

Keywords Wnt signaling · Cancer · Genome instability · TERT · Telomeres · Metastasis · Angiogenesis · Metabolism · Inflammation · Immune evasion

1 Introduction

Wnt signaling has been present in animals since the first metazoans. Wnt signaling has evolved to perform diverse functions in normal development and physiology [1–3]. Our scientific understanding of Wnt/β-catenin signaling has long been intertwined with cancer biology. Wnts in mammals were first discovered because overexpressed *Wnt1* caused mouse mammary tumors [4]. The *adenomatous polyposis coli (APC)* gene truncating mutations that stabilize β-catenin are highly prevalent in colorectal cancer, making *APC* one of the most mutated genes in human cancers [5]. β-catenin accumulation driven by active Wnt signaling results in the formation of a transcriptional complex, together with T cell factor/lymphoid enhancer factor (TCF/LEF) family members, that directly binds to the promoters of Wnt target genes and regulate their expression. The specifics and mechanisms of the Wnt signaling transduction cascade are well studied and well reviewed elsewhere [5–7]. However, how activated Wnt/β-catenin signaling contributes to multiple facets of cancer development is less clear. Our goal here is to highlight the myriad ways that aberrant Wnt signaling contributes to the development and progression of human cancers. One outstanding conceptual framework for understanding how signaling pathways contribute to cancer is the "Hallmarks of Cancer," starting with the review of Hanahan and Weinberg in 2000 [8], and updated in 2011 [9]. In this review, we evaluate some of the contributions of aberrant Wnt signaling to each of the hallmark pathways that are critical to cancer development, highlighting the complexity of the Wnt pathways and crosstalk with other signaling networks (Fig. 1).

2 Wnts and proliferative signaling

Cell proliferation is the process during which a cell grows, replicates its genetic information (DNA), and divides into two daughter cells. Cells respond to mitogenic signals and initiate the cell cycle, tightly driven by cell cycle proteins such as Cyclins and Cyclin-Dependent Kinases (CDKs) [10]. This process is strictly regulated by upstream and feedback signals under normal physiological conditions. While in many cases the driving force of cell proliferation comes from growth factor-stimulated signaling such as the Mitogen-Activated Protein Kinase (MAPK) cascades [11], an increasing number of studies also show the involvement of Wnt signaling in cell cycle regulation on multiple mechanistic layers [12]. First, two key cell cycle regulators, *MYC* and *Cyclin D1*, are direct

Zheng Zhong and Jia Yu contributed equally to this work.

✉ David M. Virshup
david.virshup@duke-nus.edu.sg

✉ Babita Madan
babita.madan@duke-nus.edu.sg

[1] Programme in Cancer and Stem Cell Biology, Duke-NUS Medical School, 8 College Road, Singapore 169857, Singapore

[2] Department of Pediatrics, Duke University School of Medicine, Durham, NC 27710, USA

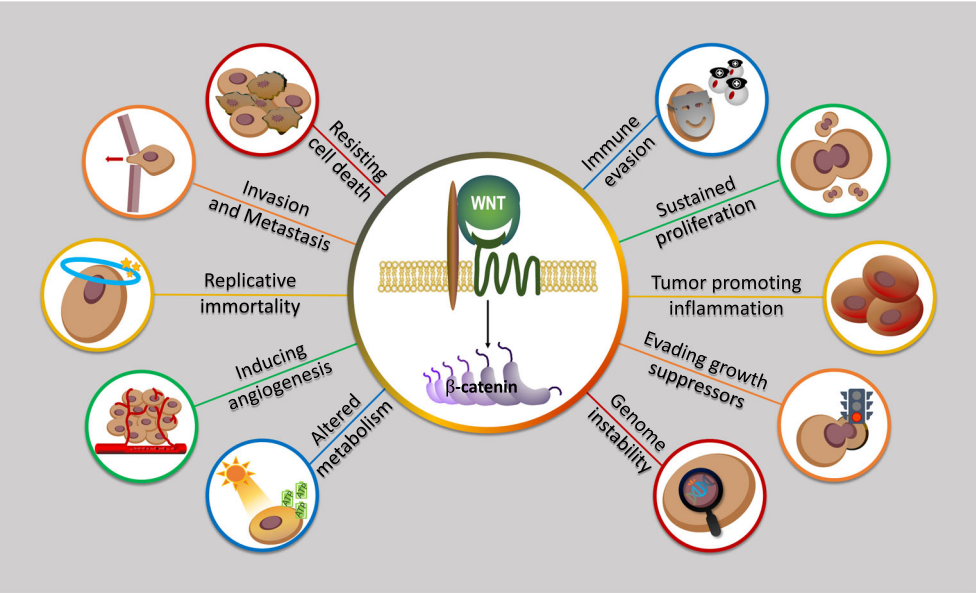

Fig. 1 This figure illustrates the multiple roles Wnt signaling plays in the pathogenesis of cancer around the conceptual framework of ten hallmarks of cancer as outlined by Hanahan and Weinberg

target genes of the β-catenin/TCF transcriptional complex [13–15]. Second, in addition to direct transcriptional regulation of cell cycle genes, the protein abundance of many important cell cycle effectors such as MYC, Cyclin D, and Cyclin E is also regulated by the GSK3-mediated Wnt/stabilization of proteins (STOP) pathway [16–19]. Finally, several Wnt pathway components, including APC, Axin2, Dishevelled (DVL), and β-catenin, are directly involved in mitotic processes such as microtubule dynamics, spindle formation, and centrosome division [12], which will be further discussed in a later section.

Although several cell cycle regulators are downstream targets of Wnt signaling, to what extent Wnt signaling regulates cell proliferation in normal cells, especially compared with the well-established mitogenic growth signaling, remains controversial. In various normal adult tissues, active Wnt signaling is enriched in tissue stem cells and maintains stemness [20, 21]. However, adult tissue stem cells are not necessarily highly proliferative and are often maintained in a quiescent state [22]. The intestine is one of the best-studied models of Wnt signaling and stemness. High Wnt/β-catenin signaling is largely restricted to the intestinal stem cells in the crypt base, whereas the highly proliferative transit-amplifying (TA) cells lack Wnt/β-catenin signaling. A recent study found that Wnt inhibition activated MAPK signaling and induced an initial burst of proliferation in the intestinal stem cells due to the conversion of the stem cells into the TA cells without stem cell self-renewal [23]. Eventually, this led to intestinal crypt ablation due to stem cell exhaustion. This finding is consistent with the observation that expression of an oncogenic *BRAF* transgene in the mouse intestine hyperactivated MAPK signaling and led to the conversion of the stem cells into proliferative TA cells and stem cell loss, and this could be antagonized by artificially enhancing Wnt/β-catenin signaling [24]. These observations support the stem cell quiescence model: excess proliferation results in stem cell exhaustion [22]. And importantly, this suggests that the role of Wnt signaling, at least in the intestinal stem cell niche, is to maintain stemness rather than to promote stem cell proliferation.

However, cancer cells dysregulate multiple signal transduction pathways through genetic and epigenetic alterations to support uncontrolled cell proliferation. Several components of the Wnt pathway, such as APC, β-catenin, and RNF43, are frequently mutated in several human cancers, leading to hyperactivated Wnt signaling [25]. Numerous studies show that hyperactivated Wnt signaling can promote cancer cell proliferation. The first evidence came from the discovery of the first mammalian Wnt gene, *Int-1*, whose overexpression led to the development of mouse mammary cancer [4]. Importantly, inhibition of Wnt secretion significantly decreased cell proliferation in these tumors [26]. Similar results were observed in multiple Wnt-driven cancer models, where genetic or pharmacological inhibition of Wnt signaling decreased cell proliferation in Wnt-driven tissue neoplasia and tumors [27–30]. Mechanistically, cancer cells hijack hyperactive Wnt signaling to maintain several pro-proliferative effectors such as MYC at high level. As one of the most important oncogenic transcription factors, MYC positively regulates a large portion of the transcriptome [31, 32], including multiple key cell cycle effectors such as cyclins, CDKs, and E2F transcription factors. MYC also antagonizes cell cycle inhibitors like p21 and p27 through transcriptional, epigenetic, or post-transcriptional mechanisms [33]. Moreover, many MYC targets are involved in protein, lipid, and nucleotide synthesis and energy metabolism pathways that are fundamental to the growth and proliferation of cancer cells [34–36]. Collectively,

hyperactive Wnt signaling upregulates the abundance of MYC and promotes cancer cell proliferation (Fig. 2). Notably, genetic deletion of *Myc* reversed the neoplasia caused by hyperactive β-catenin signaling in the *Apc*-deficient mouse intestine [37], whereas expressing a stabilized MYC partially rescued the proliferation suppression upon Wnt blockade in a Wnt-addicted pancreatic cancer orthotopic xenograft model [30], suggesting MYC as one of the critical mediators of hyperactive Wnt/β-catenin signaling-driven proliferation.

Wnt/β-catenin signaling appears to be less critical in cancers that are not driven by hyperactivated Wnt signaling. This is demonstrated, for example, by recent large-scale CRISPR or shRNA screens that found that loss of β-catenin generally has no obvious effect on *in vitro* cell survival and proliferation of non-Wnt-driven cancer cell lines [38–40]. In contrast, loss of MYC significantly impacted cell survival or proliferation in the majority of the cancer cell lines across multiple tissues, reflecting that high MYC activity is regulated and maintained by diverse oncogenic signaling pathways beyond Wnt in the non-Wnt-driven cancers. This suggests that the amplified Wnt/MYC axis, as well as the Wnt regulation of cancer cell proliferation, is part of the "oncogene addiction" phenomenon in Wnt-driven cancers.

Last but not least, even though Wnt signaling may not be the main driving force of cell proliferation in non-Wnt-driven cancers, it can sustain proliferative signaling under situations where the original proliferative pathways are blocked, e.g. by anti-cancer drugs, and thereby mediate drug resistance [25]. For example, bromodomain and extra terminal protein (BET) inhibitors like JQ1 disrupt the BRD4-chromatin interaction and repress BRD4-dependent transcription of genes such as *MYC* to suppress cancer progression. However, two independent studies on leukemia found that in the presence of BET inhibitors, β-catenin binds to the promoter loci originally occupied by BRD4 and maintains the expression of key target genes including *MYC* [41, 42]. Therefore Wnt/β-catenin signaling can promote resistance to BET inhibitors in leukemia by sustaining proliferative signaling.

3 Evading growth suppressors

Normal cells rely on several growth suppression mechanisms to avoid aberrant and uncontrolled cell growth and proliferation. Evading these growth suppressors is necessary for tumorigenesis and is important during cancer progression. As mentioned previously, the cell cycle is positively driven by cyclins and CDKs, but it is also tightly regulated by multiple negative regulators, including the Cyclin-Dependent Kinase Inhibitor proteins (CKIs, or *CDKNs*). CKIs antagonize the kinase activity of the Cyclin/CDK complexes and maintain the CDK substrate Retinoblastoma (RB) protein in a hypo-phosphorylated state. The hypo-phosphorylated RB binds to E2F transcription factors, masking their transcriptional activation domain and, in some cases, converting them into transcription repressors [43–45]. As the E2F-mediated transcription of cell cycle genes, such as *Cyclin E (CCNE1/2)* [46], is important for progression from G1 to S phase during a cell cycle [47], activation of CKIs eventually arrests the cell cycle in G1 phase.

Importantly, several CKIs are directly or indirectly repressed by Wnt/β-catenin signaling. For example, p16, encoded by $p16^{INK4A}$ (*CDKN2A*), is an inhibitor of CDK4. β-catenin/LEF1 complex directly binds to the promoter of $p16^{INK4A}$ and suppresses its transcription in melanoma [48]. Expression of stabilized β-catenin in melanocytes promoted their immortalization and prevented senescence *in vitro* by suppressing $p16^{INK4A}$ expression [48]. Moreover, the Wnt target, MYC, suppresses the transcription of multiple other CKIs including *CDKN1A* (p21), *CDKN1B* (p27), and *CDKN2B* (p15) [33]. Collectively, Wnt signaling enhances the cell cycle progression by downregulating several important CKIs. Consistent with this, inhibition of Wnt signaling by a PORCN inhibitor in Wnt-addicted pancreatic cancer models upregulated CKIs such as CDKN1A (p21) and CDKN2B (p15), contributing to the slowdown of cancer cell proliferation [30, 49, 50].

Cellular differentiation provides an additional layer of proliferation suppression. In normal adult tissues, the stem

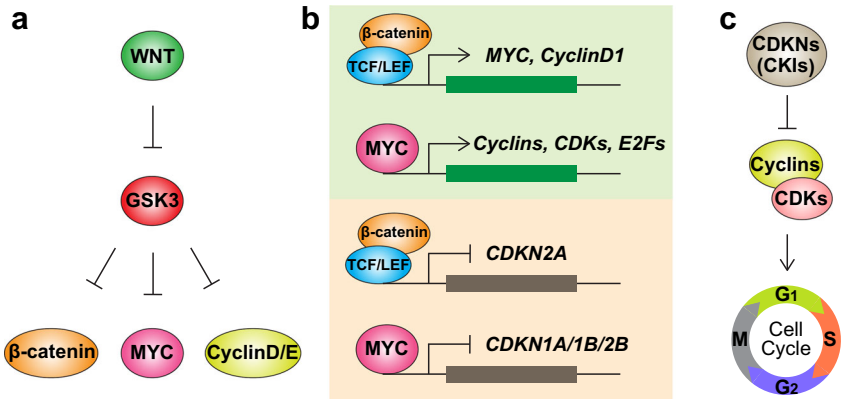

Fig. 2 Wnt signaling promotes cancer cell proliferation. **a** The Wnt/GSK3 axis regulates the protein abundance of β-catenin, MYC, and Cyclin D/E. **b**, **c** β-catenin and MYC activate the transcription of pro-proliferation genes and suppress the transcription of anti-proliferation genes (*CDKNs*)

cell pool maintains itself at a constant size. Stem cells and/or progenitor cells give rise to specified daughter cells through differentiation. While the stem cells and progenitor cells can replicate, the terminally differentiated cells are normally nonproliferative. Therefore, differentiation can limit a cell's proliferation potential. Similarly, within a tumor cell population, a subset of the cancer stem cells undergo self-renewal and generate differentiated cancer cells, contributing to the intratumor heterogeneity [51]. Different from the differentiated cells in normal tissues, non-stem cancer cell populations of the tumors can be highly proliferative due to the accumulated genetic alterations and aberrant signaling networks. But compared with the cancer stem cells, non-stem cancer cells are generally more susceptible to conventional chemo- and/or radiotherapy and lack robust tumor-initiating capacity. Therefore, it is the cancer stem cells that frequently mediate therapy resistance and cancer relapse [52, 53]. It is well established that Wnt signaling maintains stemness in various adult tissues and in cancers [20, 21, 25]. Blockade of Wnt signaling in Wnt-addicted cancers, such as the *RSPO3*-fusion colorectal tumors and *RNF43*-mutant pancreatic tumors, did not induce acute apoptosis but mainly led to cell cycle arrest and cellular differentiation [29, 30, 50, 54, 55]. Therefore, through maintaining stemness, Wnt signaling protects cancer cells from the differentiation program and helps them to evade growth suppression.

Of note, a recent study found that doxorubicin-induced senescence activated Wnt/β-catenin signaling in several cancer models [56]. Even though there is no evidence showing that the activated Wnt signaling could promote escape from cell cycle arrest in these doxorubicin-induced senescent cells, the activated Wnt signaling conferred a stemness signature on these senescent cells and significantly enhanced the tumor-initiating capacity of cancer cells released or escaping from senescence [56].

4 Wnt signaling in resisting cell death

Programmed cell death is an elegant system that safeguards the "community" by selective "suicide" of cells during development or when they are damaged beyond repair. Cancer cells experience various stresses during tumorigenesis due to imbalanced proliferative signals from oncogenes as well as high mutational burden [9]. These signals can trigger apoptosis in normal cells but are exploited by cancer cells to overcome the death signals to allow progression to higher grade malignancy. Other forms of cell death, such as autophagy, have also been recognized as playing an important role in tumorigenesis. However, the opposing effects of autophagy on cancer cells have been reported (reviewed in [57]).

4.1 Wnt signaling and apoptosis

Given the role of Wnt signaling in promoting cellular proliferation as described above, it is reasonable to speculate that the Wnt signaling can support cell survival through inhibiting apoptosis. Indeed, an early report showed that overexpression of *WNT1* inhibits chemotherapy-induced apoptosis, by reducing cytochrome c release from the mitochondria and subsequent caspase 9 activation [58]. The Bcl-2 family of proteins convey the apoptotic signals from the sensors to the effectors, regulated by a counterbalance between anti- and pro-apoptotic Bcl-2 members. For example, in developing thymocytes, stabilized β-catenin binds to the promoter of an anti-apoptotic factor Bcl-X_L to increase its levels and support thymocyte survival [59].

The role of Wnt signaling in apoptosis seems to be context-dependent, especially in the case of Wnt inhibitors secreted Frizzled-related proteins (sFRPs). It has been reported that in breast cancer cell line MCF7, expression of sFPR1, also known as SARP2 (secreted apoptosis-related protein 2), sensitizes the cells to ceramide-induced apoptosis [60]. On the other hand, in glioma cells, both sFRP1 and sFRP2 promote survival after serum starvation but do not alter apoptosis induced by cytotoxic agents [61]. sFRP1 also protects periodontal ligament fibroblasts (PDLFs) against ceramide or force-induced apoptosis, through downregulation of the pro-apoptotic proteins Bax and Bik [62]. To complicate matters, recombinant sFRP1 shows biphasic effects on β-catenin stabilization *in vitro*, with low concentration of sFPR1 stabilizing β-catenin but high concentration having the opposite effects [63].

4.2 Wnt signaling and autophagy

Autophagy is a cellular adaptive response, in which cells digest their own components, degrading them in autophagosomes to recycle the nutrients. Autophagy is upregulated during nutrient starvation or when cells are trying to get rid of damaged proteins or organelles (reviewed in [64]). Specific cargos can be targeted to autophagosomes with the help of receptor proteins such as p62 (SQSTM1). p62 recognizes polyubiquitinated proteins and binds to Atg8/MAP1LC3 (microtubule associated protein 1 light-chain 3 or LC3) [65]. The levels of membrane-bound LC3 or LC3-II are often correlated with the number of autophagosomes [66].

The involvement of autophagy in tumorigenesis is complex as it can both prevent and promote tumor growth. It has been shown that induction of autophagy, either by the mTOR inhibitor rapamycin or by nutrient deprivation, reduces Wnt signaling by promoting DVL2 degradation [67]. Upon starvation, DVL2 is ubiquitinated by an E3 ligase complex containing Von Hippel-Lindau protein (pVHL) that increases the association of DVL2 with p62 and its subsequent binding to

LC3. [67]. In addition to regulating the abundance of DVL2, the β-catenin/TCF4 complex is shown to occupy the p62 promoter to repress its transcription in colorectal cell line HT29 [68]. Upon autophagy induction, β-catenin undergoes proteasome-independent degradation through direct binding to LC3, which leads to p62 activation [68]. Consistent with this study, induction of autophagy is shown to decrease Wnt signaling in multiple cancers types, including colorectal cancers, non-small cell lung cancer (NSCLC) and glioblastoma (GBM) [69] [68, 70]. Supporting the reverse relationship between β-catenin and p62, inhibition of Wnt signaling by either DKK1 or gene silencing of *TCF4* or *CTNNB1* upregulates p62 and increases autophagic flux in GBM cells [71]. Furthermore, dual inhibition of TCF and autophagy sensitizes GBM cells to apoptosis through activation of caspase 8 [71]. Similarly in NSCLCs, Wnt inhibitory factor (WIF1) induces autophagy, through the activation of PI3K/mTOR pathway, leading to DVL2 degradation and β-catenin downregulation [69]. However, unlike GBM, blocking autophagy in WIF1-expressing NSCLCs attenuates apoptosis [69]. This implies that the level of autophagy tolerated by cells is tightly regulated and is also cell type dependent.

5 Enhancing replicative immortality

Cancers, being immortal, require active maintenance of telomeres that cap the ends of chromosomes [72]. Telomeres are synthesized by telomerase, which is made up of reverse transcriptase (TERT) and the telomerase template, *TR*. The expression of *TERT* is the rate-limiting step for the activation of telomerase and is observed in the vast majority of human cancers. Consistent with this, colorectal cancers driven by β-catenin-stabilizing *APC* mutations have upregulation of telomerase activity. Substantial evidence in mice and humans suggests this can be directly through β-catenin activation of *TERT* expression. Increased telomerase activity is found in both the intestinal mucosa and pre-malignant polyps in individuals with familial adenomatous polyposis (FAP) [73–75]. Mechanistically, Wnt/β-catenin signaling can regulate the expression of mouse TERT in diverse settings including embryonic stem cells, intestinal crypts, and neural stem cells, where knockout of β-catenin markedly decreases *mTERT* expression and activity. This appears to be a direct effect, as Wnt-stabilized β-catenin is found at the promoter of mouse *TERT* in chromatin immunoprecipitation studies [76]. Knockdown of β-catenin also markedly decreased *hTERT* expression and telomerase activity in human cancer cells, working through TCF4(TCF7L2) binding to the *hTERT* promoter [77]. Finally, MYC, a direct target of Wnt/β-catenin signaling, independently activates *TERT* expression [78]. Indeed, both Wnt/β-catenin signaling and MYC expression regulated *TERT* expression in our recent studies of Wnt-addicted pancreatic and colorectal cancers [30, 54] (Fig. 3).

Is the converse true? Do telomeres and telomerase regulate Wnt signaling? While it had been proposed that TERT protein regulates Wnt target gene expression, more recent studies have not borne this out [79, 80]. However, telomere dysfunction can influence the Wnt pathway. Critical shortening of telomeres in the mouse intestine triggered a p53-dependent pathway that decreased Wnt/β-catenin signaling. Interventions such as RSPO1 or LiCl therapy that increased Wnt signaling ameliorated both the defects in telomere capping and the accompanying crypt dysfunction [81]. In summary, Wnt/β-catenin signaling in cancer activates telomerase to allow replicative immortality.

6 Wnt signaling and angiogenesis

Angiogenesis is the process where new blood vessels are formed through sprouting from existing vessels. Angiogenesis occurs during embryonic development and in physiological as well as pathological conditions, including tumorigenesis. Absence of vascularization would limit tumor growth due to the lack of nutrient transport and waste clearance. Angiogenesis starts with breakdown of the basement membrane, followed by proliferation and migration of the endothelial cells that finally proceeds to vessel formation and recruitment of other cell types.

The breakdown of the basement membrane is mediated by metalloproteases (MMPs) to facilitate the sprouting of newly formed blood vessels. MMP-7, also known as matrilysine, is upregulated in the majority of the benign intestinal adenomas formed in $APC^{min/+}$ mice [82]. *MMP7* is a direct target of β-catenin/TCF4 and has two TCF binding sites in its promoter [83]. Another Wnt target, chemokine interleukin 8 (*IL8*),

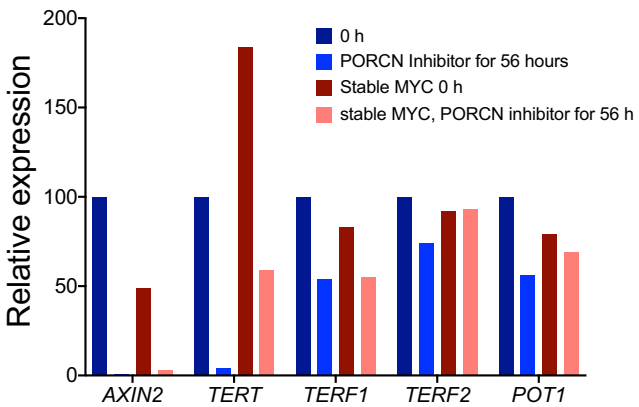

Fig. 3 Telomerase components *TERT*, *TERF*, and *POT1* are targets of the Wnt/β-catenin and MYC signaling. Relative gene expression in a Wnt-addicted pancreatic cancer xenograft before and 56 h after the inhibition of Wnt secretion. One set of xenografts expressed stabilized MYC (T58A) from a Wnt-independent promoter. *AXIN2* is included as a positive control. Primary data reported in [30]

induces MMP2 and MMP9 production, facilitating endothelial cell migration and subsequent capillary tube formation [84, 85]. In addition to regulating MMP, IL8 also reduces apoptosis of the human umbilical vein endothelial cells (HUVEC) by upregulating anti-apoptotic proteins Bcl-$_{XL}$ and Bcl-2 as well as downregulating Bax [85].

Vascular endothelial growth factor (or VEGF-A) is a key pro-angiogenic molecule that promotes endothelial proliferation, migration, and survival (reviewed in [86]) by binding to VEGF receptor tyrosine kinases expressed on the endothelium. Of the two VEGF receptors VEGFR1 (Flt-1) and VEGFR2 (Flk-1/KDR), VEGFR2 is the primary mediator for the mitogenic and permeability enhancing effects of VEGF [86]. VEGF expression is under multiple layers of modulation. For example, it can be induced by hypoxia-inducible factor 1 (HIF1), a major mediator of hypoxia response [87]. *VEGF* promoter contains TCF4 binding sites [88], and its levels are increased upon overexpression of activated β-catenin in normal colon epithelial cells [89]. The role of Wnts in regulating angiogenesis is also substantiated by the studies showing that conditional deletion of WNT7 co-receptor *Gpr124* in adult mice results in blood-brain barrier (BBB) malfunction and tumor hemorrhage in a GBM mouse model. This phenotype could be rescued by constitutive activation of β-catenin signaling in endothelial cells [90, 91]. This WNT-GPR124-FZD cell surface receptor complex is further modulated by RECK, a GPI-anchored membrane protein that binds to the extracellular domain of GPR124. Genetic studies clearly show that RECK is required for central nervous system (CNS) angiogenesis and BBB integrity and function through the assembly of the WNT7A/WNT7B/Frizzled-GPR124-RECK complex [92].

7 Wnt signaling in activating invasion and metastasis

During cancer progression, cancer cells from the primary tumor invade adjacent normal tissues, metastasize to distant sites, and form new colonies. A total of 90% of cancer-associated mortality is estimated to be due to metastasis [93]. Cancer cells exhibit phenotypic plasticity of their epithelial/mesenchymal status. Cancer cells with a more epithelial phenotype maintain cell-cell contact and are relatively well organized in a polarized layer, whereas cancer cells that are more mesenchymal lose cell-cell adhesion and gain increased motility. Although questioned in some studies [94–96], epithelial-to-mesenchymal transition (EMT) has been proposed to play a crucial role in the invasion of cancer cells into normal tissues and circulatory system. After distant colonization of circulating tumor cells, the reverse process, mesenchymal-to-epithelial transition (MET) restores the epithelial phenotype facilitating the formation of metastatic tumors [97]. Wnt signaling is involved in both EMT and MET processes to promote cancer metastasis.

Soon after the discovery of *APC* mutations in the 1990s, it was found that β-catenin accumulation and localization is very heterogeneous within the same colorectal tumor. Even though all the cancer cells within the same tumor harbored the same oncogenic mutation, β-catenin was mainly localized to the cell membrane and cytoplasm in the central areas whereas nuclear β-catenin was found at the invasive front of the tumor [98, 99]. In line with this, tumor cells in the central areas were maintained as a polarized epithelium expressing membrane E-cadherin, while tumor cells at the invasive front showed dedifferentiated mesenchymal-like features and lacking membrane E-cadherin [99]. It was subsequently found that in colorectal tumors, the tumor-adjacent myofibroblast-secreted factors, specifically hepatocyte growth factor (HGF), further stimulate Wnt/β-catenin signaling in the cancer cells and confer cancer stem cell traits that are closely related to EMT [100]. This explains the high β-catenin signaling observed at the invasive front of colorectal tumors. To date, the relationship between the β-catenin distribution pattern within the colorectal tumor and the cancer prognosis remains controversial [101–104]. But in one study, the presence of nuclear β-catenin at the invasive front of colorectal tumors was significantly linked to aggressive tumor histological features, metastatic status, and poor cancer prognosis [104]. In addition to colorectal cancer, in hepatocellular carcinoma (HCC), high levels of β-catenin are associated with enhanced metastasis and poor prognosis. In HCC, hypoxia induced downregulation of GSK3β protein abundance increased β-catenin accumulation and activation. This promoted both EMT and *in vivo* metastasis, and could be reversed by β-catenin knockdown. In a HCC tissue microarray, positive expression of β-catenin was associated with the expression of HIF-1α, a marker of tumor hypoxia [105]. Collectively, these findings suggest that Wnt/β-catenin signaling in colorectal cancers and HCC is regulated by the tumor microenvironment and is involved in EMT and cancer cell invasion and metastasis.

Mechanistically, how Wnt signaling promotes EMT remains largely unclear. While it has been demonstrated that Wnt/GSK3β axis regulates the phosphorylation/degradation of Snail, an important mediator of EMT [106], little is known about how the Wnt/β-catenin transcriptional activity regulates the EMT programs. In the HCC study mentioned previously [105], β-catenin knockdown downregulated the mRNA levels of EMT regulators Slug and Twist, but whether it is directly controlled by the β-catenin/TCF transcription complex or not remains to be elucidated.

Wnt signaling is also involved in MET and metastatic outgrowth. A recent study found that E-selectin in the bone vascular niche directly binds to cancer cells and induces MET. E-selectin-induced MET downregulated secreted Wnt repressors such as DKK1, thus activating Wnt/β-catenin signaling that

confers cancer stemness. Collectively, this promoted metastatic colonization and outgrowth in the bone. Importantly, treatment with LF3, a small molecule that disrupts the interaction between β-catenin and TCF4, reduced the bone metastasis burden, further supporting the role of Wnt/β-catenin signaling in the process of bone metastasis [107].

In addition to these well-documented roles of Wnt signaling in EMT/MET that contribute to metastasis, Wnt signaling is also reported to promote metastasis in several cancer types *via* additional mechanisms. In prostate cancer, TBX2 expression level correlates with the potential of metastasis. TBX2 directly binds to the *WNT3A* promoter and activates its transcription. WNT3A upregulates the downstream targets *MMP2*, *MMP9*, and *IL-6* that promote bone metastasis [108]. In lung adenocarcinoma, activation of the canonical Wnt/TCF pathway was identified as a determinant of metastasis to brain and bone. Metastatic subpopulations isolated from lymph node–derived lung adenocarcinoma cell lines harbor hyperactive Wnt/TCF signaling, while reduction of the TCF activity in these cells attenuated their ability to form brain and bone metastases in mice. It was found that the Wnt/TCF target genes *HOXB9* and *LEF1* are mediators of the chemotactic invasion and colony outgrowth [109]. Wnt/β-catenin signaling is regulated by multiple factors including SOX30, miR-128-3p, and miR-150-5p to regulate metastasis in lung cancer [110–112]. In breast cancer, loss of p53 in cancer cells induced the secretion of Wnt ligands that stimulate tumor-associated macrophages to produce IL-1β. This drove systemic inflammation and promoted metastasis [113]. In colon cancer, high nuclear concentrations of both FOXO3A and β-catenin correlated with metastatic stage. FOXO3A and β-catenin co-regulate metastasis-relevant genes such as *IQGAP2*, *CYR61*, and *CLDN1*, which are involved in cell-to-cell contacts, cell scattering, and/or cell motility. Of note, there was no increase of the key EMT transcription factors *SNAIL*, *SLUG*, or *ZEB1* upon co-expression of FOXO3A and β-catenin in the *in vitro* cell line model, suggesting that this could be a canonical EMT-independent process [114].

Besides canonical Wnt signaling, the noncanonical Wnt pathways are also involved in cancer metastasis. In breast cancer, fibroblast-secreted exosomes mobilized breast cancer cell-secreted WNT11 and promoted breast cancer cell protrusive activity and motility *via* Wnt/planar cell polarity (PCP) signaling, enhancing metastasis [115]. In pancreatic cancer and prostate cancer, RNA-seq of the circulating tumor cells (CTCs) revealed activation of noncanonical Wnt signaling, mediated mainly by WNT2 and WNT5A/7B, respectively. WNT2 signaling promoted anchorage-independent cell survival and metastasis in pancreatic cancer, while WNT5A promoted resistance to androgen receptor inhibition in prostate cancer [116, 117].

Last but not least, Wnt signaling maintains cancer stemness in multiple human cancers [25], and an increasing number of studies reveal that cancer stem cells may directly and/or indirectly contribute to metastasis [118, 119], while the underlying mechanisms can be diverse and remain to be studied. This provides yet another mechanism whereby Wnt signaling promotes cancer metastasis.

8 Wnt signaling and genome instability and mutation

The various growth advantages acquired during tumorigenesis, including the above mentioned hallmarks, are gained mostly through genomic alterations of important oncogenes and tumor suppressor genes, leading to subsequent clonal expansion of the most "fit" cells. This increased mutation rate allows the cells to evolve and/or to evade the surveillance mechanism and avoid senescence or programmed cell death. With the advancement of nextGen DNA sequencing, the analysis of the human cancer genome reveals that there are distinct patterns of DNA mutations in different types of cancer [9, 120]. This implies that each cell type of origin is subjected to different DNA damaging agents and utilizes certain cellular pathways for damage repair or to overcome the growth restriction imposed by checkpoint activation.

8.1 Wnt pathway and chromosome instability

Genome instability can take various forms, ranging from single nucleotide mutations to chromosome rearrangement and aneuploidy. A few reports have suggested a role of Wnt signaling in chromosome instability (CIN), mainly through the function of APC (reviewed in [121]). Besides the well-known function of APC as the scaffolding protein and negative regulator in the β-catenin destruction complex, APC is also involved in microtubule dynamics (reviewed in [122]). Full-length APC protein binds to microtubules stabilizing them both *in vivo* and *in vitro* [123–125]. Interestingly, GSK3β, whose activity is partially regulated by the interaction of Wnts with their receptors, phosphorylates APC, and decreases the interaction between APC and microtubules [125]. During mitosis, APC is localized to kinetochores [126] and centrosomes [127]. There are some discrepancies among different studies regarding whether depleting APC affects spindle assembly checkpoint (SAC) function [121]. Another defect that has been observed is reduced interkinetochore distance in cells either expressing an N-terminal fragment of APC [128] or following APC depletion [129]. Shortened interkinetochore distance implies weaker kinetochore-microtubule interaction and thus reduced interkinetochore tension. Moreover, spindle misorientation in metaphase was also observed in APC-depleted HeLa cells, resulting in defects in chromosome segregation [129].

Another mechanism by which APC can induce chromosomal instability is through AXIN2 (also called Conductin)

upregulation [130]. AXIN2 binds to polo-like kinase 1 (PLK1) and may compromise the mitotic spindle checkpoint function [130]. The product of a near-universal Wnt target gene, AXIN2, has also been found at the centrosome, where it regulates centrosomal β-catenin phosphorylation and promotes centrosome cohesion [131]. Interestingly, this function of β-catenin is independent of its transcriptional activity [131]. AXIN, a homolog of AXIN2 and an important component of the β-catenin destruction complex, is also localized to the centrosome. It interacts with γ-tubulin and is involved in microtubule nucleation at the centrosome [132]. An *in vivo* animal study found increased aneuploidy and tetraploidy in the intestines of the $APC^{min/+}$ mice, which could be attributed to cytokinesis failure caused by the APC mutation [133].

One important question to ask is whether Wnt/β-catenin signaling is required for Chromosomal instability (CIN) since APC's best-understood function is a negative regulator of β-catenin. β-catenin transcriptional activity seems to be required for CIN, as assessed by anaphase chromosome bridge index (ABI), because dominant-negative forms of TCF transcription factor rescued anaphase bridges induced by $APC^{+/D716}$ or $\beta\text{-catenin}^{Dex3}$ mutation in mouse ES cells and intestine polyps [134].

Another issue that remains difficult to reconcile is that APC is known to be one of the earliest events in the progression of cancer, but chromosome aberrations and LOH are more frequent in later stage and higher-grade tumors. We should note that earlier studies commonly used APC knockdown or overexpression, while in human pathogenesis, a truncated form of APC is usually present that has some interesting residual functions [135]. APC deficiency is permissive for CIN, but additional stresses during cancer progression may be required for this to be clinically manifest.

8.2 Wnt signaling and DNA repair pathway

DNA damage response and repair systems play a key role in maintaining genome integrity. Besides environmental genotoxic agents, various endogenous stimuli and metabolic by-products can also damage DNA. For example, reactive oxygen species (ROS)–induced oxidative stress as well as UV and ionizing radiation result in changes including base modifications, single-strand breaks (SSB), and double-strand breaks (DSBs). In response to damage, cell cycle checkpoints are activated so that DNA repair can be completed before cells enter the next cycle (reviewed in [136]). Many studies have suggested that stem cells and tumor-initiating cells are relatively resistant to genotoxic insults, probably though their increased DNA damage response activity [137, 138]. In this section, we focus on two specific aspects of the clinical relevance of Wnt signaling in DNA repair. First, we discuss how Wnt signaling modulation can affect tumors with mutations in the homologous recombination (HR) DNA repair pathway. Second, we review the evidence that Wnt signaling is implicated in tumor resistance to radiotherapy.

HR is an error-free process to repair DNA DSBs. Cells with mutations in *BRCA1/2* where HR is compromised are more prone to DSBs. If other DNA repair pathways are also compromised, such as the base excision repair (BER) pathway, then they are more susceptible to cell death. This provides the basic principle for synthetic lethality therapy. Poly(ADP-ribose) polymerases (PARPs) catalyze the transfer of ADP-ribose groups to target proteins. Among the PARP family members, activation of PARP1 is the best understood (reviewed in [139]). PARP1 zinc finger domains bind DNA nicks and are required for both BER and single-strand break repair (SSBR) [140]. The PARP inhibitor, olaparib is effective in HR deficient ovarian carcinomas [141, 142]. However, resistance can occur following PARP inhibitor therapy by various mechanisms. Recently, it has been reported that in high grade serous ovarian carcinoma, there is increased Wnt activation in olaparib-resistant cancer cells compared with their matched controls [143]. Interestingly, these Wnt-high olaparib-resistant cells have an elevated DNA repair capacity, including both HR and nonhomologous end joining (NHEJ). However, a combination of Wnt pathway inhibitor pyrvinium pamoate, that functions through activating casein kinase 1a (CSNK1A, CK1α) [144] with olaparib was not highly beneficial in preventing growth of these cells [143]. One caveat of this study is that pyrvinium pamoate is not a specific Wnt inhibitor, and it also inhibits Hedgehog (Hh) signaling by reducing the stability of the transcription factor Gli1 [145]. On the other hand, supporting the role of Wnts in DNA repair we observe that inhibition of Wnt signaling with a Wnt secretion inhibitor ETC-159 and Olaparib synergistically prevents the growth of several Wnt-high cancers (Kaur et al., manuscript in preparation, 2020).

Wnt signaling is also involved in preventing tissue damage induced by ionizing radiation (IR). DSBs are the molecular lesions caused by IR and are mainly repaired by nonhomologous end joining (NHEJ). It has been reported that β-catenin expression directly activates DNA ligase 4 (LIG4) that is required for NHEJ, driving radioresistance in intestinal stem cells (ISC) and colorectal cancers (CRC) [146]. Interestingly, LIG4 is highly enriched in crypts, particularly in LGR5+ crypt base columnar cells (CBCs) and + 4 position intestinal stem cells (ISCs), and is upregulated in CRC. Blocking β-catenin binding to TCF using iCRT14 or inhibiting LIG4 activity using the small molecule SCR7 re-sensitized CRCs to radiation [146]. Collectively, these studies suggest that high Wnt signaling is associated with enhanced capacity of the cells to repair DSBs by HR or NHEJ. Therefore, combination therapies inhibiting Wnt signaling might be useful for the treatment of cancers that have acquired Wnt-driven resistance to radiation and PARP inhibitors.

9 Wnt signaling and reprogramming energy metabolism

Wnt signaling was first identified as an oncogenic pathway in mammals and is well known for its roles in cancer and stem cell biology. More recently, it has become clear that this developmental pathway is also involved in metabolic regulation in normal tissues as well as in metabolic diseases such as obesity and diabetes [147–150]. This has been extensively reviewed previously [151].

Cancer cells maintain different cellular metabolism compared with normal tissues. In the early last century, it was observed that cancer cells have elevated glycolysis even in aerobic conditions, an inefficient mechanism compared with oxidative phosphorylation in terms of ATP production. This phenomenon was termed the Warburg effect after its discoverer. Decades of research has demonstrated that the Warburg effect is a mechanism to support the biosynthetic requirements of highly proliferative cancer cells. The increased products of glucose catabolism can be used as a carbon source for the biosynthesis of nucleotides, amino acids, and lipids that are essential for rapidly dividing cells [152]. Such reprogramming of energy metabolism is closely associated with core cancer hallmarks such as sustained proliferation and evasion of growth suppression. Due to its widespread importance in cancer, metabolic reprogramming has been proposed to be an additional hallmark of cancer [9]. Mechanistically, reprogramming of energy metabolism in cancer can be mediated by activation of oncogenes (e.g. *KRAS* and *MYC*) and/or silencing of tumor suppressor genes (e.g. *TP53*).

Recent studies reveal that Wnt signaling is also involved in this process [153]. β-catenin is frequently activated in human liver cancer [25]. Proteomic analysis in mouse liver showed that oncogenic β-catenin activation by *Apc* deletion led to differential expression of many metabolic pathway components. β-catenin activation upregulated lactate dehydrogenase (LDH) but downregulated two mitochondrial ATPase subunits (ATP5a1 and ATP5b), consistent with a metabolic switch from oxidative phosphorylation to glycolysis [154]. In colon cancer, blocking Wnt/β-catenin signaling by expressing dominant-negative TCF/LEF mutants altered the expression of multiple metabolism-related genes. Interestingly, interference with Wnt signaling downregulated glucose consumption and glycolysis but increased ATP production, suggesting an increased utilization of oxidative phosphorylation over glycolysis upon Wnt inhibition [155]. Mechanistically, *pyruvate dehydrogenase kinase 1* (*PDK1*) is a direct transcriptional target of Wnt/β-catenin. Wnt/β-catenin activates PDK1 to inhibit pyruvate flux to mitochondrial respiration, thereby promoting glycolysis [155]. Moreover, as mentioned previously, Wnt/GSK3 signaling controls the protein abundance of Snail [106]. A Wnt/Snail axis inhibits the expression of three subunits of cytochrome C oxidase (COXVIc, COXVIIa, and COXVIIc), suppressing mitochondrial respiration and promoting glycolysis in breast cancer cells [156]. Recently, our group also found that inhibiting Wnt signaling *via* a PORCN inhibitor in Wnt-driven pancreatic cancer xenografts downregulated the expression of multiple glycolysis-related genes (*GPI*, *GAPDH*, *PGK1*, *PGAM1*, *ENO1*, and *PKM*) and reduced the glucose metabolic flux [50].

While a number of these abovementioned metabolism-related genes are directly bound and regulated by the β-catenin/TCF or Snail factors, others can be indirectly regulated by the Wnt signaling, e.g., through the Wnt target MYC. MYC regulates a large fraction of the transcriptome and is a master regulator of cellular metabolism. It regulates glycolysis, glutaminolysis, mitochondrial bioenergetics, nucleotide synthesis, and/or lipid synthesis in both normal tissues and tumors. This has been extensively reviewed previously [35, 36, 157]. MYC can promote the Warburg effect by upregulating the expression of glycolysis-related genes including the glucose transporter and glycolytic enzymes. MYC also promotes mitochondrial biogenesis and function. In a word, MYC regulates glucose metabolism *via* complex and diverse mechanisms.

10 Wnts and tumor-promoting inflammation

The tumor microenvironment consists of a variety of cell types, and multiple complex interactions occur between the tumor cells, extracellular matrix components, and the host cells including immune infiltrating cells and stromal cells. With the success of immunotherapies including immune checkpoint inhibitors to treat cancers, studies that enhance our understanding of the role of Wnts in two emerging hallmarks of cancer, promoting inflammation and evading immune destruction, have grown.

Inflammation is part of the body's response to internal and foreign stimuli such as infection and injury and serves to eliminate the causative agents and restore normal tissue physiology. A short-lived inflammatory response is usually beneficial, but chronic inflammation has pathological consequences such as cardiovascular diseases, diabetes, and rheumatoid arthritis. The role of chronic inflammation in cancer has long been recognized as important. More than one-fourth of all cancers are related to chronic infections and inflammation such as hepatitis, asthma, and colitis [158]. Virtually all neoplastic lesions contain immune cells. Cancers hijack the immune cells and inflammation to enhance tumorigenesis and its progression. Supporting this, a positive correlation exists between the prolonged use of nonsteroidal anti-inflammatory drugs and reduced risk of developing cancers [159]. Importantly, cancers promote immune tolerance, helping them to evade immune destruction. The inflammation present in the tumor microenvironment is characterized by the infiltration of tumor-associated macrophages (TAM), mast cells, dendritic cells,

natural killer cells, neutrophils, eosinophils, and lymphocytes. These cells produce a variety of cytotoxic mediators such as reactive oxygen and nitrogen species, proteases, matrix metalloproteinase, cytokines, and chemokines such as tumor necrosis factor α and interleukins (IL-1, IL-6, IL-8), among others, to facilitate cancer progression by promoting angiogenesis and cancer metastasis. They can also contribute to tumorigenesis through immune suppression. Here we briefly review the role of Wnt signaling in regulating the differentiation, maturation, and/or function of some of these immune cells.

10.1 Macrophages

Macrophages help in defense against infection and facilitate wound healing. They migrate to the sites of inflammation in response to chemoattractants such as monocyte chemotactic protein (MCP-1) to promote wound healing and maintain tissue homeostasis by releasing cytokines and growth factors. Monocytes are recruited from the circulation in response to cytokines and chemokines produced by T cells and other tumor cells and differentiate into macrophages, known as tumor-associated macrophages (TAMs). TAMs secrete angiogenic factors such as vascular endothelial cell growth factor (VEGF), contributing to vascularization of the tumors that allows their continued growth. TAMs also help in remodeling the extracellular matrix by secreting collagen, proteases, and matrix metalloproteinases (MMPs) that promote extravasation hence promote metastasis. Macrophages have historically been classified into two types: proinflammatory M1 type and anti-inflammatory M2 macrophages. However, the phenotypes of macrophages are more complex and a spectrum of macrophage types exist. The tumor microenvironment is critical in shaping the identity and functionality of the TAMs, and in this, Wnt signaling gradients present in the cancers play an important role. Macrophages, in addition to responding to Wnt signaling, also produce multiple Wnts, hence contributing to tumor cell progression.

Several studies support the role of macrophage-produced Wnts in tumorigenesis. Conditional deletion of *Wnt7b* in macrophages using colony-stimulating factor 1 receptor (*Csf1r-icre*) in a mouse mammary tumor model reduced both tumor growth and lung metastasis. This was attributed to a failure of angiogenesis due to reduced *Vegf* mRNA expression in the endothelial cells, demonstrating the importance of macrophage-produced Wnts in tumor growth and invasion [160]. As mentioned before, *Vegf* is a Wnt target gene. In another study, it was shown that the Wnt signaling pathway is specifically activated in the subpopulation of TAMs at the invasive front of the tumors that promote tumor metastasis and angiogenesis. These TAMs also expressed higher levels of *Wnt5a* and *Wnt7b* compared with other tumor macrophages [161]. In a cholangiocarcinoma model, depletion of macrophages or systemic inhibition of Wnt signaling with PORCN inhibitors reduced the tumor burden *in vivo* [28]. Increased infiltration of macrophages was observed in gastric cancers with high Wnt signaling. Moreover, macrophages were required for the development of intestinal polyposis caused by Wnt activation, and the loss of macrophages reduced both the size and number of polyps [162]. WNT5A levels were high in macrophages co-cultured with breast cancer cells and were shown to be essential for macrophage-induced invasiveness by increasing tumor cell proteolytic activity and migration [163]. In another study, it was shown that intra-epithelial macrophages in mammary tumors respond to CCL2, which, in turn, can stimulate macrophages to produce Wnt-1. This leads to the disruption of E-cadherin junctions between early cancer cells, resulting in early dissemination of cancer cells to the lung [164]. All these studies highlight that one role of Wnt signaling in tumor progression is to shape the identity and activity of the macrophages.

10.2 Dendritic cells

Dendritic cells (DCs) are professional antigen-presenting cells that process and present antigens to T cells, leading to their activation, expansion, and differentiation. DCs differentiate into distinct subsets including peripheral CD103+ DCs that are essential for the recruitment of effector T cells into the tumors. Conversely, some DCs suppress T cell responses by promoting T cell apoptosis and enhancing development of regulatory T cells (Tregs) and hence help in maintaining immunological tolerance to self-antigens. Manicassamy et al. reported that β-catenin signaling programs DCs to a tolerogenic state, limiting the inflammatory response by promoting the expression of the immunosuppressive cytokines, TGF-β and IL-10, while also suppressing the expression of the proinflammatory cytokines IL-6 and IL-12 by the DCs. They showed that genetic ablation of β-catenin expression in DCs led to a significant reduction in Tregs within the intestinal epithelium and an enhanced inflammatory response in a mouse model of inflammatory bowel disease [165]. Follow-up studies showed that Wnts in the tumor microenvironment activate β-catenin in dendritic cells to condition them to a regulatory state that suppresses antitumor immunity. Both canonical (WNT3A) and noncanonical (WNT5A) Wnts induce a tolerogenic DC phenotype but *via* distinct patterns of cytokine production to promote $FOXP3^{+}$ regulatory T cell generation. WNT3A preferentially induces TGF-β and VEGF production, whereas WNT5A induces IL-10. Further, WNT5A, but not WNT3A, inhibits IL-6 production by DCs in response to the viral mimic polyinosinic:polycytidylic acid [166]. Consistent with this being a direct immunosuppressive effect of Wnt signaling, dendritic cell–specific deletion of Wnt co-receptors LRP5/6 delayed tumor growth and enhanced antitumor immunity. This block in Wnt signaling resulted in increased production of proinflammatory cytokines and

decreased production of IL-10, TGF-β, and retinoic acid (RA) leading to enhanced effector T cell differentiation and decreased regulatory T cell differentiation [167].

Melanomas have very active immune suppression *via* immune checkpoints. Melanomas with active β-catenin signaling have an absence of T cell infiltrate. This T cell exclusion was due to failure of CD103+ DC migration into the melanomas, thus excluding the host immune response. In these tumors, β-catenin activation reduced CCL4 chemokine expression that was required for the recruitment of the DCs *via* repression of ATF3-dependent transcription [168]. Further, increased β-catenin signaling upregulated IL-12 levels in melanomas that impaired DC maturation and induced formation of regulatory DCs leading to T cell exclusion. In line with these studies, analysis of T cell inflamed gene expression and activated Wnt/β-catenin signaling in cancers reported in The Cancer Genome Atlas revealed that non–T cell inflamed tumors had high activated Wnt/β-catenin signaling. High β-catenin signaling was defined by somatic mutation or copy number alterations in the Wnt pathway elements including β-catenin, APC, AXIN1, and AXIN2 [169].

Additional Wnt-dependent mechanisms also operate in melanomas to establish an immune-suppressed microenvironment. Melanoma cells were found to secrete WNT5A that metabolically reprogramed the DCs to produce indoleamine 2,3-dioxgenase-1 (IDO) enzyme in a β-catenin-dependent manner [170]. IDO converts the tryptophan amino acid into kynurenine, a compound capable of directly driving Treg differentiation [171]. Further delineating the mechanism of WNT5A induced tolerance, the authors found that β-catenin and PPAR-γ form a co-transcriptional activator complex in DCs that drives fatty acid oxidation (FAO) in DCs by upregulating the expression of the fatty acid transporter, carnitine palmitoyltransferase-1A (CPT1A). This FAO shift increases the synthesis of protoporphyrin IX prosthetic group required for regulation of IDO activity while suppressing IL-6 and IL-12 cytokine expression [172]. Further supporting the role of Wnts, the authors also showed that pharmacological inhibition of Wnt signaling with PORCN inhibitors synergistically enhanced the activity of anti-CTLA-4 antibody immunotherapy in murine melanoma model [170].

10.3 Mast cells

Mast cells are associated with promoting tumor growth and immune evasion [173]. They originate from pluripotent progenitor cells in the bone marrow and function as both positive and negative regulators of immune responses. Mast cells express CD34, KIT, and CD13 and produce matrix metalloproteinases and gelatinases that facilitate their migration to the tissues. The KIT ligand stem cell factor (SCF) is normally expressed by the stromal cells and fibroblasts in the tumors. Upon activation, mast cells release various proinflammatory factors such as IL-6 and TNF-α to modulate immune responses. They also secrete VEGF to facilitate tumor growth by promoting angiogenesis [173]. While facilitating tumor growth mast cells secrete immunosuppressive cytokines such as IL-10 that favor expansion of regulatory T cells to promote immune tolerance. Mast cells also contribute to tumor cell proliferation and invasion by secreting matrix metalloproteinases (MMPs). Only a few studies have recently explored the role of Wnt signaling in regulating mast cell activity. In DCs, the importance of Wnts in regulating the expression of VEGF and IL-10 is well established. MMPs are also known Wnt target genes. Whether Wnts regulate the expression of these genes in mast cells remains to be established.

Recent studies show that both murine and human mast cells express Wnt ligands and Wnt receptors. Murine bone marrow–derived mast cells express FZD4 and treatment of these cells with WNT5A promotes terminal differentiation *via* the Wnt/β-catenin pathway [174]. However, these data were not replicable with human mast cells. Human mast cells express Frizzleds 1, 2, 3, and 7 and LRP5/6, and in these cells, WNT3A activates mature mast cells to produce the chemokines IL-8 and CCL8 [175]. *In vitro*, the interaction between mast cells and pancreatic cancer cells has been shown to promote tumor growth and invasion and tumor-infiltrating mast cells are associated with poor prognosis in pancreatic cancer [176]. Further studies elucidating the role of Wnts in regulating differentiation and activity of mast cells in cancers would be useful and may elucidate a path to modulating the immune response in cancers.

10.4 Neutrophils

Tumor-associated neutrophils also play a central role in tumor inflammation and are increasingly recognized for their ability to promote tumor progression, mediate resistance to therapy, and regulate immunosuppression. They promote tumor initiation by the release of reactive oxygen and nitrogen species and proteases. High levels of neutrophils in tumors have been linked to poor prognosis in renal cell, pancreatic, head and neck, and esophageal carcinomas [177, 178]. Conversely, in gastric and colorectal cancers, they are associated with better survival [179, 180]. Evidence from various murine models shows that tumor and/or stromal cells express the ligands CXCL1, CXCL2, and CXCL5 that interact with the chemokine receptor CXCR2, expressed on neutrophils, to facilitate their invasion into tumors. Inhibition of neutrophil infiltration by genetic ablation of chemokine receptor CXCR2 in pancreatic tumors led to a T cell–dependent suppression of tumor growth [181].

WNT5A, a noncanonical Wnt, stimulates the production of CXCL8 and CCL2, which are potent chemoattractants for neutrophils and monocytes. Lymphoid enhancer-binding factor 1 (LEF1), which directs the binding of the Wnt effector β-

catenin to its target genes, mediates the proliferation, survival, and differentiation of granulocyte progenitor cells to neutrophils by regulating the expression of cell cycle regulators such as CCND1 and MYC. Additionally, LEF1 also directly regulates C/EBPα (CEBPA), a key transcription factor required for neutrophil differentiation. Hence, reduced levels of LEF1 lead to "differentiation block" at the promyelocyte stage of myelopoiesis leading to neutrophil cytopenia [182].

Similar to mast cells, future studies delineating the role of tumor-associated Wnts in regulating neutrophil function and differentiation are required for improving our understanding of Wnts in shaping the tumor immune microenvironment.

10.5 T cells

T cell infiltrates in cancers modify the disease progression as well as the response to immunotherapies. The simplest distinction between T cells is the CD4+ and CD8+ T cell subsets. CD8+ T cells are cytotoxic T cells that are activated by the DCs and co-stimulatory factors and are required for killing the target cancer cells. Tumor cells evade the immune response by excluding or inactivating CD8+ T cells. Wnt signaling is well known to have an important role in T cell development and differentiation [183, 184]. T cell factor (TCF), the effector transcription factor for the Wnt signaling pathway was so named due to its indispensable role in T cell development in the thymus [183]. TCF1 and Wnt/β-catenin signaling is highly activated in naive CD8+ T cells and memory T cells, while TCF1 is downregulated upon their differentiation and expansion into effector CD8+ T cells. Upon stimulation by the antigen presenting cells, the naive CD8+ T cells proliferate in the lymph nodes and spleen, and this process is attenuated by activation of Wnt/β-catenin signaling that leads to apoptosis of mature CD8+ T cells. This apoptosis requires the activation of the Wnt target gene *MYC* since *MYC* depletion inhibits the apoptosis of CD8+ T cells [185, 186].

Different CD4+ T cell (T helper) subsets have been identified to play an important role in tumors by either promoting or inhibiting antitumor responses. Conventionally, the CD4+ Th1 cells facilitate an antitumor response by secreting IFN-γ, hence stimulating the CD8+ T cells and NK cells. In contrast, the tumor resident regulatory T cells (CD4+ CD25+ FOXP3+) counteract tumor-specific immune responses by suppressing the infiltration and activity of CD8+ T cells and macrophages. CD4+ T helper cells are maintained at higher levels through activation of the key transcription factor GATA3. High levels of TCF1 and β-catenin support the polarization of T Cells to CD4+ helper cells by driving high expression of GATA3 [187, 188]. The expression of negative immunomodulators of T cells such as FOXP3 can be reduced by blocking the Wnt/β-catenin signaling pathway [189]. β-catenin expression in CD4+CD25 - naive T cells extends survival of regulatory T cells and induces unresponsiveness in effector T cells [190]. Another subset of CD4+ T cells are the Th17 cells that secrete IL-17A. The development of these Th17 cells is controlled by transcription factor RAR related orphan receptor C (RORγt), and their cell function is maintained by IL-23 signaling. Sustained activation of β-catenin in CD4+ T cells results in upregulation of RORγt and hence their polarization to Th17 type cells. Th17 cells express high levels of TCF1 and LEF1, downstream targets and effectors of Wnt/β-catenin pathway. These Th17 cells secrete a repertoire of cytokines that favors tumorigenesis. Enforced expression of β-catenin in intratumoral CD4+ T cells increased the expression of IL-17A, contributing to enhanced proliferation and inhibition of apoptosis of colorectal cancer cells [191, 192].

In summary, Wnt/β-catenin signaling regulates diverse stages of T cell development and lineage fate decisions. Overall, Wnt signaling favors the polarization of CD4+ Treg and Th17 subtypes, promoting the immunosuppressive microenvironment in tumors. Collectively these studies suggest that both canonical and noncanonical Wnts regulate differentiation, maturation, and infiltration of inflammatory cells and hence have an important role in regulating tumor immune microenvironment. While the role of Wnts in T cells, DCs, and macrophages is well established, further studies elucidating the role of Wnts in the functioning of neutrophils and mast cells would be useful for developing targeted immunotherapies.

11 Wnt signaling and evading immune destruction

The primary role of immune effectors such as macrophages, T cells, and DCs discussed in the previous section is to discriminate between healthy cells *vs.* the pathogens or tumor cells through TLR, MHC, and other receptors expressed on their cell surface. The immune cells mount an adaptive or innate immune response and protect healthy cells by adjusting the balance of activation *versus* inhibition of the immune response. However, cancer cells can evade detection by these immune cells through expression of cell surface molecules that mimic those that are expressed by the healthy cells. This prevents the infiltration of effector cells into the tumors. Furthermore, if the effector cells do infiltrate, tumor cells can induce their inactivation or death. The ligands expressed by the tumor cells to keep the immune system under control are called checkpoints. These "immune checkpoints" are used by tumors to evade the immune system. Hence, these tumors with active checkpoints lack the T cell inflamed immune microenvironment [193].

As mentioned in the previous section, a positive correlation between the activation of Wnt/β-catenin signaling and T cell exclusion was first identified in melanomas [168]. Tumor intrinsic activation of β-catenin signaling and non–T cell

inflamed phenotype was confirmed across multiple cancer types [169]. It was observed that almost half of the non–T cell inflamed tumor subsets show increased activation of Wnt/β-catenin signaling. The exclusion of T cells phenotype was partly due to the failure in the recruitment of DCs or the generation of suppressor DCs [194]. One mechanism by which tumors avoid immunity is by disrupting secretion of chemokines required for the recruitment of DCs and effector T cells. Among these chemokines, CCL4 is important for the recruitment of DCs. Stabilization of β-catenin was shown to reduce the expression of CCL4, hence impairing the activation of DCs and infiltration and priming of effector T cells [190]. In urothelial bladder cancers, an inverse correlation of WNT7B expression and the presence of CD8 positive T cells were also observed [195].

Other Wnt-regulated immune escape mechanisms involve the expression of checkpoint inhibitors such as programmed death ligand (PDL1) and CD47 that regulate the activity of T cells and macrophages in the tumors. CD47 is a transmembrane glycoprotein expressed on normal healthy cells and mediates a "self" or "do not eat me" signal. CD47 communicates with signal regulatory protein (SIRP-α) expressed on macrophages and DCs to prevent phagocytosis. The upregulation of CD47 in cancers facilitates immune evasion and resistance to immune surveillance [196]. Blockade of antiphagocytic CD47-SIRP-α interactions using anti-CD47 antibodies has shown promise in several solid tumors including gliomas. Wnt/β-catenin signaling has been shown to transcriptionally regulate CD47 expression. Gowda et al. showed that pyruvate kinase isoform M2 (PKM2) - β-catenin BRG-1 complex is recruited to the TCF4 site to regulate CD47 expression in glioma cells. High levels of β-catenin are associated with high-grade gliomas and inhibition of β-catenin abrogates the CD47 expression [197].

PDL1 is a "do not find me" ligand that may be regulated by Wnt signaling in some cancers. It is a single-pass trans-membrane protein, a member of the B7/CD28 family of costimulatory receptors. It regulates T cell activation through binding to its ligands, programmed death ligand 1 and 2 (PDL1 and PDL2) [198]. PDL1 is found on many cells including macrophages, DCs, and in several tissues such as heart lung and placenta. PDL1/PD1 interaction keeps the balance between immune tolerance and autoimmunity. Interaction of PDL1 with PD1 on cytotoxic T cells leads to the formation of PD1 complexes with TCR and CD28 that leads to inactivation of cytotoxic T cells [199, 200]. Tumor cells of the lung, bladder, head, and neck colon cancer and melanoma among others express PDL1 leading to elimination of the immune cells. Activated Wnt/β-catenin signaling was associated with high PDL1 expression and poor prognosis in testicular germ cell tumors [201]. Triple-negative breast cancer stem cells also express high levels of PDL1 that is activated by Wnt/β-catenin. In line with that, a small molecule that might act in part as a Wnt agonist, CAS 853220-52-7, significantly increased PDL1 expression both at transcript and protein levels in multiple breast cancer cell lines [202].

Oncogene MYC is regulated both transcriptionally and posttranslationally by Wnt signaling. MYC also regulates expression of both PDL1 and CD47 checkpoint inhibitors in various human cancers including T cell–acute lymphoblastic leukemia (T-ALL), non-small cell lung cancer, hepatocellular carcinomas, and melanoma. Suppression of MYC in mouse tumors and human tumor cells caused a reduction in CD47 and PD-L1 messenger RNA and protein levels [203]. In an animal model of T-ALL, MYC overexpression resulted in upregulation of both PDL1 and CD47 on tumor cells and their levels were decreased upon MYC inactivation leading to enhanced antitumor response [203].

CTLA4 (CD152) is constitutively expressed in regulatory T cells and is another negative regulator of the T cell-mediated immune response that can be influenced by Wnt/β-catenin signaling. CTLA4 interacts with CD80 or CD86 expressed on the antigen-presenting cells to switch off the immune response. Hence, widespread efforts have been made to block CTLA4 using antibodies to boost antitumor immunity. Several anti-CTLA4 antibodies have shown promising results in clinical trials and are hence approved for treatment of certain cancers alone or in combination with anti-PDL1 antibodies [204, 205]. CTLA4 has TCF/LEF1 binding sites in its promoter and its expression is strongly induced by Wnt/β-catenin signaling in melanomas [206].

Cancer-associated fibroblasts (CAFs) also play a vital role in shaping the immunosuppressive microenvironment within the tumor. In addition to being a source of Wnts such as WNT2 in colorectal cancers, they make the stroma dense, preventing the infiltration of immune cells. In pancreatic cancers, stromal cells constitute 70% of the tumor cells compared with 30% in the melanomas [207]. This dense stroma, excluding immune cells, may explain why immune checkpoint inhibitors have reported minimal benefit in pancreatic cancers. Elevated Wnt signaling is associated with tissue fibrosis and therapies targeting Wnt signaling have shown benefit in preventing fibrosis in lung, kidney, heart, and skin [208] [209]. The Wnts expressed in the pancreatic cancer stroma may therefore contribute to the dense stroma and the poor immune cell infiltration.

These data indicate that there is a positive correlation between high Wnt signaling and an immunosuppressive microenvironment. In addition, there is a lack of response of Wnt/β-catenin-high tumors to anti-CTLA4/anti-PD-1 immunotherapy. Taken together these studies suggest that pharmacologic inhibition of Wnt/β-catenin signaling may be a therapeutic option to restore T cell infiltration and potentially expand immunotherapy efficacy in the clinic.

12 Conclusion

Wnt/β-catenin signaling contributes to multiple pathways and hallmarks that underlie cancer development and progression. Wnt signaling in cancer was first associated with the ability to support cell proliferation and maintain stemness. However, multiple studies over many years also support the role of this developmental pathway in regulating multiple facets of carcinogenesis including angiogenesis, genome instability, glycolysis, and metastasis. With the advent of immunotherapies, an indispensable role of Wnts in regulating T cell development and differentiation has been revisited, and new studies have now firmly established the importance of this pathway in regulating the expression of various checkpoints on the immune cells as well as tumor cells to promote immune evasion.

Given the diverse role of Wnts in regulating tumor growth and progression, targeting Wnt signaling alone or in combination with other small molecule inhibitors would be highly beneficial. Small molecules and antibodies targeting various components of the Wnt signaling pathway have been developed (reviewed in [210]). These include modulators of AXIN stability such as Tankyrase inhibitors and small molecules that directly interfere with the binding of β-catenin with various components of transcriptional complex. While these inhibitors regulate Wnt/β-catenin signaling, accumulating evidence also supports β-catenin independent roles of Wnt signaling in cancer. The development of Wnt secretion inhibitors that regulate the activity of O-acyltransferase PORCN required for secretion of all Wnts allows these β-catenin independent pathways to be targeted as well. Additionally, decoy receptors that prevent interaction of Wnts with their receptors, antibodies targeting Wnt receptors Frizzleds, and the Wnt agonists R-spondins have also been developed. These modulators of Wnt signaling hold promise for the treatment of cancers, either acting alone or by contributing to the efficacy of already existing drugs. Wnt pathway modulation could potentially improve clinical outcomes by interfering with many of the hallmarks of cancer.

Acknowledgments We gratefully acknowledge the assistance of Nakeisha Tan for the illustrations.

Funding information The authors received support from the Singapore Ministry of Health's National Medical Research Council Open Fund–Independent Research grant NMRC/OFIRG/0055/2017 (to Babita Madan) and the STAR Award Program MOH-000155 (to David Virshup).

References

1. Srivastava, M., Begovic, E., Chapman, J., Putnam, N. H., Hellsten, U., Kawashima, T., et al. (2008). The Trichoplax genome and the nature of placozoans. *Nature, 454*(7207), 955–960. https://doi.org/10.1038/nature07191.
2. Nichols, S. A., Dirks, W., Pearse, J. S., & King, N. (2006). Early evolution of animal cell signaling and adhesion genes. *Proceedings of the National Academy of Sciences of the United States of America, 103*(33), 12451–12456. https://doi.org/10.1073/pnas.0604065103.
3. Loh, K. M., van Amerongen, R., & Nusse, R. (2016). Generating cellular diversity and spatial form: Wnt signaling and the evolution of multicellular animals. *Developmental Cell, 38*(6), 643–655. https://doi.org/10.1016/j.devcel.2016.08.011.
4. Nusse, R., & Varmus, H. E. (1982). Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell, 31*(1), 99–109.
5. Aoki, K., & Taketo, M. M. (2007). Adenomatous polyposis coli (APC): a multi-functional tumor suppressor gene. *Journal of Cell Science, 120*(Pt 19), 3327–3335. https://doi.org/10.1242/jcs.03485.
6. Yu, J., & Virshup, D. M. (2014). Updating the Wnt pathways. *Bioscience Reports, 34*(5). https://doi.org/10.1042/BSR20140119.
7. Steinhart, Z., & Angers, S. (2018). Wnt signaling in development and tissue homeostasis. *Development, 145*(11), dev146589. https://doi.org/10.1242/dev.146589.
8. Hanahan, D., & Weinberg, R. A. (2000). The hallmarks of cancer. *Cell, 100*(1), 57–70. https://doi.org/10.1016/s0092-8674(00)81683-9.
9. Hanahan, D., & Weinberg, R. A. (2011). Hallmarks of cancer: the next generation. *Cell, 144*(5), 646–674. https://doi.org/10.1016/j.cell.2011.02.013.
10. Obaya, A. J., & Sedivy, J. M. (2002). Regulation of cyclin-Cdk activity in mammalian cells. *Cellular and Molecular Life Sciences, 59*(1), 126–142. https://doi.org/10.1007/s00018-002-8410-1.
11. Massagué, J. (2004). G1 cell-cycle control and cancer. *Nature, 432*(7015), 298–306. https://doi.org/10.1038/nature03094.
12. Niehrs, C., & Acebron, S. P. (2012). Mitotic and mitogenic Wnt signalling. *The EMBO Journal, 31*(12), 2705–2713. https://doi.org/10.1038/emboj.2012.124.
13. He, T. C., Sparks, A. B., Rago, C., Hermeking, H., Zawel, L., da Costa, L. T., et al. (1998). Identification of c-MYC as a target of the APC pathway. *Science, 281*(5382), 1509–1512. https://doi.org/10.1126/science.281.5382.1509.
14. Tetsu, O., & McCormick, F. (1999). Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature, 398*(6726), 422–426. https://doi.org/10.1038/18884.
15. Shtutman, M., Zhurinsky, J., Simcha, I., Albanese, C., D'Amico, M., Pestell, R., & Ben-Ze'ev, A. (1999). The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proceedings of the National Academy of Sciences of the United States of America, 96*(10), 5522–5527. https://doi.org/10.1073/pnas.96.10.5522.
16. Diehl, J. A., Cheng, M., Roussel, M. F., & Sherr, C. J. (1998). Glycogen synthase kinase-3beta regulates cyclin D1 proteolysis and subcellular localization. *Genes & Development, 12*(22), 3499–3511. https://doi.org/10.1101/gad.12.22.3499.
17. Welcker, M., Singer, J., Loeb, K. R., Grim, J., Bloecher, A., Gurien-West, M., et al. (2003). Multisite phosphorylation by Cdk2 and GSK3 controls cyclin E degradation. *Molecular Cell, 12*(2), 381–392. https://doi.org/10.1016/s1097-2765(03)00287-9.
18. Welcker, M., Orian, A., Jin, J., Grim, J. E., Grim, J. A., Harper, J. W., et al. (2004). The Fbw7 tumor suppressor regulates glycogen synthase kinase 3 phosphorylation-dependent c-Myc protein degradation. *Proceedings of the National Academy of Sciences of the United States of America, 101*(24), 9085–9090. https://doi.org/10.1073/pnas.0402770101.
19. Acebron, S. P., Karaulanov, E., Berger, B. S., Huang, Y.-L., & Niehrs, C. (2014). Mitotic wnt signaling promotes protein

stabilization and regulates cell size. *Molecular Cell, 54*(4), 663–674. https://doi.org/10.1016/j.molcel.2014.04.014.

20. Reya, T., & Clevers, H. (2005). Wnt signalling in stem cells and cancer. *Nature, 434*(7035), 843–850. https://doi.org/10.1038/nature03319.
21. Leung, C., Tan, S. H., & Barker, N. (2018). Recent advances in Lgr5+ stem cell research. *Trends in Cell Biology, 28*(5), 380–391. https://doi.org/10.1016/j.tcb.2018.01.010.
22. Orford, K. W., & Scadden, D. T. (2008). Deconstructing stem cell self-renewal: Genetic insights into cell-cycle regulation. *Nature reviews* Genetics, 9(2), 115–128. https://doi.org/10.1038/nrg2269.
23. Kabiri, Z., Greicius, G., Zaribafzadeh, H., Hemmerich, A., Counter, C. M., & Virshup, D. M. (2018). Wnt signaling suppresses MAPK-driven proliferation of intestinal stem cells. *The Journal of Clinical Investigation, 128*(9). https://doi.org/10.1172/JCI99325.
24. Riemer, P., Sreekumar, A., Reinke, S., Rad, R., Schäfer, R., Sers, C., et al. (2015). Transgenic expression of oncogenic BRAF induces loss of stem cells in the mouse intestine, which is antagonized by β-catenin activity. *Oncogene, 34*(24), 3164–3175. https://doi.org/10.1038/onc.2014.247.
25. Zhong, Z., & Virshup, D. M. (2020). Wnt signaling and drug resistance in cancer. *Molecular Pharmacology, 97*(2), 72–89. https://doi.org/10.1124/mol.119.117978.
26. Proffitt, K. D., Madan, B., Ke, Z., Pendharkar, V., Ding, L., Lee, M. A., et al. (2013). Pharmacological inhibition of the Wnt acyltransferase PORCN prevents growth of WNT-driven mammary cancer. *Cancer Research, 73*(2), 502–507. https://doi.org/10.1158/0008-5472.CAN-12-2258.
27. Koo, B.-K., van Es, J. H., van den Born, M., & Clevers, H. (2015). Porcupine inhibitor suppresses paracrine Wnt-driven growth of Rnf43;Znrf3-mutant neoplasia. *Proceedings of the National Academy of Sciences of the United States of America*, 201508113. https://doi.org/10.1073/pnas.1508113112.
28. Boulter, L., Guest, R. V., Kendall, T. J., Wilson, D. H., Wojtacha, D., Robson, A. J., et al. (2015). WNT signaling drives cholangiocarcinoma growth and can be pharmacologically inhibited. *The Journal of Clinical Investigation, 125*(3), 1269–1285. https://doi.org/10.1172/JCI76452.
29. Storm, E. E., Durinck, S., de Sousa e Melo, F., Tremayne, J., Kljavin, N., Tan, C., et al. (2016). Targeting PTPRK-RSPO3 colon tumours promotes differentiation and loss of stem-cell function. *Nature, 529*(7584), 97–100. https://doi.org/10.1038/nature16466.
30. Madan, B., Harmston, N., Nallan, G., Montoya, A., Faull, P., Petretto, E., & Virshup, D. M. (2018). Temporal dynamics of Wnt-dependent transcriptome reveal an oncogenic Wnt/MYC/ribosome axis. *The Journal of Clinical Investigation, 128*(12), 5620–5633. https://doi.org/10.1172/JCI122383.
31. Lin, C. Y., Lovén, J., Rahl, P. B., Paranal, R. M., Burge, C. B., Bradner, J. E., et al. (2012). Transcriptional amplification in tumor cells with elevated c-Myc. *Cell, 151*(1), 56–67. https://doi.org/10.1016/j.cell.2012.08.026.
32. Nie, Z., Hu, G., Wei, G., Cui, K., Yamane, A., Resch, W., et al. (2012). c-Myc is a universal amplifier of expressed genes in lymphocytes and embryonic stem cells. *Cell, 151*(1), 68–79. https://doi.org/10.1016/j.cell.2012.08.033.
33. Bretones, G., Delgado, M. D., & León, J. (2015). Myc and cell cycle control. *Biochimica et Biophysica Acta, 1849*(5), 506–516. https://doi.org/10.1016/j.bbagrm.2014.03.013.
34. van Riggelen, J., Yetil, A., & Felsher, D. W. (2010). MYC as a regulator of ribosome biogenesis and protein synthesis. *Nature Reviews. Cancer, 10*(4), 301–309. https://doi.org/10.1038/nrc2819.
35. Dang, C. V. (2013). MYC, metabolism, cell growth, and tumorigenesis. *Cold Spring Harbor Perspectives in Medicine, 3*(8), a014217–a014217. https://doi.org/10.1101/cshperspect.a014217.
36. Wahlström, T., & Henriksson, M. A. (2015). Impact of MYC in regulation of tumor cell metabolism. *Biochimica et Biophysica Acta, 1849*(5), 563–569. https://doi.org/10.1016/j.bbagrm.2014.07.004.
37. Sansom, O. J., Meniel, V. S., Muncan, V., Phesse, T. J., Wilkins, J. A., Reed, K. R., et al. (2007). Myc deletion rescues Apc deficiency in the small intestine. *Nature, 446*(7136), 676–679. https://doi.org/10.1038/nature05674.
38. Meyers, R. M., Bryan, J. G., McFarland, J. M., Weir, B. A., Sizemore, A. E., Xu, H., et al. (2017). Computational correction of copy number effect improves specificity of CRISPR–Cas9 essentiality screens in cancer cells. *Nature Genetics, 49*(12), 1779–1784. https://doi.org/10.1038/ng.3984.
39. McDonald, E. R., de Weck, A., Schlabach, M. R., Billy, E., Mavrakis, K. J., Hoffman, G. R., et al. (2017). Project DRIVE: a compendium of cancer dependencies and synthetic lethal relationships uncovered by large-scale, deep RNAi screening. *Cell, 170*(3), 577–592.e10. https://doi.org/10.1016/j.cell.2017.07.005.
40. Behan, F. M., Iorio, F., Picco, G., Gonçalves, E., Beaver, C. M., Migliardi, G., et al. (2019). Prioritization of cancer therapeutic targets using CRISPR-Cas9 screens. *Nature, 31*, 1806. https://doi.org/10.1038/s41586-019-1103-9.
41. Fong, C. Y., Gilan, O., Lam, E. Y. N., Rubin, A. F., Ftouni, S., Tyler, D., et al. (2015). BET inhibitor resistance emerges from leukaemia stem cells. *Nature, 525*(7570), 538–542. https://doi.org/10.1038/nature14888.
42. Rathert, P., Roth, M., Neumann, T., Muerdter, F., Roe, J.-S., Muhar, M., et al. (2015). Transcriptional plasticity promotes primary and acquired resistance to BET inhibition. *Nature, 525*(7570), 543–547. https://doi.org/10.1038/nature14898.
43. Harbour, J. W., & Dean, D. C. (2000). Rb function in cell-cycle regulation and apoptosis. *Nature Cell Biology, 2*(4), E65–E67. https://doi.org/10.1038/35008695.
44. Giacinti, C., & Giordano, A. (2006). RB and cell cycle progression. *Oncogene, 25*(38), 5220–5227. https://doi.org/10.1038/sj.onc.1209615.
45. Henley, S. A., & Dick, F. A. (2012). The retinoblastoma family of proteins and their regulatory functions in the mammalian cell division cycle. *Cell Division, 7*(1), 10–14. https://doi.org/10.1186/1747-1028-7-10.
46. Ohtani, K., DeGregori, J., & Nevins, J. R. (1995). Regulation of the cyclin E gene by transcription factor E2F1. *Proceedings of the National Academy of Sciences of the United States of America, 92*(26), 12146–12150. https://doi.org/10.1073/pnas.92.26.12146.
47. Bracken, A. P., Ciro, M., Cocito, A., & Helin, K. (2004). E2F target genes: unraveling the biology. *Trends in Biochemical Sciences, 29*(8), 409–417. https://doi.org/10.1016/j.tibs.2004.06.006.
48. Delmas, V., Beermann, F., Martinozzi, S., Carreira, S., Ackermann, J., Kumasaka, M., et al. (2007). Beta-catenin induces immortalization of melanocytes by suppressing p16INK4a expression and cooperates with N-Ras in melanoma development. *Genes & Development, 21*(22), 2923–2935. https://doi.org/10.1101/gad.450107.
49. Jiang, X., Hao, H.-X., Growney, J. D., Woolfenden, S., Bottiglio, C., Ng, N., et al. (2013). Inactivating mutations of RNF43 confer Wnt dependency in pancreatic ductal adenocarcinoma. *Proceedings of the National Academy of Sciences of the United States of America, 110*(31), 12649–12654. https://doi.org/10.1073/pnas.1307218110.
50. Zhong, Z., Sepramaniam, S., Chew, X. H., Wood, K., Lee, M. A., Madan, B., & Virshup, D. M. (2019). PORCN inhibition

synergizes with PI3K/mTOR inhibition in Wnt-addicted cancers. *Oncogene, 67*(12), 7. https://doi.org/10.1038/s41388-019-0908-1.
51. Visvader, J. E., & Lindeman, G. J. (2008). Cancer stem cells in solid tumours: accumulating evidence and unresolved questions. *Nature Reviews. Cancer, 8*(10), 755–768. https://doi.org/10.1038/nrc2499.
52. Chang, J. C. (2016). Cancer stem cells: role in tumor growth, recurrence, metastasis, and treatment resistance. *Medicine, 95*(1 Suppl 1), S20–S25. https://doi.org/10.1097/MD.0000000000004766.
53. Peitzsch, C., Tyutyunnykova, A., Pantel, K., & Dubrovska, A. (2017). Cancer stem cells: the root of tumor recurrence and metastases. *Seminars in Cancer Biology, 44*, 10–24. https://doi.org/10.1016/j.semcancer.2017.02.011.
54. Madan, B., Ke, Z., Harmston, N., Ho, S. Y., Frois, A. O., Alam, J., et al. (2016). Wnt addiction of genetically defined cancers reversed by PORCN inhibition. *Oncogene, 35*(17), 2197–2207. https://doi.org/10.1038/onc.2015.280.
55. Steinhart, Z., Pavlovic, Z., Chandrashekhar, M., Hart, T., Wang, X., Zhang, X., et al. (2017). Genome-wide CRISPR screens reveal a Wnt-FZD5 signaling circuit as a druggable vulnerability of RNF43-mutant pancreatic tumors. *Nature Medicine, 23*(1), 60–68. https://doi.org/10.1038/nm.4219.
56. Milanovic, M., Fan, D. N. Y., Belenki, D., Däbritz, J. H. M., Zhao, Z., Yu, Y., et al. (2017). Senescence-associated reprogramming promotes cancer stemness. *Nature, 15*, 482. https://doi.org/10.1038/nature25167.
57. White, E., & DiPaola, R. S. (2009). The double-edged sword of autophagy modulation in cancer. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 15*(17), 5308–5316. https://doi.org/10.1158/1078-0432.CCR-07-5023.
58. Chen, S., Guttridge, D. C., You, Z., Zhang, Z., Fribley, A., Mayo, M. W., et al. (2001). Wnt-1 signaling inhibits apoptosis by activating beta-catenin/T cell factor-mediated transcription. *The Journal of Cell Biology, 152*(1), 87–96. https://doi.org/10.1083/jcb.152.1.87.
59. Xie, H., Huang, Z., Sadim, M. S., & Sun, Z. (2005). Stabilized beta-catenin extends thymocyte survival by up-regulating Bcl-xL. *The Journal of Immunology, 175*(12), 7981–7988. https://doi.org/10.4049/jimmunol.175.12.7981.
60. Melkonyan, H. S., Chang, W. C., Shapiro, J. P., Mahadevappa, M., Fitzpatrick, P. A., Kiefer, M. C., et al. (1997). SARPs: a family of secreted apoptosis-related proteins. *Proceedings of the National Academy of Sciences of the United States of America, 94*(25), 13636–13641. https://doi.org/10.1073/pnas.94.25.13636.
61. Roth, W., Wild-Bode, C., Platten, M., Grimmel, C., Melkonyan, H. S., Dichgans, J., & Weller, M. (2000). Secreted frizzled-related proteins inhibit motility and promote growth of human malignant glioma cells. *Oncogene, 19*(37), 4210–4220. https://doi.org/10.1038/sj.onc.1203783.
62. Han, X., & Amar, S. (2004). Secreted frizzled-related protein 1 (SFRP1) protects fibroblasts from ceramide-induced apoptosis. *The Journal of Biological Chemistry, 279*(4), 2832–2840. https://doi.org/10.1074/jbc.M308102200.
63. Uren, A., Reichsman, F., Anest, V., Taylor, W. G., Muraiso, K., Bottaro, D. P., et al. (2000). Secreted frizzled-related protein-1 binds directly to wingless and is a biphasic modulator of Wnt signaling. *The Journal of Biological Chemistry, 275*(6), 4374–4382. https://doi.org/10.1074/jbc.275.6.4374.
64. Levine, B., & Kroemer, G. (2008). Autophagy in the pathogenesis of disease. *Cell, 132*(1), 27–42. https://doi.org/10.1016/j.cell.2007.12.018.
65. Sánchez-Martín, P., & Komatsu, M. (2018). p62/SQSTM1 - steering the cell through health and disease. *Journal of Cell Science, 131*(21), jcs222836. https://doi.org/10.1242/jcs.222836.
66. Lee, Y.-K., & Lee, J.-A. (2016). Role of the mammalian ATG8/LC3 family in autophagy: differential and compensatory roles in the spatiotemporal regulation of autophagy. *BMB Reports, 49*(8), 424–430. https://doi.org/10.5483/bmbrep.2016.49.8.081.
67. Gao, C., Cao, W., Bao, L., Zuo, W., Xie, G., Cai, T., et al. (2010). Autophagy negatively regulates Wnt signalling by promoting Dishevelled degradation. *Nature Cell Biology, 12*(8), 781–790. https://doi.org/10.1038/ncb2082.
68. Petherick, K. J., Williams, A. C., Lane, J. D., Ordóñez-Morán, P., Huelsken, J., Collard, T. J., et al. (2013). Autolysosomal β-catenin degradation regulates Wnt-autophagy-p62 crosstalk. *The EMBO Journal, 32*(13), 1903–1916. https://doi.org/10.1038/emboj.2013.123.
69. Luo, X., Ye, S., Jiang, Q., Gong, Y., Yuan, Y., Hu, X., et al. (2018). Wnt inhibitory factor-1-mediated autophagy inhibits Wnt/β-catenin signaling by downregulating dishevelled-2 expression in non-small cell lung cancer cells. *International Journal of Oncology, 53*(2), 904–914. https://doi.org/10.3892/ijo.2018.4442.
70. Colella, B., Faienza, F., Carinci, M., D'Alessandro, G., Catalano, M., Santoro, A., et al. (2019). Autophagy induction impairs Wnt/β-catenin signalling through β-catenin relocalisation in glioblastoma cells. *Cellular Signalling, 53*, 357–364. https://doi.org/10.1016/j.cellsig.2018.10.017.
71. Nàger, M., Sallán, M. C., Visa, A., Pushparaj, C., Santacana, M., Macià, A., et al. (2018). Inhibition of WNT-CTNNB1 signaling upregulates SQSTM1 and sensitizes glioblastoma cells to autophagy blockers. *Autophagy, 14*(4), 619–636. https://doi.org/10.1080/15548627.2017.1423439.
72. Low, K. C., & Tergaonkar, V. (2013). Telomerase: central regulator of all of the hallmarks of cancer. *Trends in Biochemical Sciences, 38*(9), 426–434. https://doi.org/10.1016/j.tibs.2013.07.001.
73. Tang, R., Cheng, A. J., Wang, J. Y., & Wang, T. C. (1998). Close correlation between telomerase expression and adenomatous polyp progression in multistep colorectal carcinogenesis. *Cancer Research, 58*(18), 4052–4054.
74. Tahara, H., Kuniyasu, H., Yokozaki, H., Yasui, W., Shay, J. W., Ide, T., & Tahara, E. (1995). Telomerase activity in preneoplastic and neoplastic gastric and colorectal lesions. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 1*(11), 1245–1251.
75. Mizumoto, I., Ogawa, Y., Niiyama, H., Nagai, E., Sato, I., Urashima, T., et al. (2001). Possible role of telomerase activation in the multistep tumor progression of periampullary lesions in patients with familial adenomatous polyposis. *The American Journal of Gastroenterology, 96*(4), 1261–1265. https://doi.org/10.1111/j.1572-0241.2001.03710.x.
76. Hoffmeyer, K., Raggioli, A., Rudloff, S., Anton, R., Hierholzer, A., Del Valle, I., et al. (2012). Wnt/β-catenin signaling regulates telomerase in stem cells and cancer cells. *Science, 336*(6088), 1549–1554. https://doi.org/10.1126/science.1218370.
77. Zhang, Y., Toh, L., Lau, P., & Wang, X. (2012). Human telomerase reverse transcriptase (hTERT) is a novel target of the Wnt/β-catenin pathway in human cancer. *The Journal of Biological Chemistry, 287*(39), 32494–32511. https://doi.org/10.1074/jbc.M112.368282.
78. Wang, J., Xie, L. Y., Allan, S., Beach, D., & Hannon, G. J. (1998). Myc activates telomerase. *Genes & Development, 12*(12), 1769–1774. https://doi.org/10.1101/gad.12.12.1769.
79. Strong, M. A., Vidal-Cardenas, S. L., Karim, B., Yu, H., Guo, N., & Greider, C. W. (2011). Phenotypes in mTERT$^{+/-}$ and mTERT$^{-/-}$ mice are due to short telomeres, not telomere-independent functions of telomerase reverse transcriptase. *Molecular and Cellular Biology, 31*(12), 2369–2379. https://doi.org/10.1128/MCB.05312-11.

80. Listerman, I., Gazzaniga, F. S., & Blackburn, E. H. (2014). An investigation of the effects of the core protein telomerase reverse transcriptase on Wnt signaling in breast cancer cells. *Molecular and Cellular Biology, 34*(2), 280–289. https://doi.org/10.1128/MCB.00844-13.
81. Yang, T.-L. B., Chen, Q., Deng, J. T., Jagannathan, G., Tobias, J. W., Schultz, D. C., et al. (2017). Mutual reinforcement between telomere capping and canonical Wnt signalling in the intestinal stem cell niche. *Nature Communications, 8*(1), 14766–14710. https://doi.org/10.1038/ncomms14766.
82. Wilson, C. L., Heppner, K. J., Labosky, P. A., Hogan, B. L., & Matrisian, L. M. (1997). Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. *Proceedings of the National Academy of Sciences of the United States of America, 94*(4), 1402–1407. https://doi.org/10.1073/pnas.94.4.1402.
83. Brabletz, T., Jung, A., Dag, S., Hlubek, F., & Kirchner, T. (1999). Beta-catenin regulates the expression of the matrix metalloproteinase-7 in human colorectal cancer. *The American Journal of Pathology, 155*(4), 1033–1038. https://doi.org/10.1016/s0002-9440(10)65204-2.
84. Lévy, L., Neuveut, C., Renard, C.-A., Charneau, P., Branchereau, S., Gauthier, F., et al. (2002). Transcriptional activation of interleukin-8 by beta-catenin-Tcf4. *The Journal of Biological Chemistry, 277*(44), 42386–42393. https://doi.org/10.1074/jbc.M207418200.
85. Li, A., Dubey, S., Varney, M. L., Dave, B. J., & Singh, R. K. (2003). IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *The Journal of Immunology, 170*(6), 3369–3376. https://doi.org/10.4049/jimmunol.170.6.3369.
86. Ferrara, N., Hillan, K. J., Gerber, H.-P., & Novotny, W. (2004). Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nature Reviews Drug Discovery, 3*(5), 391–400. https://doi.org/10.1038/nrd1381.
87. Krock, B. L., Skuli, N., & Simon, M. C. (2011). Hypoxia-induced angiogenesis: good and evil. *Genes & Cancer, 2*(12), 1117–1133. https://doi.org/10.1177/1947601911423654.
88. Zhang, X., Gaspard, J. P., & Chung, D. C. (2001). Regulation of vascular endothelial growth factor by the Wnt and K-ras pathways in colonic neoplasia. *Cancer Research, 61*(16), 6050–6054.
89. Easwaran, V., Lee, S. H., Inge, L., Guo, L., Goldbeck, C., Garrett, E., et al. (2003). Beta-catenin regulates vascular endothelial growth factor expression in colon cancer. *Cancer Research, 63*(12), 3145–3153.
90. Posokhova, E., Shukla, A., Seaman, S., Volate, S., Hilton, M. B., Wu, B., et al. (2015). GPR124 functions as a WNT7-specific coactivator of canonical β-catenin signaling. *Cell Reports, 10*(2), 123–130. https://doi.org/10.1016/j.celrep.2014.12.020.
91. Chang, J., Mancuso, M. R., Maier, C., Liang, X., Yuki, K., Yang, L., et al. (2017). Gpr124 is essential for blood-brain barrier integrity in central nervous system disease. *Nature Medicine, 23*(4), 450–460. https://doi.org/10.1038/nm.4309.
92. Cho, C., Smallwood, P. M., & Nathans, J. (2017). Reck and Gpr124 are essential receptor cofactors for Wnt7a/Wnt7b-specific signaling in mammalian CNS angiogenesis and blood-brain barrier regulation. *Neuron, 95*(5), 1056–1073.e5. https://doi.org/10.1016/j.neuron.2017.07.031.
93. Chaffer, C. L., & Weinberg, R. A. (2011). A perspective on cancer cell metastasis. *Science, 331*(6024), 1559–1564. https://doi.org/10.1126/science.1203543.
94. Fischer, K. R., Durrans, A., Lee, S., Sheng, J., Li, F., Wong, S. T. C., et al. (2015). Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature, 527*(7579), 472–476. https://doi.org/10.1038/nature15748.
95. Zheng, X., Carstens, J. L., Kim, J., Scheible, M., Kaye, J., Sugimoto, H., et al. (2015). Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature, 527*(7579), 525–530. https://doi.org/10.1038/nature16064.
96. Somarelli, J. A., Schaeffer, D., Marengo, M. S., Bepler, T., Rouse, D., Ware, K. E., et al. (2016). Distinct routes to metastasis: plasticity-dependent and plasticity-independent pathways. *Oncogene*. https://doi.org/10.1038/onc.2015.497.
97. Lambert, A. W., Pattabiraman, D. R., & Weinberg, R. A. (2017). Emerging biological principles of metastasis. *Cell, 168*(4), 670–691. https://doi.org/10.1016/j.cell.2016.11.037.
98. Brabletz, T., Jung, A., Hermann, K., Günther, K., Hohenberger, W., & Kirchner, T. (1998). Nuclear overexpression of the oncoprotein beta-catenin in colorectal cancer is localized predominantly at the invasion front. *Pathology, Research and Practice, 194*(10), 701–704. https://doi.org/10.1016/s0344-0338(98)80129-5.
99. Brabletz, T., Jung, A., Reu, S., Porzner, M., Hlubek, F., Kunz-Schughart, L. A., et al. (2001). Variable beta-catenin expression in colorectal cancers indicates tumor progression driven by the tumor environment. *Proceedings of the National Academy of Sciences of the United States of America, 98*(18), 10356–10361. https://doi.org/10.1073/pnas.171610498.
100. Vermeulen, L., de Sousa e Melo, F., van der Heijden, M., Cameron, K., de Jong, J. H., Borovski, T., et al. (2010). Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nature Cell Biology, 12*(5), 468–476. https://doi.org/10.1038/ncb2048.
101. Maruyama, K., Ochiai, A., Akimoto, S., Nakamura, S., Baba, S., Moriya, Y., & Hirohashi, S. (2000). Cytoplasmic beta-catenin accumulation as a predictor of hematogenous metastasis in human colorectal cancer. *Oncology, 59*(4), 302–309. https://doi.org/10.1159/000012187.
102. Hörkkö, T. T., Klintrup, K., Mäkinen, J. M., Näpänkangas, J. B., Tuominen, H. J., Mäkelä, J., et al. (2006). Budding invasive margin and prognosis in colorectal cancer–no direct association with beta-catenin expression. *European Journal of Cancer, 42*(7), 964–971. https://doi.org/10.1016/j.ejca.2006.01.017.
103. Horst, D., Reu, S., Kriegl, L., Engel, J., Kirchner, T., & Jung, A. (2009). The intratumoral distribution of nuclear beta-catenin is a prognostic marker in colon cancer. *Cancer, 115*(10), 2063–2070. https://doi.org/10.1002/cncr.24254.
104. Gao, Z.-H., Lu, C., Wang, M.-X., Han, Y., & Guo, L.-J. (2014). Differential β-catenin expression levels are associated with morphological features and prognosis of colorectal cancer. *Oncology Letters, 8*(5), 2069–2076. https://doi.org/10.3892/ol.2014.2433.
105. Liu, L., Zhu, X.-D., Wang, W.-Q., Shen, Y., Qin, Y., Ren, Z.-G., et al. (2010). Activation of beta-catenin by hypoxia in hepatocellular carcinoma contributes to enhanced metastatic potential and poor prognosis. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 16*(10), 2740–2750. https://doi.org/10.1158/1078-0432.CCR-09-2610.
106. Yook, J. I., Li, X.-Y., Ota, I., Fearon, E. R., & Weiss, S. J. (2005). Wnt-dependent regulation of the E-cadherin repressor snail. *The Journal of Biological Chemistry, 280*(12), 11740–11748. https://doi.org/10.1074/jbc.M413878200.
107. Esposito, M., Mondal, N., Greco, T. M., Wei, Y., Spadazzi, C., Lin, S.-C., et al. (2019). Bone vascular niche E-selectin induces mesenchymal-epithelial transition and Wnt activation in cancer cells to promote bone metastasis. *Nature Cell Biology, 21*(5), 627–639. https://doi.org/10.1038/s41556-019-0309-2.
108. Nandana, S., Tripathi, M., Duan, P., Chu, C.-Y., Mishra, R., Liu, C., et al. (2017). Bone metastasis of prostate cancer can be therapeutically targeted at the TBX2-WNT signaling axis. *Cancer*

Research, 77(6), 1331–1344. https://doi.org/10.1158/0008-5472.CAN-16-0497.

109. Nguyen, D. X., Chiang, A. C., Zhang, X. H.-F., Kim, J. Y., Kris, M. G., Ladanyi, M., et al. (2009). WNT/TCF signaling through LEF1 and HOXB9 mediates lung adenocarcinoma metastasis. *Cell, 138*(1), 51–62. https://doi.org/10.1016/j.cell.2009.04.030.
110. Han, F., Liu, W.-B., Shi, X.-Y., Yang, J.-T., Zhang, X., Li, Z.-M., et al. (2018). SOX30 inhibits tumor metastasis through attenuating Wnt-signaling via transcriptional and posttranslational regulation of β-catenin in lung cancer. *EBioMedicine, 31*, 253–266. https://doi.org/10.1016/j.ebiom.2018.04.026.
111. Cai, J., Fang, L., Huang, Y., Li, R., Xu, X., Hu, Z., et al. (2017). Simultaneous overactivation of Wnt/β-catenin and TGFβ signalling by miR-128-3p confers chemoresistance-associated metastasis in NSCLC. *Nature Communications, 8*(1), 15870–15819. https://doi.org/10.1038/ncomms15870.
112. Dai, F.-Q., Li, C.-R., Fan, X.-Q., Tan, L., Wang, R.-T., & Jin, H. (2019). miR-150-5p inhibits non-small-cell lung cancer metastasis and recurrence by targeting HMGA2 and β-catenin signaling. *Molecular Therapy–Nucleic Acids, 16*, 675–685. https://doi.org/10.1016/j.omtn.2019.04.017.
113. Wellenstein, M. D., Coffelt, S. B., Duits, D. E. M., van Miltenburg, M. H., Slagter, M., de Rink, I., et al. (2019). Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. *Nature, 15*, e493. https://doi.org/10.1038/s41586-019-1450-6.
114. Tenbaum, S. P., Ordóñez-Morán, P., Puig, I., Chicote, I., Arqués, O., Landolfi, S., et al. (2012). β-catenin confers resistance to PI3K and AKT inhibitors and subverts FOXO3a to promote metastasis in colon cancer. *Nature Medicine, 18*(6), 892–901. https://doi.org/10.1038/nm.2772.
115. Luga, V., Zhang, L., Viloria-Petit, A. M., Ogunjimi, A. A., Inanlou, M. R., Chiu, E., et al. (2012). Exosomes mediate stromal mobilization of autocrine Wnt-PCP signaling in breast cancer cell migration. *Cell, 151*(7), 1542–1556. https://doi.org/10.1016/j.cell.2012.11.024.
116. Yu, M., Ting, D. T., Stott, S. L., Wittner, B. S., Ozsolak, F., Paul, S., et al. (2012). RNA sequencing of pancreatic circulating tumour cells implicates WNT signalling in metastasis. *Nature, 487*(7408), 510–513. https://doi.org/10.1038/nature11217.
117. Miyamoto, D. T., Zheng, Y., Wittner, B. S., Lee, R. J., Zhu, H., Broderick, K. T., et al. (2015). RNA-Seq of single prostate CTCs implicates noncanonical Wnt signaling in antiandrogen resistance. *Science, 349*(6254), 1351–1356. https://doi.org/10.1126/science.aab0917.
118. Li, F., Tiede, B., Massagué, J., & Kang, Y. (2007). Beyond tumorigenesis: cancer stem cells in metastasis. *Cell Research, 17*(1), 3–14. https://doi.org/10.1038/sj.cr.7310118.
119. Shiozawa, Y., Nie, B., Pienta, K. J., Morgan, T. M., & Taichman, R. S. (2013). Cancer stem cells and their role in metastasis. *Pharmacology & Therapeutics, 138*(2), 285–293. https://doi.org/10.1016/j.pharmthera.2013.01.014.
120. Alexandrov, L. B., Kim, J., Haradhvala, N. J., Huang, M. N., Tian Ng, A. W., Wu, Y., et al. (2020). The repertoire of mutational signatures in human cancer. *Nature, 578*(7793), 94–101. https://doi.org/10.1038/s41586-020-1943-3.
121. Rusan, N. M., & Peifer, M. (2008). Original CIN: reviewing roles for APC in chromosome instability. *The Journal of Cell Biology, 181*(5), 719–726. https://doi.org/10.1083/jcb.200802107.
122. Näthke, I. S. (2004). The adenomatous polyposis coli protein: the Achilles heel of the gut epithelium. *Annual Review of Cell and Developmental Biology, 20*(1), 337–366. https://doi.org/10.1146/annurev.cellbio.20.012103.094541.
123. Munemitsu, S., Souza, B., Müller, O., Albert, I., Rubinfeld, B., & Polakis, P. (1994). The APC gene product associates with microtubules in vivo and promotes their assembly in vitro. *Cancer Research, 54*(14), 3676–3681.
124. Smith, K. J., Levy, D. B., Maupin, P., Pollard, T. D., Vogelstein, B., & Kinzler, K. W. (1994). Wild-type but not mutant APC associates with the microtubule cytoskeleton. *Cancer Research, 54*(14), 3672–3675.
125. Zumbrunn, J., Kinoshita, K., Hyman, A. A., & Näthke, I. S. (2001). Binding of the adenomatous polyposis coli protein to microtubules increases microtubule stability and is regulated by GSK3 beta phosphorylation. *Current Biology: CB, 11*(1), 44–49. https://doi.org/10.1016/s0960-9822(01)00002-1.
126. Fodde, R., Kuipers, J., Rosenberg, C., Smits, R., Kielman, M., Gaspar, C., et al. (2001). Mutations in the APC tumour suppressor gene cause chromosomal instability. *Nature Cell Biology, 3*(4), 433–438. https://doi.org/10.1038/35070129.
127. Banks, J. D., & Heald, R. (2004). Adenomatous polyposis coli associates with the microtubule-destabilizing protein XMCAK. *Current Biology: CB, 14*(22), 2033–2038. https://doi.org/10.1016/j.cub.2004.10.049.
128. Tighe, A., Johnson, V. L., & Taylor, S. S. (2004). Truncating APC mutations have dominant effects on proliferation, spindle checkpoint control, survival and chromosome stability. *Journal of Cell Science, 117*(Pt 26), 6339–6353. https://doi.org/10.1242/jcs.01556.
129. Draviam, V. M., Shapiro, I., Aldridge, B., & Sorger, P. K. (2006). Misorientation and reduced stretching of aligned sister kinetochores promote chromosome missegregation in EB1- or APC-depleted cells. *The EMBO Journal, 25*(12), 2814–2827. https://doi.org/10.1038/sj.emboj.7601168.
130. Hadjihannas, M. V., Brückner, M., Jerchow, B., Birchmeier, W., Dietmaier, W., & Behrens, J. (2006). Aberrant Wnt/beta-catenin signaling can induce chromosomal instability in colon cancer. *Proceedings of the National Academy of Sciences of the United States of America, 103*(28), 10747–10752. https://doi.org/10.1073/pnas.0604206103.
131. Hadjihannas, M. V., Brückner, M., & Behrens, J. (2010). Conductin/axin2 and Wnt signalling regulates centrosome cohesion. *EMBO Reports, 11*(4), 317–324. https://doi.org/10.1038/embor.2010.23.
132. Fumoto, K., Kadono, M., Izumi, N., & Kikuchi, A. (2009). Axin localizes to the centrosome and is involved in microtubule nucleation. *EMBO Reports, 10*(6), 606–613. https://doi.org/10.1038/embor.2009.45.
133. Caldwell, C. M., Green, R. A., & Kaplan, K. B. (2007). APC mutations lead to cytokinetic failures in vitro and tetraploid genotypes in Min mice. *The Journal of Cell Biology, 178*(7), 1109–1120. https://doi.org/10.1083/jcb.200703186.
134. Aoki, K., Aoki, M., Sugai, M., Harada, N., Miyoshi, H., Tsukamoto, T., et al. (2007). Chromosomal instability by beta-catenin/TCF transcription in APC or beta-catenin mutant cells. *Oncogene, 26*(24), 3511–3520. https://doi.org/10.1038/sj.onc.1210141.
135. Voloshanenko, O., Erdmann, G., Dubash, T. D., Augustin, I., Metzig, M., Moffa, G., et al. (2013). Wnt secretion is required to maintain high levels of Wnt activity in colon cancer cells. *Nature Communications, 4*(1), 2610. https://doi.org/10.1038/ncomms3610.
136. Srinivas, U. S., Tan, B. W. Q., Vellayappan, B. A., & Jeyasekharan, A. D. (2019). ROS and the DNA damage response in cancer. *Redox Biology, 25*, 101084. https://doi.org/10.1016/j.redox.2018.101084.
137. Tichy, E. D., Pillai, R., Deng, L., Tischfield, J. A., Hexley, P., Babcock, G. F., & Stambrook, P. J. (2012). The abundance of Rad51 protein in mouse embryonic stem cells is regulated at multiple levels. *Stem Cell Research, 9*(2), 124–134. https://doi.org/10.1016/j.scr.2012.05.004.

138. Bao, S., Wu, Q., McLendon, R. E., Hao, Y., Shi, Q., Hjelmeland, A. B., et al. (2006). Glioma stem cells promote radioresistance by preferential activation of the DNA damage response. *Nature, 444*(7120), 756–760. https://doi.org/10.1038/nature05236.
139. Gibson, B. A., & Kraus, W. L. (2012). New insights into the molecular and cellular functions of poly(ADP-ribose) and PARPs. *Nature Reviews. Molecular Cell Biology, 13*(7), 411–424. https://doi.org/10.1038/nrm3376.
140. Eustermann, S., Videler, H., Yang, J.-C., Cole, P. T., Gruszka, D., Veprintsev, D., & Neuhaus, D. (2011). The DNA-binding domain of human PARP-1 interacts with DNA single-strand breaks as a monomer through its second zinc finger. *Journal of Molecular Biology, 407*(1), 149–170. https://doi.org/10.1016/j.jmb.2011.01.034.
141. Armstrong, A. C., & Clay, V. (2019). Olaparib in germline-mutated metastatic breast cancer: implications of the OlympiAD trial. *Future Oncology (London, England), 15*(20), 2327–2335. https://doi.org/10.2217/fon-2018-0067.
142. Gelmon, K. A., Tischkowitz, M., Mackay, H., Swenerton, K., Robidoux, A., Tonkin, K., et al. (2011). Olaparib in patients with recurrent high-grade serous or poorly differentiated ovarian carcinoma or triple-negative breast cancer: a phase 2, multicentre, open-label, non-randomised study. *The Lancet Oncology, 12*(9), 852–861. https://doi.org/10.1016/S1470-2045(11)70214-5.
143. Yamamoto, T. M., McMellen, A., Watson, Z. L., Aguilera, J., Ferguson, R., Nurmemmedov, E., et al. (2019). Activation of Wnt signaling promotes olaparib resistant ovarian cancer. *Molecular Carcinogenesis, 68*, 7. https://doi.org/10.1002/mc.23064.
144. Thorne, C. A., Hanson, A. J., Schneider, J., Tahinci, E., Orton, D., Cselenyi, C. S., et al. (2010). Small-molecule inhibition of Wnt signaling through activation of casein kinase 1α. *Nature Chemical Biology, 6*(11), 829–836. https://doi.org/10.1038/nchembio.453.
145. Li, B., Fei, D. L., Flaveny, C. A., Dahmane, N., Baubet, V., Wang, Z., et al. (2014). Pyrvinium attenuates Hedgehog signaling downstream of smoothened. *Cancer Research, 74*(17), 4811–4821. https://doi.org/10.1158/0008-5472.CAN-14-0317.
146. Jun, S., Jung, Y.-S., Suh, H. N., Wang, W., Kim, M. J., Oh, Y. S., et al. (2016). LIG4 mediates Wnt signalling-induced radioresistance. *Nature Communications, 7*(1), 10994–10913. https://doi.org/10.1038/ncomms10994.
147. Kanazawa, A., Tsukada, S., Sekine, A., Tsunoda, T., Takahashi, A., Kashiwagi, A., et al. (2004). Association of the gene encoding wingless-type mammary tumor virus integration-site family member 5B (WNT5B) with type 2 diabetes. *American Journal of Human Genetics, 75*(5), 832–843. https://doi.org/10.1086/425340.
148. Zeve, D., Seo, J., Suh, J. M., Stenesen, D., Tang, W., Berglund, E. D., et al. (2012). Wnt signaling activation in adipose progenitors promotes insulin-independent muscle glucose uptake. *Cell Metabolism, 15*(4), 492–504. https://doi.org/10.1016/j.cmet.2012.03.010.
149. Mori, H., Prestwich, T. C., Reid, M. A., Longo, K. A., Gerin, I., Cawthorn, W. P., et al. (2012). Secreted frizzled-related protein 5 suppresses adipocyte mitochondrial metabolism through WNT inhibition. *The Journal of Clinical Investigation, 122*(7), 2405–2416. https://doi.org/10.1172/JCI63604.
150. Behari, J., Li, H., Liu, S., Stefanovic-Racic, M., Alonso, L., O'Donnell, C. P., et al. (2014). β-catenin links hepatic metabolic zonation with lipid metabolism and diet-induced obesity in mice. *The American Journal of Pathology, 184*(12), 3284–3298. https://doi.org/10.1016/j.ajpath.2014.08.022.
151. Sethi, J. K., & Vidal-Puig, A. (2010). Wnt signalling and the control of cellular metabolism. *The Biochemical Journal, 427*(1), 1–17. https://doi.org/10.1042/BJ20091866.
152. Liberti, M. V., & Locasale, J. W. (2016). The Warburg effect: how does it benefit cancer cells? *Trends in Biochemical Sciences, 41*(3), 211–218. https://doi.org/10.1016/j.tibs.2015.12.001.
153. Sherwood, V. (2015). WNT signaling: an emerging mediator of cancer cell metabolism? *Molecular and Cellular Biology, 35*(1), 2–10. https://doi.org/10.1128/MCB.00992-14.
154. Chafey, P., Finzi, L., Boisgard, R., Caüzac, M., Clary, G., Broussard, C., et al. (2009). Proteomic analysis of beta-catenin activation in mouse liver by DIGE analysis identifies glucose metabolism as a new target of the Wnt pathway. *Proteomics, 9*(15), 3889–3900. https://doi.org/10.1002/pmic.200800609.
155. Pate, K. T., Stringari, C., Sprowl-Tanio, S., Wang, K., TeSlaa, T., Hoverter, N. P., et al. (2014). Wnt signaling directs a metabolic program of glycolysis and angiogenesis in colon cancer. *The EMBO Journal, 33*(13), 1454–1473. https://doi.org/10.15252/embj.201488598.
156. Lee, S. Y., Jeon, H. M., Ju, M. K., Kim, C. H., Yoon, G., Han, S. I., et al. (2012). Wnt/Snail signaling regulates cytochrome C oxidase and glucose metabolism. *Cancer Research, 72*(14), 3607–3617. https://doi.org/10.1158/0008-5472.CAN-12-0006.
157. Dang, C. V., Le, A., & Gao, P. (2009). MYC-induced cancer cell energy metabolism and therapeutic opportunities. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 15*(21), 6479–6483. https://doi.org/10.1158/1078-0432.CCR-09-0889.
158. Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. *Nature, 420*(6917), 860–867. https://doi.org/10.1038/nature01322.
159. Crusz, S. M., & Balkwill, F. R. (2015). Inflammation and cancer: advances and new agents. *Nature Reviews. Clinical Oncology, 12*(10), 584–596. https://doi.org/10.1038/nrclinonc.2015.105.
160. Yeo, E.-J., Cassetta, L., Qian, B.-Z., Lewkowich, I., Li, J.-F., Stefater, J. A., et al. (2014). Myeloid WNT7b mediates the angiogenic switch and metastasis in breast cancer. *Cancer Research, 74*(11), 2962–2973. https://doi.org/10.1158/0008-5472.CAN-13-2421.
161. Ojalvo, L. S., Whittaker, C. A., Condeelis, J. S., & Pollard, J. W. (2010). Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. *The Journal of Immunology, 184*(2), 702–712. https://doi.org/10.4049/jimmunol.0902360.
162. Oguma, K., Oshima, H., Aoki, M., Uchio, R., Naka, K., Nakamura, S., et al. (2008). Activated macrophages promote Wnt signalling through tumour necrosis factor-alpha in gastric tumour cells. *The EMBO Journal, 27*(12), 1671–1681. https://doi.org/10.1038/emboj.2008.105.
163. Pukrop, T., Klemm, F., Hagemann, T., Gradl, D., Schulz, M., Siemes, S., et al. (2006). Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proceedings of the National Academy of Sciences of the United States of America, 103*(14), 5454–5459. https://doi.org/10.1073/pnas.0509703103.
164. Linde, N., Casanova-Acebes, M., Sosa, M. S., Mortha, A., Rahman, A., Farias, E., et al. (2018). Macrophages orchestrate breast cancer early dissemination and metastasis. *Nature Communications, 9*(1), 21. https://doi.org/10.1038/s41467-017-02481-5.
165. Manicassamy, S., Reizis, B., Ravindran, R., Nakaya, H., Salazar-Gonzalez, R. M., Wang, Y.-C., & Pulendran, B. (2010). Activation of beta-catenin in dendritic cells regulates immunity versus tolerance in the intestine. *Science, 329*(5993), 849–853. https://doi.org/10.1126/science.1188510.
166. Oderup, C., LaJevic, M., & Butcher, E. C. (2013). Canonical and noncanonical Wnt proteins program dendritic cell responses for

tolerance. *The Journal of Immunology, 190*(12), 6126–6134. https://doi.org/10.4049/jimmunol.1203002.

167. Hong, Y., Manoharan, I., Suryawanshi, A., Shanmugam, A., Swafford, D., Ahmad, S., et al. (2016). Deletion of LRP5 and LRP6 in dendritic cells enhances antitumor immunity. *Oncoimmunology, 5*(4), e1115941. https://doi.org/10.1080/2162402X.2015.1115941.
168. Spranger, S., Bao, R., & Gajewski, T. F. (2015). Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature, 523*(7559), 231–235. https://doi.org/10.1038/nature14404.
169. Luke, J. J., Bao, R., Sweis, R. F., Spranger, S., & Gajewski, T. F. (2019). WNT/β-catenin pathway activation correlates with immune exclusion across human cancers. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research*, clincanres.1942.2018. https://doi.org/10.1158/1078-0432.CCR-18-1942.
170. Holtzhausen, A., Zhao, F., Evans, K. S., Tsutsui, M., Orabona, C., Tyler, D. S., & Hanks, B. A. (2015). Melanoma-derived Wnt5a promotes local dendritic-cell expression of IDO and immunotolerance: opportunities for pharmacologic enhancement of immunotherapy. *Cancer Immunology Research, 3*(9), 1082–1095. https://doi.org/10.1158/2326-6066.CIR-14-0167.
171. Fallarino, F., Grohmann, U., You, S., McGrath, B. C., Cavener, D. R., Vacca, C., et al. (2006). The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *The Journal of Immunology, 176*(11), 6752–6761. https://doi.org/10.4049/jimmunol.176.11.6752.
172. Zhao, F., Xiao, C., Evans, K. S., Theivanthiran, T., DeVito, N., Holtzhausen, A., et al. (2018). Paracrine Wnt5a-β-catenin signaling triggers a metabolic program that drives dendritic cell tolerization. *Immunity, 48*(1), 147–160.e7. https://doi.org/10.1016/j.immuni.2017.12.004.
173. Khazaie, K., Blatner, N. R., Khan, M. W., Gounari, F., Gounaris, E., Dennis, K., et al. (2011). The significant role of mast cells in cancer. *Cancer Metastasis Reviews, 30*(1), 45–60. https://doi.org/10.1007/s10555-011-9286-z.
174. Yamaguchi, T., Nishijima, M., Tashiro, K., & Kawabata, K. (2016). Wnt-β-catenin signaling promotes the maturation of mast cells. *BioMed Research International, 2016*(7, article 1378), 2048987–2048988. https://doi.org/10.1155/2016/2048987.
175. Tebroke, J., Lieverse, J. E., Säfholm, J., Schulte, G., Nilsson, G., & Rönnberg, E. (2019). Wnt-3a induces cytokine release in human mast cells. *Cells, 8*(11), 1372. https://doi.org/10.3390/cells8111372.
176. Strouch, M. J., Cheon, E. C., Salabat, M. R., Krantz, S. B., Gounaris, E., Melstrom, L. G., et al. (2010). Crosstalk between mast cells and pancreatic cancer cells contributes to pancreatic tumor progression. *Clinical Cancer Research: An Official Journal of the American Association for Cancer Research, 16*(8), 2257–2265. https://doi.org/10.1158/1078-0432.CCR-09-1230.
177. Jensen, H. K., Donskov, F., Marcussen, N., Nordsmark, M., Lundbeck, F., & von der Maase, H. (2009). Presence of intratumoral neutrophils is an independent prognostic factor in localized renal cell carcinoma. *Journal of Clinical Oncology : Official Journal of the American Society of Clinical Oncology, 27*(28), 4709–4717. https://doi.org/10.1200/JCO.2008.18.9498.
178. Trellakis, S., Bruderek, K., Dumitru, C. A., Gholaman, H., Gu, X., Bankfalvi, A., et al. (2011). Polymorphonuclear granulocytes in human head and neck cancer: enhanced inflammatory activity, modulation by cancer cells and expansion in advanced disease. *International Journal of Cancer, 129*(9), 2183–2193. https://doi.org/10.1002/ijc.25892.
179. Galdiero, M. R., Bianchi, P., Grizzi, F., Di Caro, G., Basso, G., Ponzetta, A., et al. (2016). Occurrence and significance of tumor-associated neutrophils in patients with colorectal cancer. *International Journal of Cancer, 139*(2), 446–456. https://doi.org/10.1002/ijc.30076.
180. Caruso, R. A., Bellocco, R., Pagano, M., Bertoli, G., Rigoli, L., & Inferrera, C. (2002). Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in northern Italy. *Modern pathology : an official journal of the United States and Canadian Academy of Pathology, Inc, 15*(8), 831–837. https://doi.org/10.1097/01.MP.0000020391.98998.6B.
181. Chao, T., Furth, E. E., & Vonderheide, R. H. (2016). CXCR2-dependent accumulation of tumor-associated neutrophils regulates T-cell immunity in pancreatic ductal adenocarcinoma. *Cancer Immunology Research, 4*(11), 968–982. https://doi.org/10.1158/2326-6066.CIR-16-0188.
182. Skokowa, J., Cario, G., Uenalan, M., Schambach, A., Germeshausen, M., Battmer, K., et al. (2006). LEF-1 is crucial for neutrophil granulocytopoiesis and its expression is severely reduced in congenital neutropenia. *Nature Medicine, 12*(10), 1191–1197. https://doi.org/10.1038/nm1474.
183. Verbeek, S., Izon, D., Hofhuis, F., Robanus-Maandag, E., te Riele, H., van de Wetering, M., et al. (1995). An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature, 374*(6517), 70–74. https://doi.org/10.1038/374070a0.
184. Staal, F. J., Meeldijk, J., Moerer, P., Jay, P., van de Weerdt, B. C., Vainio, S., et al. (2001). Wnt signaling is required for thymocyte development and activates Tcf-1 mediated transcription. *European Journal of Immunology, 31*(1), 285–293. https://doi.org/10.1002/1521-4141(200101)31:1<285::AID-IMMU285>3.0.CO;2-D.
185. Wang, B., Tian, T., Kalland, K.-H., Ke, X., & Qu, Y. (2018). Targeting Wnt/β-catenin signaling for cancer immunotherapy. *Trends in Pharmacological Sciences, 39*(7), 648–658. https://doi.org/10.1016/j.tips.2018.03.008.
186. Wong, C., Chen, C., Wu, Q., Liu, Y., & Zheng, P. (2015). A critical role for the regulated wnt-myc pathway in naive T cell survival. *The Journal of Immunology, 194*(1), 158–167. https://doi.org/10.4049/jimmunol.1401238.
187. Yu, Q., Sharma, A., Oh, S. Y., Moon, H.-G., Hossain, M. Z., Salay, T. M., et al. (2009). T cell factor 1 initiates the T helper type 2 fate by inducing the transcription factor GATA-3 and repressing interferon-gamma. *Nature Immunology, 10*(9), 992–999. https://doi.org/10.1038/ni.1762.
188. Notani, D., Gottimukkala, K. P., Jayani, R. S., Limaye, A. S., Damle, M. V., Mehta, S., et al. (2010). Global regulator SATB1 recruits beta-catenin and regulates T(H)2 differentiation in Wnt-dependent manner. *PLoS Biology, 8*(1), e1000296. https://doi.org/10.1371/journal.pbio.1000296.
189. Dai, W., Liu, F., Li, C., Lu, Y., Lu, X., Du, S., et al. (2016). Blockade of Wnt/β-catenin pathway aggravated silica-induced lung inflammation through Tregs regulation on Th immune responses. *Mediators of Inflammation, 2016*(6), 6235614–6235614. https://doi.org/10.1155/2016/6235614.
190. Ding, Y., Shen, S., Lino, A. C., Curotto de Lafaille, M. A., & Lafaille, J. J. (2008). Beta-catenin stabilization extends regulatory T cell survival and induces anergy in nonregulatory T cells. *Nature Medicine, 14*(2), 162–169. https://doi.org/10.1038/nm1707.
191. Sun, X., Liu, S., Wang, D., Zhang, Y., Li, W., Guo, Y., et al. (2017). Colorectal cancer cells suppress CD4+ T cells immunity through canonical Wnt signaling. *Oncotarget, 8*(9), 15168–15181. https://doi.org/10.18632/oncotarget.14834.
192. Keerthivasan, S., Aghajani, K., Dose, M., Molinero, L., Khan, M. W., Venkateswaran, V., et al. (2014). β-Catenin promotes colitis and colon cancer through imprinting of proinflammatory properties in T cells. *Science Translational Medicine, 6*(225), 225ra28–225ra28. https://doi.org/10.1126/scitranslmed.3007607.

193. Trujillo, J. A., Sweis, R. F., Bao, R., & Luke, J. J. (2018). T cell-inflamed versus non-T cell-inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. *Cancer Immunology Research, 6*(9), 990–1000. https://doi.org/10.1158/2326-6066.CIR-18-0277.
194. Spranger, S., & Gajewski, T. F. (2018). Impact of oncogenic pathways on evasion of antitumour immune responses. *Nature Reviews. Cancer, 18*(3), 139–147. https://doi.org/10.1038/nrc.2017.117.
195. Sweis, R. F., Spranger, S., Bao, R., Paner, G. P., Stadler, W. M., Steinberg, G., & Gajewski, T. F. (2016). Molecular drivers of the non-T-cell-inflamed tumor microenvironment in urothelial bladder cancer. *Cancer Immunology Research, 4*(7), 563–568. https://doi.org/10.1158/2326-6066.CIR-15-0274.
196. Murata, Y., Saito, Y., Kotani, T., & Matozaki, T. (2018). CD47-signal regulatory protein α signaling system and its application to cancer immunotherapy. *Cancer Science, 109*(8), 2349–2357. https://doi.org/10.1111/cas.13663.
197. Gowda, P., Patrick, S., Singh, A., Sheikh, T., & Sen, E. (2018). Mutant isocitrate dehydrogenase 1 disrupts PKM2-β-catenin-BRG1 transcriptional network-driven CD47 expression. *Molecular and Cellular Biology, 38*(9), 597. https://doi.org/10.1128/MCB.00001-18.
198. Buchbinder, E. I., & Desai, A. (2016). CTLA-4 and PD-1 pathways: similarities, differences, and implications of their inhibition. *American Journal of Clinical Oncology, 39*(1), 98–106. https://doi.org/10.1097/COC.0000000000000239.
199. Hui, E., Cheung, J., Zhu, J., Su, X., Taylor, M. J., Wallweber, H. A., et al. (2017). T cell costimulatory receptor CD28 is a primary target for PD-1-mediated inhibition. *Science, 355*(6332), 1428–1433. https://doi.org/10.1126/science.aaf1292.
200. Yokosuka, T., Takamatsu, M., Kobayashi-Imanishi, W., Hashimoto-Tane, A., Azuma, M., & Saito, T. (2012). Programmed cell death 1 forms negative costimulatory microclusters that directly inhibit T cell receptor signaling by recruiting phosphatase SHP2. *The Journal of Experimental Medicine, 209*(6), 1201–1217. https://doi.org/10.1084/jem.20112741.
201. Chovanec, M., Cierna, Z., Miskovska, V., Machalekova, K., Kalavska, K., Rejlekova, K., et al. (2018). βcatenin is a marker of poor clinical characteristics and suppressed immune infiltration in testicular germ cell tumors. *BMC Cancer, 18*(1), 1062–1010. https://doi.org/10.1186/s12885-018-4929-x.
202. Castagnoli, L., Cancila, V., Cordoba-Romero, S. L., Faraci, S., Talarico, G., Belmonte, B., et al. (2019). WNT signaling modulates PD-L1 expression in the stem cell compartment of triple-negative breast cancer. *Oncogene, 38*(21), 4047–4060. https://doi.org/10.1038/s41388-019-0700-2.
203. Casey, S. C., Tong, L., Li, Y., Do, R., Walz, S., Fitzgerald, K. N., et al. (2016). MYC regulates the antitumor immune response through CD47 and PD-L1. *Science, 352*(6282), 227–231. https://doi.org/10.1126/science.aac9935.
204. Kalbasi, A., & Ribas, A. (2020). Tumour-intrinsic resistance to immune checkpoint blockade. *Nature Reviews. Immunology, 20*(1), 25–39. https://doi.org/10.1038/s41577-019-0218-4.
205. Rotte, A. (2019). Combination of CTLA-4 and PD-1 blockers for treatment of cancer. *Journal of Experimental and Clinical Cancer Research, 38*(1), 255–212. https://doi.org/10.1186/s13046-019-1259-z.
206. Shah, K. V., Chien, A. J., Yee, C., & Moon, R. T. (2008). CTLA-4 is a direct target of Wnt/beta-catenin signaling and is expressed in human melanoma tumors. *The Journal of Investigative Dermatology, 128*(12), 2870–2879. https://doi.org/10.1038/jid.2008.170.
207. Blando, J., Sharma, A., Higa, M. G., Zhao, H., Vence, L., Yadav, S. S., et al. (2019). Comparison of immune infiltrates in melanoma and pancreatic cancer highlights VISTA as a potential target in pancreatic cancer. *Proceedings of the National Academy of Sciences of the United States of America, 116*(5), 1692–1697. https://doi.org/10.1073/pnas.1811067116.
208. Guo, Y., Xiao, L., Sun, L., & Liu, F. (2012). Wnt/beta-catenin signaling: a promising new target for fibrosis diseases. *Physiological Research, 61*(4), 337–346.
209. Madan, B., Patel, M. B., Zhang, J., Bunte, R. M., Rudemiller, N. P., Griffiths, R., et al. (2016). Experimental inhibition of porcupine-mediated Wnt O-acylation attenuates kidney fibrosis. *Kidney International*. https://doi.org/10.1016/j.kint.2016.01.017.
210. Madan, B., & Virshup, D. M. (2015). Targeting Wnts at the source–new mechanisms, new biomarkers, new drugs. *Molecular Cancer Therapeutics, 14*(5), 1087–1094. https://doi.org/10.1158/1535-7163.MCT-14-1038.