ANNUAL REVIEWS

Annual Review of Cancer Biology

WNT and β-Catenin in Cancer: Genes and Therapy

Rene Jackstadt,[1,*] Michael Charles Hodder,[1,*] and Owen James Sansom[1,2]

[1]Cancer Research UK Beatson Institute, Glasgow G61 1BD, United Kingdom; email: o.sansom@beatson.gla.ac.uk

[2]Institute of Cancer Sciences, University of Glasgow, Glasgow G61 1QH, United Kingdom

Annu. Rev. Cancer Biol. 2020. 4:177–96

First published as a Review in Advance on November 25, 2019

The *Annual Review of Cancer Biology* is online at cancerbio.annualreviews.org

https://doi.org/10.1146/annurev-cancerbio-030419-033628

*These authors contributed equally to this article

Keywords

WNT, cancer, β-catenin, APC, microenvironment, stem cells

Abstract

The WNT pathway is a pleiotropic signaling pathway that controls developmental processes, tissue homeostasis, and cancer. The WNT pathway is commonly mutated in many cancers, leading to widespread research into the role of WNT signaling in carcinogenesis. Understanding which cancers are reliant upon WNT activation and which components of the WNT signaling pathway are mutated is paramount to advancing therapeutic strategies. In addition, building holistic insights into the role of WNT signaling in not only tumor cells but also the tumor microenvironment is a vital area of research and may be a promising therapeutic strategy in multiple immunologically inert cancers. Novel compounds aimed at modulating the WNT signaling pathway using diverse mechanisms are currently under investigation in preclinical/early clinical studies. Here, we review how the WNT pathway is activated in multiple cancers and discuss current strategies to target aberrant WNT signaling.

INTRODUCTION

The WNT pathway is an evolutionarily conserved signaling pathway. *Wnt* was initially discovered as an essential developmental gene in *Drosophila* (*Wingless*) (Nusslein-Volhard & Wieschaus 1980), and shortly thereafter as a gene ectopically expressed in mice following infection with murine mammary tumor virus (*Int-1*) (Nusse & Varmus 1982). Once it was recognized that *Wingless* and *Int-1* are the same gene, the portmanteau "Wnt" was coined to recognize both discoveries.

Since these studies almost 40 years ago, a huge amount of work has been undertaken to dissect the functional impact of this pathway in development, tissue homeostasis, and cancer. Of particular relevance for this review is the importance of WNT signaling in normal stem cells (SCs) and homeostasis, as toxicity to normal systems has always been a potential concern with WNT pathway inhibitors. Finding an effective therapeutic window for targeting the WNT pathway requires that tumors be more reliant upon WNT than normal SCs.

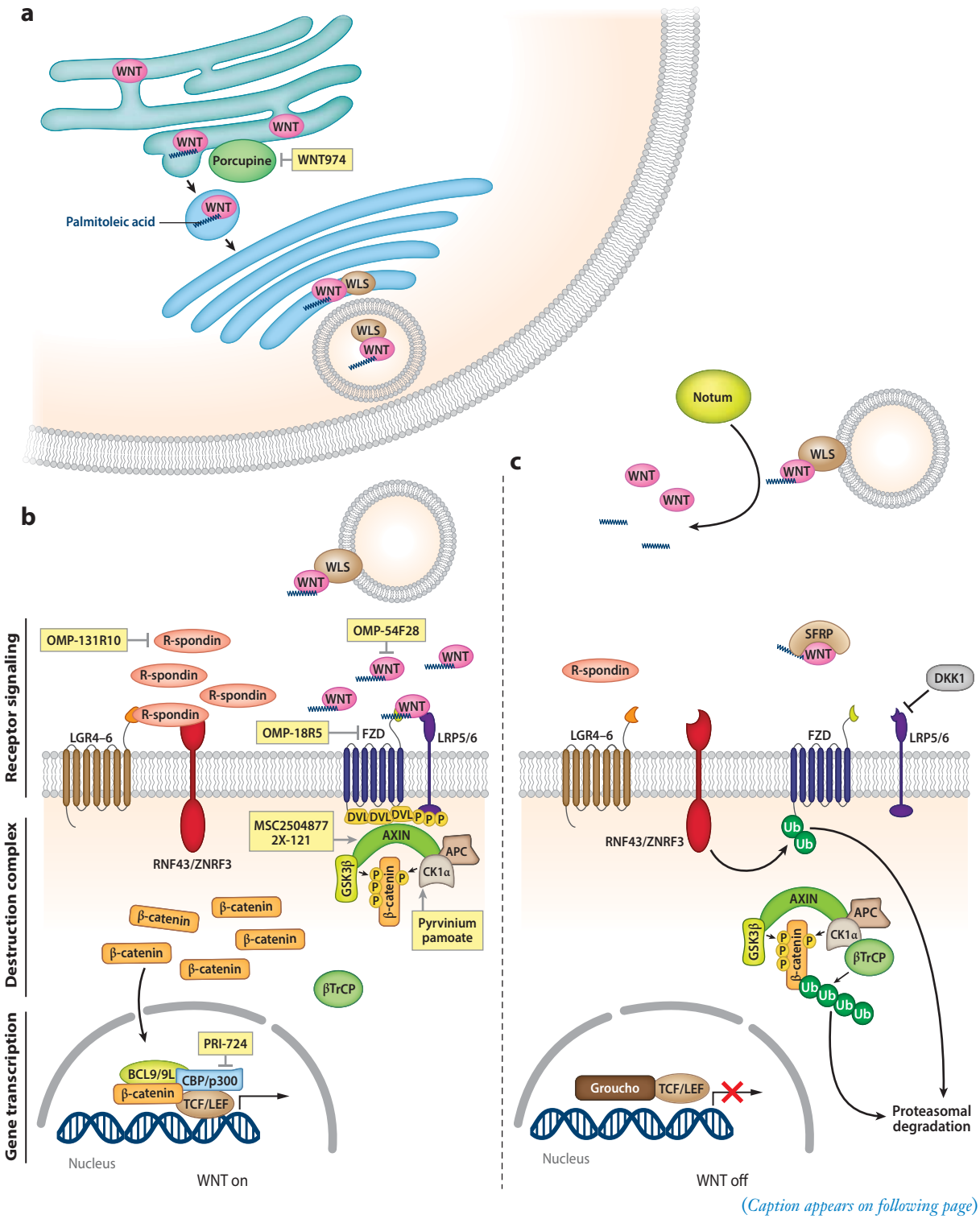
The identification of WNT targets as markers of adult SCs has highlighted the importance of WNT signaling in adult tissue homeostasis. The WNT target LGR5 (leucine-rich repeat–containing G protein–coupled receptor 5; also known as GPR49) is highly expressed in adult intestinal stem cells (ISCs) and was initially identified by lineage-tracing experiments using an *Lgr5*-EGFP-IRES-creERT2 knock-in mouse (Barker et al. 2007). This reporter facilitated the identification and characterization of adult SCs in multiple tissues such as the stomach (Barker et al. 2010), ovary (Ng et al. 2014), hair follicle (Jaks et al. 2008), and liver (Huch et al. 2013). Further transgenic strategies including creating an *Axin2*^{CreERT2} allele showed that the Wnt target Axin2 marks epidermal (Lim et al. 2013), liver (Wang et al. 2015), and mammary SCs (van Amerongen et al. 2012). All these approaches demonstrate that adult SCs have high levels of WNT signaling.

Adenomatous polyposis coli (*APC*), a WNT pathway gene frequently mutated in cancer, was first identified during investigations into familial adenomatous polyposis (FAP). Patients with FAP commonly have a truncating mutation in one copy of *APC*, predisposing them to developing intestinal polyps (Groden et al. 1991, Kinzler et al. 1991, Nishisho et al. 1991). *APC* was later linked with the WNT signaling pathway when the interaction between β-catenin and *APC* was discovered (Rubinfeld et al. 1993, Su et al. 1993). Mutations in *APC*, which occur most frequently in the mutation cluster region (between codons 1,286 and 1,513) lead to loss of AXIN binding motifs and subsequently to stabilization of β-catenin (Minde et al. 2011). Interestingly, *APC* mutations are particularly frequent in colorectal cancer (CRC), highlighted by the enrichment of CRC cases in FAP patients when compared with other cancers. The spectrum of dependency of different cancers upon WNT signaling activation is a crucial area of research that will define the utility of therapeutics targeting the WNT signaling pathway.

Here we discuss tissue-specific effects of WNT pathway alterations in cancer with a focus on CRC, endometrial cancer, hepatocellular carcinoma (HCC), gastric cancer (GC), pancreatic ductal adenocarcinoma (PDAC), and melanoma. We describe how WNT-activating mutations contribute to tumorigenesis in a tissue-specific context, how WNT activation may influence cancer stem cells (CSCs) and the tumor immune milieu, and current strategies to target WNT signaling in cancer.

THE WNT PATHWAY AT A GLANCE

The WNT signaling pathway acts in a paracrine or autocrine fashion to control gene expression and other cellular functions. Under homeostatic conditions, WNT ligands (hereafter termed WNTs), which are ~40-kDa lipid-modified glycoproteins, act as morphogens to control long- and short-distance activation of the WNT pathway by binding to Frizzled (FZD) receptors (**Figure 1**) (Farin et al. 2016, Mulligan et al. 2012).



(Caption appears on following page)

Figure 1 (*Figure appears on preceding page*)

The WNT signaling pathway. (*a*) WNTs are modified by Porcupine and then transported by WLS. WNTs can interact with an FZD receptor to activate canonical WNT signaling. (*b*) WNT on. Once activated, β-catenin is no longer degraded and can accumulate in the cytoplasm and translocate to the nucleus. Once in the nucleus, β-catenin displaces Groucho, binds to TCF/LEF transcription factors, and subsequently binds to WNT-responsive elements, driving transcription of a range of WNT target genes. The WNT signaling pathway can be modulated by the R-spondin axis, whereby R-spondin interacts with LGRs and RNF43/ZNRF3, preventing RNF43 and ZNRF3 from ubiquitinating FZD, resulting in its internalization. (*c*) WNT off. In the absence of WNTs, β-catenin is sequestered by the destruction complex containing AXIN, APC, CK1α, and GSK3β. β-catenin is then sequentially phosphorylated and ubiquitinated, targeting it for proteasomal degradation. Secreted factors can inhibit WNT signaling at the ligand and receptor level. NOTUM removes the palmitoleate moiety from WNTs, inhibiting their interaction with FZD. SFRPs bind WNTs, preventing WNT-FZD interactions, and DKK1 inhibits the ability of LRP5/6 to dimerize with FZDs. Multiple drug targets under investigation to modulate the WNT signaling pathway are indicated on the figure (*yellow boxes*). These drugs target three main levels of the pathway: receptor signaling, the β-catenin destruction complex, and WNT target gene transcription. Abbreviations: FZD, Frizzled; LEF, lymphoid enhancer–binding factor; LGR, leucine-rich repeat–containing G protein–coupled receptor; SFRP, soluble FZD-related protein; TCF, T cell factor; WLS, WNTLESS; WNTs, WNT ligands.

WNT signaling is divided into canonical and noncanonical signaling involving the interaction of one or more of the 19 WNTs with one of the 10 FZD receptors and an associated coreceptor. Once activated, FZDs recruit Dishevelled (DVL) to the cell membrane, allowing it to act as a key node for further signaling. Noncanonical signaling results in activation of the planar cell polarity pathway or the WNT-dependent Ca^{2+} pathway, as described elsewhere (Niehrs 2012). This review focuses upon the canonical WNT signaling pathway because of its importance in both homeostasis and cancer.

Canonical WNT signaling is dependent upon β-catenin-mediated gene transcription. During conditions of low-WNT activity, cytoplasmic β-catenin is targeted for degradation by its destruction complex (Nusse & Clevers 2017), which contains, in brief, APC, axis inhibitor (AXIN), casein kinase 1α (CK1α), and glycogen synthase kinase 3β (GSK3β). APC and AXIN are essential scaffold proteins in the destruction complex (Behrens et al. 1998), and AXIN is required for the phosphorylation of β-catenin by both CK1α and GSK3β. CK1α primes β-catenin by phosphorylating the Ser45 (serine 45) residue, allowing GSK3β to phosphorylate Ser33, Ser37, and Thr41 (threonine 41) (Amit et al. 2002, Ikeda et al. 1998, Liu et al. 2002). Phosphorylation of β-catenin at these residues marks β-catenin for ubiquitination by E3 ligases, such as β-transducin repeat–containing protein (βTrCP), and subsequent proteasomal degradation (Winston et al. 1999).

WNTs bind to a heterodimeric complex of FZD and a coreceptor to mediate signaling. Low-density lipoprotein receptor–related proteins 5 and 6 (LRP5/6) are common coreceptors, and upon a WNT-FZD-LRP interaction, the cytoplasmic tail of LRP is phosphorylated, recruiting AXIN and the destruction complex to the cell membrane. The mutation patterns between *AXIN1* and *AXIN2* vary. While *AXIN1* shows mutations along the entire coding sequence, with changes in alterations between tumors types, *AXIN2* shows recurrent mutations in exon 7 (nucleotides 637 to 714), which lead to protein truncations (Salahshor & Woodgett 2005). This prevents degradation of β-catenin and allows cytoplasmic accumulation and nuclear translocation of β-catenin (Nusse & Clevers 2017). Once in the nucleus, β-catenin binds to the transcription factors T cell factor (TCF) and lymphoid enhancer–binding factor 1 (LEF1), displacing the transcriptional repressor Groucho and mediating binding to WNT-responsive elements on DNA (Nusse & Clevers 2017). At this stage, multiple cofactors can bind β-catenin to modify histone marks, leading to chromatin remodeling (**Figure 1**) (Behrens et al. 1996, Li et al. 2012, Molenaar et al. 1996, Riese et al. 1997). The transcriptional complex of β-catenin/TCF can interact with BCL9/BCL9L, which links the N terminus of β-catenin with Pygopus; this complex can control nuclear localization and transcriptional activation (Hoffmans et al. 2005, Townsley et al. 2004). On the C terminus of β-catenin, cofactors like CBP, BRG1, and Hyrax bind and acetylate histones to activate transcription

(Stadeli et al. 2006). Eventually activation of WNT targets leads to dramatic changes in cellular characteristics in multiple organs, while target genes can be context and tissue specific (Sansom 2009).

Roof plate–specific spondins (R-spondins) 1–4 can bind to LGR4–6 and modulate WNT signaling (de Lau et al. 2011, Kazanskaya et al. 2004). LGR4–6 are type B members of the rhodopsin superfamily of G protein–coupled receptors (GPCRs) and have a large extracellular domain containing multiple leucine-rich repeats (de Lau et al. 2014). R-spondins form a complex between LGRs and the transmembrane E3 ubiquitin ligases ring finger protein 43 (RNF43) or zinc and ring finger 3 (ZNRF3), resulting in the internalization of this complex. RNF43 and ZNRF3 are otherwise able to ubiquitinate FZDs, promoting their destruction and limiting WNT signaling (**Figure 1**) (Hao et al. 2012, Koo et al. 2012). Since *LGR5*, *RNF43*, and *ZNRF3* are all WNT target genes, these represent an intricate feedback mechanism with which WNT signaling can be modulated.

There are multiple negative feedback loops that aim to constrain WNT signaling and limit overactivation of the pathway. AXIN1 and 2 are WNT target genes that once produced will dampen WNT signaling by destabilizing β-catenin via the destruction complex (Behrens et al. 1998). Notum, another negative regulator of WNT signaling, is a secreted carboxylesterase that removes an essential palmitoleate moiety from WNTs, preventing their signaling (Kakugawa et al. 2015).

The Dickkopf-related protein family (DKK) consists of five members, of which DKK1 is the most thoroughly investigated. DKK1 is a secreted protein that inhibits the ability of LRP5/6 to dimerize with FZDs and thus inhibits canonical WNT signaling (Glinka et al. 1998). Soluble FZD-related proteins (SFRPs) are secreted WNT signaling inhibitors that bind to WNTs, preventing WNT-FZD interactions (Logan & Nusse 2004). Overall the WNT signaling pathway has multiple feedback loops that aim to modulate WNT signaling in diverse settings; dysregulation of these pathways can perturb homeostasis and result in cancer.

WNT DYSREGULATION ACROSS CANCER

The WNT pathway is activated in several cancers; however, the precise mechanism and frequency of deregulation is tissue specific. In CRC, around 70% of tumors carry *APC* mutations, whereas approximately one third of HCCs carry WNT pathway mutations, with β-catenin mutations being most common (**Figure 2**). The precise reason for the tissue specificity of WNT pathway mutations remains unclear, although it is possible that differing levels of WNT signaling associated with different mutations influence the fitness of their host cells and that this varies between organs. For example, mouse models have demonstrated that a single activating β-catenin mutation is less efficient at transforming the colon than biallelic *Apc* mutations (Huels et al. 2015).

COLORECTAL CANCER

CRC is the cancer with the highest frequency of WNT pathway mutations (**Figure 2**). The high frequency of *APC* mutations in CRC suggests a specific role for APC loss of function, whereas activating mutations in *CTNNB1* (encoding for β-catenin) only occur in a small percentage of patients (Yaeger et al. 2018). This can be explained by the ability of *Apc* mutations to efficiently transform the murine intestinal epithelium, drive tumor initiation, and impede differentiation (Korinek et al. 1997; Morin et al. 1997; Sansom et al. 2004, 2007). Further, *Apc* mutations inhibit the migration of mutated cells along the crypt-villus axis, reducing the chance that a mutated cell is sloughed off into the lumen of the gut (Sansom et al. 2004). Analysis of ISC competition shows that an *Apc*

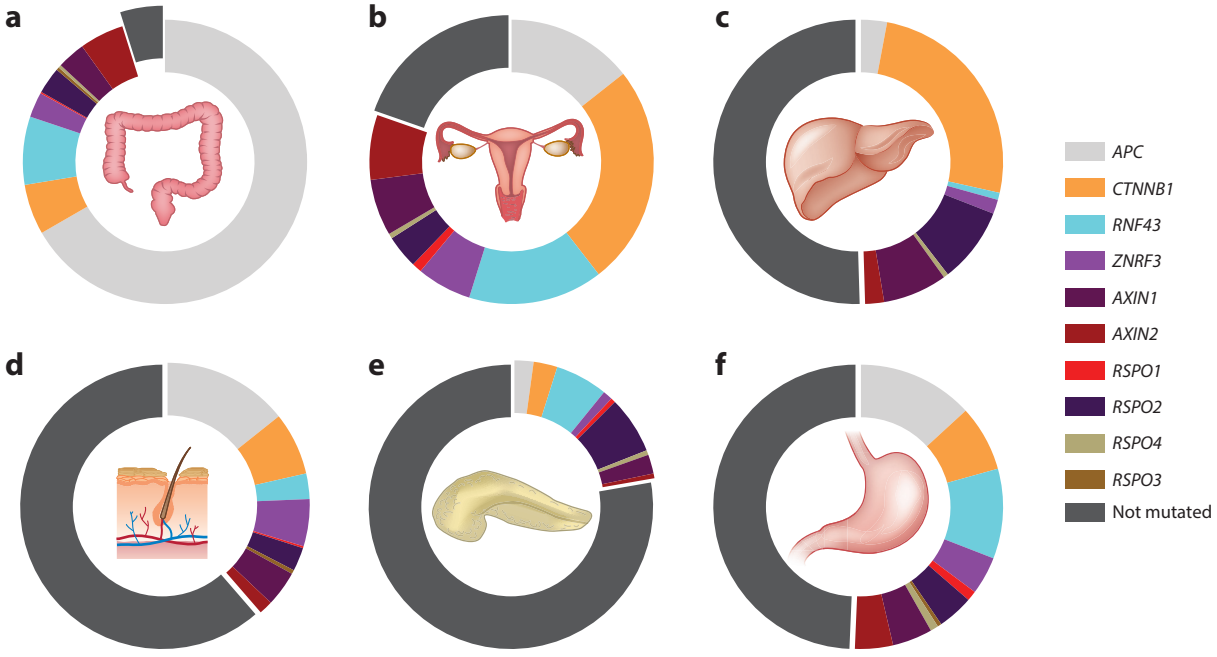


Figure 2

The abundance of potentially activating WNT pathway mutations in various cancers: (*a*) colorectal adenocarcinoma, (*b*) uterine corpus endometrial carcinoma, (*c*) hepatocellular carcinoma, (*d*) cutaneous melanoma, (*e*) pancreatic adenocarcinoma, and (*f*) stomach adenocarcinoma. Data from TCGA (The Cancer Genome Atlas) Pan-Cancer Atlas analyses, as accessed from cBioPortal (**https://www.cbioportal.org**) on March 11, 2019.

mutation is beneficial when compared with wild-type ISCs, allowing mutant cells to be more readily retained within a crypt (Vermeulen et al. 2013). Furthermore, the truncation of *Apc* in Lgr5$^+$ ISCs rapidly results in tumor formation (Barker et al. 2009). More recently, translocations and mutations that enhance ligand-dependent activation of the pathway have been identified. These include EIF3E-RSPO2 and PTPRK-RSPO3 fusions, which drive expression of the WNT-enhancing R-spondin molecules, or deleterious mutations in *RNF43* or *ZNRF3* (Koo et al. 2012, Seshagiri et al. 2012). Interestingly, mutations in *RNF43* or *ZNRF3* were associated with *BRAF*-mutant CRC. In *BRAF*-mutant microsatellite-instable (MSI) tumors, the most frequent frameshift mutation in *RNF43* is detected at nucleotide 659 in 79.6% of patients. *ZNRF3* mutations were detected with lower frequency in MSI (29.6% of patients) and microsatellite-stable (MSS) (15.2% of patients) tumors (Bond et al. 2016), indicating that mutations in *ZNRF3* and *RNF43* drive WNT pathway activation in *BRAF*-mutant tumors and might depict a strategy to target this CRC subtype.

Molecular subtyping of cancers has provided a significant contribution toward understanding the complexity of this disease. A prototype example is the generation of the consensus molecular subtypes (CMSs) of CRC (Fessler & Medema 2016, Guinney et al. 2015). This approach identified four different CMSs, which show marked differences in their stromal composition. CMS2 (the classical subtype) and CMS3 (the metabolic subtype) are characterized by high canonical WNT activation (*APC* mutations in 83% and 78% of patients, respectively) and an immunologically blunt stroma, and the mesenchymal subtype, CMS4, has the worst prognosis, high stromal infiltration, and *APC* mutations in 66% of patients (Becht et al. 2016, Guinney et al. 2015).

These results indicate that these molecular subtypes are largely independent of *APC* mutational status. Recent evidence from engineered human colonic organoids shows that WNT receptor signaling rather than deletion of *APC* is associated with poor prognosis and overlaps with the CMS4 signature (Michels et al. 2019). The first preclinical studies utilizing cell lines or patient-derived xenografts demonstrated that targeting CRC subtypes with tailored therapeutic strategies is promising (Linnekamp et al. 2018, Sveen et al. 2018, Tauriello et al. 2018). Which CRC subtypes are responsive to WNT inhibitors is an important question yet to be addressed.

While strong evidence suggests that WNT pathway activation is key at the earliest stages of tumorigenesis, recent studies have indicated a reduction in WNT pathway activation during carcinogenesis (de Sousa e Melo et al. 2011). This involves methylation of WNT target genes such as *ASCL2* and *LGR5* and is associated with a poorer prognosis (de Sousa e Melo et al. 2011). An elegant proof-of-concept study in mice highlighted that reducing Wnt signaling activity, by restoring *Apc*, triggers tumor regression (Dow et al. 2015). These data imply that targeting WNT signaling in CRC is a promising approach, but the different levels of WNT signaling in CRC require further investigation to identify the most effective therapeutic strategies.

HEPATOCELLULAR CARCINOMA

Approximately half of all HCCs contain WNT-activating mutations, with ~25% containing mutations in β-catenin. Loss-of-function mutations in *AXIN1* as well as *RSPO2* amplifications make up the majority of the other methods of WNT activation (**Figure 2**). Ser45 and Ser33 are the most commonly mutated β-catenin residues in HCC and are principally phosphorylated by CK1α and GSK3β, respectively (Amit et al. 2002, Liu et al. 2002). Overexpression of normal (Tan et al. 2005) or mutant β-catenin (Cadoret et al. 2001, Harada et al. 2002) in the livers of mice results in hepatomegaly but not tumor formation, indicating that, in contrast to the intestine, mutant β-catenin is unable to drive tumor formation in isolation.

Recently a 46.4-kilobase deletion on chromosome 8q23.1 was described in liver tumors (Longerich et al. 2019). This deletion, overlapping with exon 1 of *RSPO2*, is potentially identical to the EIF3E-RSPO2 fusion previously described in CRC and is associated with increased expression of RSPO2 and WNT signaling activation (Longerich et al. 2019). *Axin1* deletion in the murine liver also results in hepatomegaly but, unlike β-catenin mutations, can also drive tumor development over the long term (Feng et al. 2012). Reexpression of wild-type *AXIN1*, for the purpose of restraining WNT signaling, induces apoptosis in hepatocytes that have accumulated nuclear β-catenin (Satoh et al. 2000). Although mutations in *APC* are rare in HCC, genetically disrupted *Apc* in mouse models results in β-catenin-dependent hepatomegaly and disrupted liver zonation (Reed et al. 2008), as well as liver tumors (Buchert et al. 2010, Colnot et al. 2004). The ability of *Apc* and *Axin1* mutations, but not β-catenin mutations, to drive liver tumors in murine models raises the question of why β-catenin mutations predominate in HCC. It is possible that in a largely nonproliferative organ such as the liver, a single β-catenin mutation is more likely to occur than biallelic mutations in either *APC* or *AXIN1*. Conversely, the intestine may favor loss of tumor suppressors such as *APC* partly because of its high basal proliferation rate.

GASTRIC CANCER

GCs show between 10% and 50% deregulated WNT signaling, with a similar mutation rate in *APC* and *RNF43* (**Figure 2**). Loss-of-function mutations in *RNF43* are associated with microsatellite instable GC (Wang et al. 2014). Beside mutations in the WNT pathway, ligands such

as WNT1, WNT5A, and WNT6 have been reported to be increasingly expressed in gastric tumors (Kurayoshi et al. 2006, Mao et al. 2014, Yuan et al. 2013). Wnt1 overexpression in mice resulted in preneoplastic lesions and, when combined with activation of the prostaglandin pathway, led to invasive GC development (Oshima et al. 2006).

Further work in genetically engineered mouse models underscores the importance of WNT pathway alterations to trigger epithelial transformation. When we deleted *Gsk3* or *Apc* or expressed a constitutively active version of β-catenin, homeostasis of the gastric epithelium rapidly changed and tumors formed (Radulescu et al. 2013). Likewise, multiple reports have demonstrated that deletion of *Apc* is sufficient to drive GC in mice (Powell et al. 2014, Sarkar et al. 2016). However, the percentage of WNT pathway mutations in GC is relatively low compared to CRC, indicating that other drivers of tumor initiation are important in GC. Two major factors associated with GC development are germline mutations of *CDH1* (encoding for E-cadherin) and *Helicobacter pylori* infection (Tahara 1995). Interestingly, these factors have been linked to WNT signaling activation (Huels et al. 2015, Kim et al. 2010). *H. pylori* infection causes WNT pathway activation by phosphorylation of the WNT coreceptor LRP6, inhibition of GSK3, and regulation of c-MET (Gnad et al. 2010, Murata-Kamiya et al. 2007, Suzuki et al. 2009). These data imply that targeting GC via WNTs-dependent mechanisms may be a promising strategy.

PANCREATIC CANCER

Genetic alterations of WNT signaling occur relatively rarely in PDAC (**Figure 2**) (Cancer Genome Atlas Res. Netw. 2017). However, some evidence correlates nuclear β-catenin with worse overall survival in PDAC patients (Sano et al. 2016). This activation of WNT signaling might depend on the composition of SC niche factors such as WNTs or R-spondins. Seino et al. (2018) generated a patient-derived organoid biobank of 39 samples and demonstrated differential patterns of WNT ligand dependency in PDACs. Two major types of PDACs were identified, non-ligand-producing epithelial cells, which rely on fibroblast-derived WNTs, and tumors in which the epithelium produces WNTs. Interestingly, patients with epithelial WNT production show worse survival, possibly because those tumor cells can grow independently of niche factors, a process speculated to be important during metastasis. The most frequently mutated WNT pathway component in human PDAC is the negative regulator of WNT signaling, ubiquitin E3 ligase RNF43 (Cancer Genome Atlas Res. Netw. 2017). Patient-derived cell lines that harbor RNF43 mutations are responsive to Porcupine and FZD5 inhibition (Jiang et al. 2013, Steinhart et al. 2017), although it will be important to test the efficacy of these therapies in immune-competent settings.

PROSTATE AND ENDOMETRIAL CANCER

The WNT pathway is mutationally activated in the majority of uterine corpus endometrial carcinomas, with β-catenin mutations present in ~25% of cases (**Figure 2**). Endometrial cancer can be subdivided into four categories that display some resemblance to those described in CRC, such as the MLH1-associated microsatellite-instable subtype and the POLE subtypes (Cancer Genome Atlas Res. Netw. et al. 2012, 2013; Palles et al. 2013). The microsatellite-stable low–copy number endometrioid subtype is enriched for β-catenin mutations, which appear in 52% of cases. Additionally *SOX17*, which has been shown to mediate degradation of β-catenin (Sinner et al. 2007), is exclusively mutated in this subtype (Cancer Genome Atlas Res. Netw. et al. 2013). A separate study reported that high expression levels of β-catenin and the known WNT target genes *MYC* and *CCND1* were associated with worse overall survival in low-grade tumors (Liu et al. 2014). It

has been proposed that microsatellite-instable cancers could increase their WNT signaling levels via other mechanisms, such as the methylation and downregulation of *APC* or *SFRP1/4* (Risinger et al. 2005, Zysman et al. 2002). In mice, canonical Wnt signaling is essential for estrogen-induced uterine growth (Hou et al. 2004), and expression of a mutant β-catenin allele drives endometrial hyperplasia but not carcinogenesis (Jeong et al. 2009).

In addition to the commonly occurring WNT pathway mutations in endometrial cancer, downregulation of the WNT inhibitor DKK1 in an endometrial cancer cell line increased cell migration and invasion (Yi et al. 2013). Overexpression of WNT7a has been correlated with poor overall survival (Liu et al. 2013), while in vitro WNT7a has been shown to activate the canonical WNT signaling pathway, promoting proliferation of endometrial cancer cells, a phenomenon that can be abrogated by the addition of SFRP4 (Carmon & Loose 2008).

Initially, mutational studies showed very low levels of WNT pathway mutations in prostate cancers. However, when aggressive castrate-resistant prostate cancer was sequenced, a higher frequency of WNT pathway mutations was found (18% of cases, ~10% *APC* mutations) (Robinson et al. 2015). This suggests that inhibiting WNT signaling may be effective against castrate-resistant disease (Isaacsson Velho et al. 2020, Zhang et al. 2018).

MELANOMA

WNT signaling is a crucial controller of melanocyte development and migration toward the hair follicle and epidermis (Larue & Delmas 2006). Activation of Wnt signaling in mice increases melanocyte quantity, and in turn, depletion of WNTs completely ablates melanoblasts (Ikeya et al. 1997). However, WNT signaling activation is more controversial in melanoma than in other cancers. This may be partly due to difficulties in the detection of activated WNT signaling by nuclear β-catenin. Analyses of patient cohorts show contradictory results in terms of patient outcomes (Xue et al. 2016). Approximately 30% of tumors exhibit nuclear β-catenin (Larue & Delmas 2006), and the highest WNT pathway mutation frequency is 7%, detected in β-catenin (Hodis et al. 2012). Activation of β-catenin in transgenic mice did not lead to the onset of melanoma in isolation; only when intercrossed to a mutant *Nras* allele did β-catenin mutation promote melanoma onset by suppressing $p16^{INK4a}$ (Delmas et al. 2007).

TARGETING WNT IN CANCER

Currently there are no therapies specifically targeting WNT signaling that are clinically approved for the treatment of cancer. However, there has been considerable interest in this space, and many strategies to target WNT signaling have been proposed. Current therapeutic approaches can be broadly separated into three distinct categories: targeting receptor signaling, targeting the destruction complex, and targeting gene transcription. Most preclinical and clinical attempts to modulate WNT signaling are focused upon modulating receptor signaling, with inhibitors of Porcupine being the most established WNT-targeting xenobiotics.

WNT RECEPTOR SIGNALING

Porcupine is a membrane-bound O-acetyltransferase that is essential for the posttranslational modification and activation of WNTs (**Figure 1**). This palmitoylation of WNTs in a position corresponding to Ser209 of WNT3a is required for binding to WNTLESS, secretion, and interaction with FZDs (Janda et al. 2012). There are currently multiple Porcupine inhibitors in phase I/II clinical trials for cancer, with the Novartis compound WNT974 (previously LGK974)

currently in phase II trials for both CRC and head and neck squamous cell carcinoma. Porcupine inhibitors have shown promising results in preclinical models of CRC (Han et al. 2017, Koo et al. 2015). Furthermore, in a preclinical model of lung adenocarcinoma driven by *Kras*$^{G12D/+}$;*Trp53*$^{fl/fl}$ mutations, WNT974 treatment inhibited proliferation by disrupting the SC niche (Tammela et al. 2017). Interestingly, tumor cell–derived WNTs not only contribute to the SC niche of tumors but also can activate stromal components. In breast cancer models, epithelial cell–secreted WNT7a influences cancer-associated fibroblasts (CAFs) by potentiating TGF-β signaling, eventually leading to extracellular matrix remodeling and tumor progression (Avgustinova et al. 2016). Importantly, the efficacy of Porcupine inhibitors is likely limited to WNT-driven tumors that do not carry an activating mutation downstream of receptor signaling, which in CRC means that patients with R-spondin fusions or RNF43 mutations should be identified for treatment. The potential of Porcupine inhibitors is likely to be determined somewhat by an on-target side effect resulting in loss of bone density. However, efforts are underway to combine Porcupine inhibitors with antiresorptive drugs to minimize this side effect (Madan et al. 2018).

An alternative strategy for targeting WNT signaling may be at the level of receptor internalization. A recent study has demonstrated that WNT signaling can be modulated by RAL protein–mediated regulation of FZD internalization (Johansson et al. 2019).

There are multiple therapeutic antibodies aimed at preventing WNT-receptor interactions either by acting as a ligand trap or by binding receptors directly. Both OMP-18R5 (vantictumab), a pan-FZD-blocking antibody, and OMP-54F28, a WNT-ligand-trapping antibody, are currently in phase I clinical trials for multiple cancers. Interestingly, an elegant study in mouse models of GC showed that the use of OMP-18R5 was sufficient to inhibit adenoma growth, with or without *Apc* mutations (Flanagan et al. 2019). This finding demonstrates that WNT receptor–based therapies can still impact on tumor cells containing mutations in the destruction complex. It will be important to determine whether this generalizes across multiple cancer types. Additionally, a humanized monoclonal antibody against RSPO3, OMP-131R10, is currently in a dose-escalating study for metastatic CRC in combination with FOLFIRI (**https://www.clinicaltrials.gov/** identifier NCT02482441). OMP-131R10 will most likely be aimed at patients carrying RSPO3 fusion mutations, a subset of tumors that have been shown to be sensitive to anti-RSPO3 antibodies in preclinical settings (Storm et al. 2016).

The noncanonical ligand WNT5a has been reported to be tumor suppressive and possibly inhibit the canonical WNT signaling pathway by promoting the degradation of β-catenin. Consequently, Foxy-5, a formulated hexapeptide and WNT5a mimetic, is currently in phase II clinical trials as neo-adjuvant therapy for metastatic CRC (NCT03883802).

DESTRUCTION COMPLEX

Since multiple WNT-activating mutations occur downstream of receptor signaling, targeting ligand signaling may only be feasible in a subset of patients. For this reason, efforts have been made to develop intracellular inhibitors of WNT signaling. Tankyrase inhibitors prevent the PAR [poly(ADP-ribose)]-ylation of AXIN and its subsequent degradation, thereby suppressing canonical WNT signaling (Nusse & Clevers 2017). Tankyrase inhibitors have antitumorigenic effects (Huang et al. 2009), but concerns over on-target intestinal toxicity may limit the utility of these compounds (Zhong et al. 2016). Nevertheless, one dual PARP (PAR polymerase) and Tankyrase inhibitor (2X-121) is currently in clinical trials for metastatic breast cancer (NCT03562832). A small-molecule inhibitor of Tankyrase, MSC2504877, has been shown to reduce growth of *Apc*-mutant cancer cells and hyperproliferation triggered by homozygous truncation of *Apc* in the mouse intestine (Menon et al. 2019). This effect was exacerbated when combined with the

CDK4/6 inhibitor palbociclib and may offer a promising strategy to target *APC*-mutant CRC. As a critical kinase involved in β-catenin degradation, CK1α has also been targeted to modulate WNT signaling. Pyrvinium pamoate, an FDA-approved antihelminthic, has been shown to activate CK1α and attenuate intestinal polyp formation in mouse models (Li et al. 2014).

WNT TARGET GENE TRANSCRIPTION

Targeting gene transcription is a promising strategy for multiple oncogenic pathways. This is particularly important for tumors carrying WNT pathway mutations, as mutations occur mainly in the destruction complex, upstream of the transcriptional complex. However, targeting these protein-protein interfaces has proven challenging. There have been multiple attempts to target the nuclear translocation of β-catenin. For example, targeting the chaperones of β-catenin, BCL9 and BCL9L, has been shown to suppress WNT signaling using both drug treatments (Takada et al. 2012) and genetic models (Gay et al. 2019, Mieszczanek et al. 2019). ICG-001, a low-molecular-weight inhibitor that binds directly to CBP, inhibits WNT target gene expression in cancer cell line–based assays (Emami et al. 2004). Importantly, this inhibitor triggers apoptosis in tumor cells but not in normal tissues when tested in preclinical models (Emami et al. 2004). PRI-724, a compound closely related to ICG-001, was successfully tested in a phase I trial for PDAC in combination with gemcitabine (NCT01764477). PRI-724 was planned for a phase II clinical trial for metastatic CRC in combination with FOLFOX and bevacizumab (NCI-2015-00436, NCT0241385) but was withdrawn because of drug supply issues.

Targeting the WNT pathway at the appropriate signaling node within the pathway will be critical to the potential success of therapies. Although targeting ligand signaling may be efficacious in some tumors, even in the presence of *APC* mutations (Flanagan et al. 2019), this is unlikely to be true of all APC-driven tumors since the major activating mutation is downstream of the receptor signaling. Indeed, in CRC we and others have shown that Porcupine inhibition does not affect proliferation of *APC*-mutant intestinal cells (Huels et al. 2018). Identifying which WNT activating mutations are present will be critical to stratifying patients for particular treatments. It is likely that targeting the WNT pathway downstream of the activating mutation will be the most efficacious strategy for the treatment of cancer.

WNT AND IMMUNOTHERAPY

The striking results of immune checkpoint inhibition (ICI) in recent years has generated an increased interest in the tumor immune microenvironment (TIME) of solid cancers (Binnewies et al. 2018, Topalian et al. 2016). Currently, four different ICIs [ipilimumab, an anticytotoxic T lymphocyte–associated protein 4 (anti-CTLA-4) antibody; nivolumab or pembrolizumab, anti–programmed cell death 1 (PD-1) antibodies; and atezolizumab, anti–programmed cell death ligand 1 (PD-L1) antibody] are approved for over a dozen solid cancer types. However, only a fraction of patients respond to monotherapy, most likely due to the lack of T cell–rich TIMEs. WNT-dependent immune exclusion mechanisms may be promising for finding mechanisms to target these cancers. Activation of β-catenin in murine models of melanoma revealed that this epithelial oncogenic pathway leads to inhibition of $CD103^+$ dendritic cells and impaired priming of antitumor T cells. Reconstitution of $CD103^+$ dendritic cells reverses insensitivity to PD-1 treatment (Spranger et al. 2015). Interestingly, WNT activation has been identified as a major genetic mechanism for T cell/immune exclusion in CRC and other tumor types (Grasso et al. 2018, Iglesia et al. 2016, Luke et al. 2019). Another mechanism of WNT-dependent immune exclusion is *c-MYC* expression, an important WNT target gene (He et al. 1998, Sansom et al. 2007). c-MYC

drives immune exclusion by secretion of CCL9 and IL-23 in transgenic models of lung cancer, and coinhibition of CCL9 and IL-23 abrogates c-MYC-induced tumor progression (Kortlever et al. 2017). In addition, c-MYC regulates the expression of PD-L1 and CD47, which together lead to a reduced immune response (Casey et al. 2016). Inhibition of Dkk2 with a monoclonal antibody decreases the tumor burden of $Apc^{Min/+}$ mice (Xiao et al. 2018). This is interesting because this model generates intestinal polyps that are immunologically blunt. DKK2 inhibition led to activation of natural killer cells and $CD8^+$ T cells, potentiating the effect of PD-1 treatment via the LRP5-STAT5 signaling axis in immune cells (Xiao et al. 2018). These mechanisms demonstrate non-cell-autonomous communication to the TIME. It should be considered that WNT signaling is involved in virtually every step of immune cell development and education (Wang et al. 2018); thus, effects on the TIME are logical consequences that should be explored.

WNT IN STEM CELLS AND CANCER STEM CELLS

Associations between SCs and cancer have long been recognized because of SCs' innate ability to self-renew and form clones from single cells—features thought to be important for tumor initiation and metastasis. Consequently, there has been great interest in understanding the role of SCs in the evolution of cancer with a view to targeting CSCs using novel therapies.

WNT signaling is crucial for SC function in multiple species and is essential for embryonic SCs, promoting the expression of the pluripotent transcription factors *OCT4* and *NANOG* and inhibiting differentiation (Sato et al. 2004). The WNT-amplifying LGR5, originally identified as a marker of ISCs (Barker et al. 2007), is now known to mark populations of SCs or stem-like cells in multiple epithelial tissues (Leung et al. 2018).

In the stomach, *H. pylori* bacteria interact with and induce an expansion of the $LGR5^+$ population (Sigal et al. 2015). *H. pylori* infections in GC promote CSC-like properties in a WNT-dependent manner (Yong et al. 2016). In childhood liver cancer, WNT appears to be a driving force behind cancer development, with additional upregulated Myc signaling resulting in immature tumors enriched for hepatic SC/progenitor markers (Cairo et al. 2008).

The ISC compartment has been used as a prototype system for investigating the role of WNT signaling on stemness. Indeed, the reduction of WNT signaling in the intestinal epithelium results in loss of ISCs' self-renewal capacity (Yan et al. 2017), whereas hyperactivation of WNT signaling in ISCs results in adenoma formation (Barker et al. 2009). The number of $LGR5^+$ ISCs is dictated by R-spondin rather than WNT ligands, but the addition of R-spondin can only increase ISC numbers in the presence of active WNT signaling (Yan et al. 2017). Thus, WNTs are required but not sufficient for expanding the ISC zone. Reducing WNT ligand secretion using Porcupine inhibitors can influence the efficiency of tumor initiation from ISCs by altering the number of ISCs (Huels et al. 2018). These data combined suggest that WNT can limit ISC numbers in the intestine, but that WNT and R-spondin must work in tandem to promote stemness. This could be due to WNT-independent functions of R-spondin, or because R-spondin signaling is required to amplify WNT signaling to levels not possible with WNT ligands alone and because this is required for stem-like traits. The importance of WNT signaling for intestinal proliferation is crucial when considering on-target side effects of WNT-targeting therapies. As a result, cancers must be more reliant upon WNT than ISCs for an effective therapeutic window to be found.

In CRC, WNT signaling plays a crucial role in regulating stemness within tumors. In fact, normalization of the WNT signaling pathway causes differentiation and regression of established tumors even in the presence of other oncogenic mutations (Dow et al. 2015, Han et al. 2017, Storm et al. 2016). Human CRCs often have heterogeneous levels of nuclear β-catenin (Horst et al.

2012). It has been proposed that stemness is intertwined with WNT signaling, and that stemness can be modulated by secreted signals from CAFs (Lenos et al. 2018, Vermeulen et al. 2010). These reports indicate that targeting the CSC niche may be possible and offer an additional therapeutic strategy for CRC. However, there is also evidence that *LGR5*$^+$ cells are intrinsically more capable of expanding in tumor xenografts than more differentiated cells, arguing that the niche may not be all important (Shimokawa et al. 2017). It is not currently known how much these *LGR5*$^+$ CSCs rely upon niche signals to function as CSCs, but normal intestinal proliferation can be abrogated by codeletion of *LGR5* and *LGR4* but not *LGR5* alone (de Lau et al. 2011).

The ability to create clonal progeny from a single cell is a fundamental characteristic of an SC and is also thought to be critical in the tumor metastatic process. Emerging evidence indicates that targeting the WNT pathway in the metastatic niche could have therapeutic utility. *LGR5*$^+$ CSCs have been shown to be essential for metastatic liver colonization using CRC models (de Sousa e Melo et al. 2017). Further research is required to establish whether the dependence of liver metastases on *LGR5*$^+$ cells is related to the WNT-amplifying function of LGR5 or is more closely associated with loss of a fundamental cell type that *LGR5* marks. Efforts are underway to target LGR5$^+$ cells with antibody-drug conjugates, with some success in preclinical models (Gong et al. 2016, Junttila et al. 2015). The clinical utility of these therapeutics remains to be seen, but preclinical models that deplete *LGR5*$^+$ cells from tumors have shown limited evidence of tumor regression. Current phase I trials (NCT02726334, NCT03526835) using anti-LGR5 antibodies to block the R-spondin signaling axis will be closely watched by the field.

In breast cancer, canonical WNT signaling is important for metastatic lung colonization by CSCs. Periostin, an extracellular matrix protein, has been reported to sequester WNTs and foster a permissive metastatic environment for CSCs (Malanchi et al. 2011). These reports highlight the importance of WNT in regulating metastatic colonization of distant organs and suggest that targeting WNT signaling may be promising in multiple stages in the evolution of cancers.

CONCLUDING REMARKS

The WNT signaling cascade is commonly dysregulated in cancer and therefore represents a promising target for therapeutic intervention. However, it is apparent that different tissues show specific landscapes of driver mutations and are diverse in their permissiveness to oncogenic transformation. It is important to understand why certain driver mutations are preferentially mutated in specific cancer types and to know what the key contextual effects of each mutation are. If these questions are answered, it may help target the WNT pathway in a tissue-specific manner. Importantly, the level of WNT signaling varies between tissues (Buchert et al. 2010); thus, the optimal dose of a drug targeting WNT signaling might be an important parameter when tumor-type-specific therapies are tested. Targeted therapies in stratified cohorts should be concerned with the mutation status of WNT pathway components to guide therapeutic decisions. This is vital if tumors are treated with WNTs-targeting inhibitors, for which downstream mutations may render the tumor epithelium resistant to therapy.

Encouraging results from early clinical trials of Porcupine inhibitors and the increased understanding of ligand-dependent WNT signaling mean that WNTs-dependent cancers constitute a promising subset of tumors to target. Given the relatively short distance of WNTs signaling, it would be interesting to interrogate the WNT tumor-stroma interaction and map intra- and intertumor heterogeneity of WNTs, their receptors, and coreceptors.

Results from ICI therapies have shown little progress in non-hyper-mutated microsatellite-stable cancers (Wei et al. 2018). Understanding checkpoint inhibition and the dominant ability of WNT signaling to create an immunologically blunt TIME will be critical for the advancement of this field.

On-target toxicity is a longstanding complication of drugging WNT signaling, given its pivotal role in homeostasis, adult SC populations, and wound healing (Nusse & Clevers 2017). The field is working toward a holistic understanding of how WNT functions in cancer, with a view to optimizing therapies. The realization that WNT is modulated by R-spondin and that WNT is important for metastatic seeding has opened the door for novel therapeutic strategies targeting these axes.

Overall, the contribution of WNT to cancer biology is undeniable, and understanding the nuances associated with individual mutations and tissues may allow stratified therapies to be developed for a range of cancers. The first wave of phase II clinical trials specifically targeting WNT signaling are currently underway, the results of which will have significant ramifications for the field.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We would like to thank Cancer Research UK (CRUK) for core funding to Owen Sansom (A17196 and A21139) that supported Rene Jackstadt and Michael Hodder. In addition, Owen Sansom received a Starter Grant from the European Research Council (311301).

LITERATURE CITED

Amit S, Hatzubai A, Birman Y, Andersen JS, Ben-Shushan E, et al. 2002. Axin-mediated CKI phosphorylation of β-catenin at Ser 45: a molecular switch for the Wnt pathway. *Genes Dev*. 16:1066–76

Avgustinova A, Iravani M, Robertson D, Fearns A, Gao Q, et al. 2016. Tumour cell-derived Wnt7a recruits and activates fibroblasts to promote tumour aggressiveness. *Nat. Commun.* 7:10305

Barker N, Huch M, Kujala P, van de Wetering M, Snippert HJ, et al. 2010. Lgr5^{+ve} stem cells drive self-renewal in the stomach and build long-lived gastric units in vitro. *Cell Stem Cell* 6:25–36

Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, et al. 2009. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 457:608–11

Barker N, van Es JH, Kuipers J, Kujala P, van den Born M, et al. 2007. Identification of stem cells in small intestine and colon by marker gene *Lgr5*. *Nature* 449:1003–7

Becht E, de Reynies A, Giraldo NA, Pilati C, Buttard B, et al. 2016. Immune and stromal classification of colorectal cancer is associated with molecular subtypes and relevant for precision immunotherapy. *Clin. Cancer Res.* 22:4057–66

Behrens J, Jerchow BA, Wurtele M, Grimm J, Asbrand C, et al. 1998. Functional interaction of an axin homolog, conductin, with β-catenin, APC, and GSK3β. *Science* 280:596–99

Behrens J, von Kries JP, Kuhl M, Bruhn L, Wedlich D, et al. 1996. Functional interaction of β-catenin with the transcription factor LEF-1. *Nature* 382:638–42

Binnewies M, Roberts EW, Kersten K, Chan V, Fearon DF, et al. 2018. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat. Med.* 24:541–50

Bond CE, McKeone DM, Kalimutho M, Bettington ML, Pearson SA, et al. 2016. *RNF43* and *ZNRF3* are commonly altered in serrated pathway colorectal tumorigenesis. *Oncotarget* 7:70589–600

Buchert M, Athineos D, Abud HE, Burke ZD, Faux MC, et al. 2010. Genetic dissection of differential signaling threshold requirements for the Wnt/β-catenin pathway in vivo. *PLOS Genet*. 6:e1000816

Cadoret A, Ovejero C, Saadi-Kheddouci S, Souil E, Fabre M, et al. 2001. Hepatomegaly in transgenic mice expressing an oncogenic form of β-catenin. *Cancer Res*. 61:3245–49

Cairo S, Armengol C, De Reynies A, Wei Y, Thomas E, et al. 2008. Hepatic stem-like phenotype and interplay of Wnt/β-catenin and Myc signaling in aggressive childhood liver cancer. *Cancer Cell* 14:471–84

Cancer Genome Atlas Res. Netw. 2017. Integrated genomic characterization of pancreatic ductal adenocarcinoma. *Cancer Cell* 32:185–203.e13

Cancer Genome Atlas Res. Netw., Kandoth C, Schultz N, Cherniack AD, Akbani R, et al. 2013. Integrated genomic characterization of endometrial carcinoma. *Nature* 497:67–73

Cancer Genome Atlas Res. Netw., Muzny DM, Bainbridge MN, Chang K, Dinh HH, et al. 2012. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* 487:330–37

Carmon KS, Loose DS. 2008. Secreted frizzled-related protein 4 regulates two Wnt7a signaling pathways and inhibits proliferation in endometrial cancer cells. *Mol. Cancer Res.* 6:1017–28

Casey SC, Tong L, Li Y, Do R, Walz S, et al. 2016. MYC regulates the antitumor immune response through CD47 and PD-L1. *Science* 352:227–31

Colnot S, Decaens T, Niwa-Kawakita M, Godard C, Hamard G, et al. 2004. Liver-targeted disruption of *Apc* in mice activates β-catenin signaling and leads to hepatocellular carcinomas. *PNAS* 101:17216–21

de Lau W, Barker N, Low TY, Koo BK, Li VS, et al. 2011. Lgr5 homologues associate with Wnt receptors and mediate R-spondin signalling. *Nature* 476:293–97

de Lau W, Peng WC, Gros P, Clevers H. 2014. The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. *Genes Dev.* 28:305–16

de Sousa e Melo F, Colak S, Buikhuisen J, Koster J, Cameron K, et al. 2011. Methylation of cancer-stem-cell-associated Wnt target genes predicts poor prognosis in colorectal cancer patients. *Cell Stem Cell* 9:476–85

de Sousa e Melo F, Kurtova AV, Harnoss JM, Kljavin N, Hoeck JD, et al. 2017. A distinct role for $Lgr5^{+}$ stem cells in primary and metastatic colon cancer. *Nature* 543:676–80

Delmas V, Beermann F, Martinozzi S, Carreira S, Ackermann J, et al. 2007. β-catenin induces immortalization of melanocytes by suppressing $p16^{INK4a}$ expression and cooperates with N-Ras in melanoma development. *Genes Dev.* 21:2923–35

Dow LE, O'Rourke KP, Simon J, Tschaharganeh DF, van Es JH, et al. 2015. Apc restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer. *Cell* 161:1539–52

Emami KH, Nguyen C, Ma H, Kim DH, Jeong KW, et al. 2004. A small molecule inhibitor of β-catenin/CREB-binding protein transcription. *PNAS* 101:12682–87. Erratum. 2004. *PNAS* 101:16707

Farin HF, Jordens I, Mosa MH, Basak O, Korving J, et al. 2016. Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. *Nature* 530:340–43

Feng GJ, Cotta W, Wei XQ, Poetz O, Evans R, et al. 2012. Conditional disruption of Axin1 leads to development of liver tumors in mice. *Gastroenterology* 143:1650–59

Fessler E, Medema JP. 2016. Colorectal cancer subtypes: developmental origin and microenvironmental regulation. *Trends Cancer* 2:505–18

Flanagan DJ, Barker N, Costanzo NSD, Mason EA, Gurney A, et al. 2019. Frizzled-7 is required for Wnt signaling in gastric tumors with and without Apc mutations. *Cancer Res.* 79:970–81

Gay DM, Ridgway RA, Mueller M, Hodder MC, Hedley A, et al. 2019. Loss of BCL9/9l suppresses Wnt driven tumourigenesis in models that recapitulate human cancer. *Nat. Commun.* 10:723

Glinka A, Wu W, Delius H, Monaghan AP, Blumenstock C, Niehrs C. 1998. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* 391:357–62

Gnad T, Feoktistova M, Leverkus M, Lendeckel U, Naumann M. 2010. *Helicobacter pylori*-induced activation of β-catenin involves low density lipoprotein receptor-related protein 6 and Dishevelled. *Mol. Cancer* 9:31

Gong X, Azhdarinia A, Ghosh SC, Xiong W, An Z, et al. 2016. LGR5-targeted antibody–drug conjugate eradicates gastrointestinal tumors and prevents recurrence. *Mol. Cancer Ther.* 15:1580–90

Grasso CS, Giannakis M, Wells DK, Hamada T, Mu XJ, et al. 2018. Genetic mechanisms of immune evasion in colorectal cancer. *Cancer Discov.* 8:730–49

Groden J, Thliveris A, Samowitz W, Carlson M, Gelbert L, et al. 1991. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* 66:589–600

Guinney J, Dienstmann R, Wang X, de Reynies A, Schlicker A, et al. 2015. The consensus molecular subtypes of colorectal cancer. *Nat. Med.* 21:1350–56

Han T, Schatoff EM, Murphy C, Zafra MP, Wilkinson JE, et al. 2017. R-Spondin chromosome rearrangements drive Wnt-dependent tumour initiation and maintenance in the intestine. *Nat. Commun.* 8:15945

Hao HX, Xie Y, Zhang Y, Charlat O, Oster E, et al. 2012. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* 485:195–200

Harada N, Miyoshi H, Murai N, Oshima H, Tamai Y, et al. 2002. Lack of tumorigenesis in the mouse liver after adenovirus-mediated expression of a dominant stable mutant of β-catenin. *Cancer Res.* 62:1971–77

He TC, Sparks AB, Rago C, Hermeking H, Zawel L, et al. 1998. Identification of c-MYC as a target of the APC pathway. *Science* 281:1509–12

Hodis E, Watson IR, Kryukov GV, Arold ST, Imielinski M, et al. 2012. A landscape of driver mutations in melanoma. *Cell* 150:251–63

Hoffmans R, Stadeli R, Basler K. 2005. Pygopus and legless provide essential transcriptional coactivator functions to Armadillo/β-catenin. *Curr. Biol.* 15:1207–11

Horst D, Chen J, Morikawa T, Ogino S, Kirchner T, Shivdasani RA. 2012. Differential WNT activity in colorectal cancer confers limited tumorigenic potential and is regulated by MAPK signaling. *Cancer Res.* 72:1547–56

Hou X, Tan Y, Li M, Dey SK, Das SK. 2004. Canonical Wnt signaling is critical to estrogen-mediated uterine growth. *Mol. Endocrinol.* 18:3035–49

Huang SM, Mishina YM, Liu S, Cheung A, Stegmeier F, et al. 2009. Tankyrase inhibition stabilizes axin and antagonizes Wnt signalling. *Nature* 461:614–20

Huch M, Dorrell C, Boj SF, van Es JH, Li VS, et al. 2013. In vitro expansion of single Lgr5$^+$ liver stem cells induced by Wnt-driven regeneration. *Nature* 494:247–50

Huels DJ, Bruens L, Hodder MC, Cammareri P, Campbell AD, et al. 2018. Wnt ligands influence tumour initiation by controlling the number of intestinal stem cells. *Nat. Commun.* 9:1132

Huels DJ, Ridgway RA, Radulescu S, Leushacke M, Campbell AD, et al. 2015. E-cadherin can limit the transforming properties of activating β-catenin mutations. *EMBO J.* 34:2321–33

Iglesia MD, Parker JS, Hoadley KA, Serody JS, Perou CM, Vincent BG. 2016. Genomic analysis of immune cell infiltrates across 11 tumor types. *J. Natl. Cancer Inst.* 108:djw144

Ikeda S, Kishida S, Yamamoto H, Murai H, Koyama S, Kikuchi A. 1998. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3β and β-catenin and promotes GSK-3β-dependent phosphorylation of β-catenin. *EMBO J.* 17:1371–84

Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. 1997. Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* 389:966–70

Isaacsson Velho P, Fu W, Wang H, Mirkheshti N, Qazi F, et al. 2020. Wnt-pathway activating mutations are associated with resistance to first-line abiraterone and enzalutamide in castration-resistant prostate cancer. *Eur. Urol.* 77(1):14–21

Jaks V, Barker N, Kasper M, van Es JH, Snippert HJ, et al. 2008. Lgr5 marks cycling, yet long-lived, hair follicle stem cells. *Nat. Genet.* 40:1291–99

Janda CY, Waghray D, Levin AM, Thomas C, Garcia KC. 2012. Structural basis of Wnt recognition by Frizzled. *Science* 337:59–64

Jeong JW, Lee HS, Franco HL, Broaddus RR, Taketo MM, et al. 2009. β-catenin mediates glandular formation and dysregulation of β-catenin induces hyperplasia formation in the murine uterus. *Oncogene* 28:31–40

Jiang X, Hao HX, Growney JD, Woolfenden S, Bottiglio C, et al. 2013. Inactivating mutations of *RNF43* confer Wnt dependency in pancreatic ductal adenocarcinoma. *PNAS* 110:12649–54

Johansson J, Naszai M, Hodder MC, Pickering KA, Miller BW, et al. 2019. RAL GTPases drive intestinal stem cell function and regeneration through internalization of Wnt signalosomes. *Cell Stem Cell* 24:592–607

Junttila MR, Mao W, Wang X, Wang BE, Pham T, et al. 2015. Targeting LGR5$^+$ cells with an antibody-drug conjugate for the treatment of colon cancer. *Sci. Transl. Med.* 7:314ra186

Kakugawa S, Langton PF, Zebisch M, Howell S, Chang TH, et al. 2015. Notum deacylates Wnt proteins to suppress signalling activity. *Nature* 519:187–92

Kazanskaya O, Glinka A, del Barco Barrantes I, Stannek P, Niehrs C, Wu W. 2004. R-Spondin2 is a secreted activator of Wnt/β-catenin signaling and is required for *Xenopus* myogenesis. *Dev. Cell* 7:525–34

Kim B, Byun SJ, Kim YA, Kim JE, Lee BL, et al. 2010. Cell cycle regulators, APC/β-catenin, NF-κB and Epstein-Barr virus in gastric carcinomas. *Pathology* 42:58–65

Kinzler KW, Nilbert MC, Su LK, Vogelstein B, Bryan TM, et al. 1991. Identification of FAP locus genes from chromosome 5q21. *Science* 253:661–65

Koo BK, Spit M, Jordens I, Low TY, Stange DE, et al. 2012. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* 488:665–69

Koo BK, van Es JH, van den Born M, Clevers H. 2015. Porcupine inhibitor suppresses paracrine Wnt-driven growth of *Rnf43;Znrf3*-mutant neoplasia. *PNAS* 112:7548–50

Korinek V, Barker N, Morin PJ, van Wichen D, de Weger R, et al. 1997. Constitutive transcriptional activation by a β-catenin-Tcf complex in $APC^{-/-}$ colon carcinoma. *Science* 275:1784–87

Kortlever RM, Sodir NM, Wilson CH, Burkhart DL, Pellegrinet L, et al. 2017. Myc cooperates with Ras by programming inflammation and immune suppression. *Cell* 171:1301–15.e14

Kurayoshi M, Oue N, Yamamoto H, Kishida M, Inoue A, et al. 2006. Expression of Wnt-5a is correlated with aggressiveness of gastric cancer by stimulating cell migration and invasion. *Cancer Res.* 66:10439–48

Larue L, Delmas V. 2006. The WNT/β-catenin pathway in melanoma. *Front. Biosci.* 11:733–42

Lenos KJ, Miedema DM, Lodestijn SC, Nijman LE, van den Bosch T, et al. 2018. Stem cell functionality is microenvironmentally defined during tumour expansion and therapy response in colon cancer. *Nat. Cell Biol.* 20:1193–202

Leung C, Tan SH, Barker N. 2018. Recent advances in $Lgr5^{+}$ stem cell research. *Trends Cell Biol.* 28:380–91

Li B, Flaveny CA, Giambelli C, Fei DL, Han L, et al. 2014. Repurposing the FDA-approved pinworm drug pyrvinium as a novel chemotherapeutic agent for intestinal polyposis. *PLOS ONE* 9:e101969

Li VS, Ng SS, Boersema PJ, Low TY, Karthaus WR, et al. 2012. Wnt signaling through inhibition of β-catenin degradation in an intact Axin1 complex. *Cell* 149:1245–56

Lim X, Tan SH, Koh WL, Chau RM, Yan KS, et al. 2013. Interfollicular epidermal stem cells self-renew via autocrine Wnt signaling. *Science* 342:1226–30

Linnekamp JF, Hooff SRV, Prasetyanti PR, Kandimalla R, Buikhuisen JY, et al. 2018. Consensus molecular subtypes of colorectal cancer are recapitulated in in vitro and in vivo models. *Cell Death Differ.* 25:616–33

Liu C, Li Y, Semenov M, Han C, Baeg GH, et al. 2002. Control of β-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* 108:837–47

Liu Y, Meng F, Xu Y, Yang S, Xiao M, et al. 2013. Overexpression of Wnt7a is associated with tumor progression and unfavorable prognosis in endometrial cancer. *Int. J. Gynecol. Cancer* 23:304–11

Liu Y, Patel L, Mills GB, Lu KH, Sood AK, et al. 2014. Clinical significance of *CTNNB1* mutation and Wnt pathway activation in endometrioid endometrial carcinoma. *J. Natl. Cancer Inst.* 106:dju245

Logan CY, Nusse R. 2004. The Wnt signaling pathway in development and disease. *Annu. Rev. Cell Dev. Biol.* 20:781–810

Longerich T, Endris V, Neumann O, Rempel E, Kirchner M, et al. 2019. *RSPO2* gene rearrangement: a powerful driver of β-catenin activation in liver tumours. *Gut* 68:1140–42

Luke JJ, Bao R, Sweis RF, Spranger S, Gajewski TF. 2019. WNT/β-catenin pathway activation correlates with immune exclusion across human cancers. *Clin. Cancer Res.* 25:3074–83

Madan B, McDonald MJ, Foxa GE, Diegel CR, Williams BO, Virshup DM. 2018. Bone loss from Wnt inhibition mitigated by concurrent alendronate therapy. *Bone Res.* 6:17

Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, et al. 2011. Interactions between cancer stem cells and their niche govern metastatic colonization. *Nature* 481:85–89

Mao J, Fan S, Ma W, Fan P, Wang B, et al. 2014. Roles of Wnt/β-catenin signaling in the gastric cancer stem cells proliferation and salinomycin treatment. *Cell Death Dis.* 5:e1039

Menon M, Elliott R, Bowers L, Balan N, Rafiq R, et al. 2019. A novel tankyrase inhibitor, MSC2504877, enhances the effects of clinical CDK4/6 inhibitors. *Sci. Rep.* 9:201

Michels BE, Mosa MH, Grebbin BM, Yepes D, Darvishi T, et al. 2019. Human colon organoids reveal distinct physiologic and oncogenic Wnt responses. *J. Exp. Med.* 216:704–20

Mieszczanek J, van Tienen LM, Ibrahim AEK, Winton DJ, Bienz M. 2019. Bcl9 and Pygo synergise downstream of *Apc* to effect intestinal neoplasia in FAP mouse models. *Nat. Commun.* 10:724

Minde DP, Anvarian Z, Rudiger SG, Maurice MM. 2011. Messing up disorder: How do missense mutations in the tumor suppressor protein APC lead to cancer? *Mol. Cancer* 10:101

Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, et al. 1996. XTcf-3 transcription factor mediates β-catenin-induced axis formation in Xenopus embryos. *Cell* 86:391–99

Morin PJ, Sparks AB, Korinek V, Barker N, Clevers H, et al. 1997. Activation of β-catenin-Tcf signaling in colon cancer by mutations in β-catenin or APC. *Science* 275:1787–90

Mulligan KA, Fuerer C, Ching W, Fish M, Willert K, Nusse R. 2012. Secreted Wingless-interacting molecule (Swim) promotes long-range signaling by maintaining Wingless solubility. *PNAS* 109:370–77

Murata-Kamiya N, Kurashima Y, Teishikata Y, Yamahashi Y, Saito Y, et al. 2007. *Helicobacter pylori* CagA interacts with E-cadherin and deregulates the β-catenin signal that promotes intestinal transdifferentiation in gastric epithelial cells. *Oncogene* 26:4617–26

Ng A, Tan S, Singh G, Rizk P, Swathi Y, et al. 2014. Lgr5 marks stem/progenitor cells in ovary and tubal epithelia. *Nat. Cell Biol.* 16:745–57

Niehrs C. 2012. The complex world of WNT receptor signalling. *Nat. Rev. Mol. Cell Biol.* 13:767–79

Nishisho I, Nakamura Y, Miyoshi Y, Miki Y, Ando H, et al. 1991. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. *Science* 253:665–69

Nusse R, Clevers H. 2017. Wnt/β-catenin signaling, disease, and emerging therapeutic modalities. *Cell* 169:985–99

Nusse R, Varmus HE. 1982. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* 31:99–109

Nusslein-Volhard C, Wieschaus E. 1980. Mutations affecting segment number and polarity in *Drosophila*. *Nature* 287:795–801

Oshima H, Matsunaga A, Fujimura T, Tsukamoto T, Taketo MM, Oshima M. 2006. Carcinogenesis in mouse stomach by simultaneous activation of the Wnt signaling and prostaglandin E2 pathway. *Gastroenterology* 131:1086–95

Palles C, Cazier JB, Howarth KM, Domingo E, Jones AM, et al. 2013. Germline mutations affecting the proofreading domains of POLE and POLD1 predispose to colorectal adenomas and carcinomas. *Nat. Genet.* 45:136–44

Powell AE, Vlacich G, Zhao ZY, McKinley ET, Washington MK, et al. 2014. Inducible loss of one *Apc* allele in Lrig1-expressing progenitor cells results in multiple distal colonic tumors with features of familial adenomatous polyposis. *Am. J. Physiol. Gastrointest. Liver Physiol.* 307:G16–23

Radulescu S, Ridgway RA, Cordero J, Athineos D, Salgueiro P, et al. 2013. Acute WNT signalling activation perturbs differentiation within the adult stomach and rapidly leads to tumour formation. *Oncogene* 32:2048–57

Reed KR, Athineos D, Meniel VS, Wilkins JA, Ridgway RA, et al. 2008. β-catenin deficiency, but not Myc deletion, suppresses the immediate phenotypes of APC loss in the liver. *PNAS* 105:18919–23

Riese J, Yu X, Munnerlyn A, Eresh S, Hsu SC, et al. 1997. LEF-1, a nuclear factor coordinating signaling inputs from wingless and decapentaplegic. *Cell* 88:777–87

Risinger JI, Maxwell GL, Chandramouli GV, Aprelikova O, Litzi T, et al. 2005. Gene expression profiling of microsatellite unstable and microsatellite stable endometrial cancers indicates distinct pathways of aberrant signaling. *Cancer Res.* 65:5031–37

Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, et al. 2015. Integrative clinical genomics of advanced prostate cancer. *Cell* 161(5):1215–28

Rubinfeld B, Souza B, Albert I, Muller O, Chamberlain SH, et al. 1993. Association of the APC gene product with β-catenin. *Science* 262:1731–34

Salahshor S, Woodgett JR. 2005. The links between axin and carcinogenesis. *J Clin. Pathol.* 58:225–36

Sano M, Driscoll DR, DeJesus-Monge WE, Quattrochi B, Appleman VA, et al. 2016. Activation of WNT/β-catenin signaling enhances pancreatic cancer development and the malignant potential via up-regulation of Cyr61. *Neoplasia* 18:785–94

Sansom OJ. 2009. Tissue-specific tumour suppression by APC. *Adv. Exp. Med. Biol.* 656:107–18

Sansom OJ, Meniel VS, Muncan V, Phesse TJ, Wilkins JA, et al. 2007. Myc deletion rescues Apc deficiency in the small intestine. *Nature* 446:676–79

Sansom OJ, Reed KR, Hayes AJ, Ireland H, Brinkmann H, et al. 2004. Loss of Apc in vivo immediately perturbs Wnt signaling, differentiation, and migration. *Genes Dev*. 18:1385–90

Sarkar A, Huebner AJ, Sulahian R, Anselmo A, Xu X, et al. 2016. Sox2 suppresses gastric tumorigenesis in mice. *Cell Rep*. 16:1929–41

Sato N, Meijer L, Skaltsounis L, Greengard P, Brivanlou AH. 2004. Maintenance of pluripotency in human and mouse embryonic stem cells through activation of Wnt signaling by a pharmacological GSK-3-specific inhibitor. *Nat. Med.* 10:55–63

Satoh S, Daigo Y, Furukawa Y, Kato T, Miwa N, et al. 2000. *AXIN1* mutations in hepatocellular carcinomas, and growth suppression in cancer cells by virus-mediated transfer of *AXIN1*. *Nat. Genet*. 24:245–50

Seino T, Kawasaki S, Shimokawa M, Tamagawa H, Toshimitsu K, et al. 2018. Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression. *Cell Stem Cell* 22:454–67.e6

Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, et al. 2012. Recurrent R-spondin fusions in colon cancer. *Nature* 488:660–64

Shimokawa M, Ohta Y, Nishikori S, Matano M, Takano A, et al. 2017. Visualization and targeting of $LGR5^+$ human colon cancer stem cells. *Nature* 545:187–92

Sigal M, Rothenberg ME, Logan CY, Lee JY, Honaker RW, et al. 2015. *Helicobacter pylori* activates and expands $Lgr5^+$ stem cells through direct colonization of the gastric glands. *Gastroenterology* 148:1392–404.e21

Sinner D, Kordich JJ, Spence JR, Opoka R, Rankin S, et al. 2007. Sox17 and Sox4 differentially regulate β-catenin/T-cell factor activity and proliferation of colon carcinoma cells. *Mol. Cell. Biol.* 27:7802–15

Spranger S, Bao R, Gajewski TF. 2015. Melanoma-intrinsic β-catenin signalling prevents anti-tumour immunity. *Nature* 523:231–35

Stadeli R, Hoffmans R, Basler K. 2006. Transcription under the control of nuclear Arm/β-catenin. *Curr. Biol.* 16:R378–85

Steinhart Z, Pavlovic Z, Chandrashekhar M, Hart T, Wang X, et al. 2017. Genome-wide CRISPR screens reveal a Wnt–FZD5 signaling circuit as a druggable vulnerability of *RNF43*-mutant pancreatic tumors. *Nat. Med.* 23:60–68

Storm EE, Durinck S, de Sousa e Melo F, Tremayne J, Kljavin N, et al. 2016. Targeting *PTPRK-RSPO3* colon tumours promotes differentiation and loss of stem-cell function. *Nature* 529:97–100

Su LK, Vogelstein B, Kinzler KW. 1993. Association of the APC tumor suppressor protein with catenins. *Science* 262:1734–37

Suzuki M, Mimuro H, Kiga K, Fukumatsu M, Ishijima N, et al. 2009. *Helicobacter pylori* CagA phosphorylation-independent function in epithelial proliferation and inflammation. *Cell Host Microbe* 5:23–34

Sveen A, Bruun J, Eide PW, Eilertsen IA, Ramirez L, et al. 2018. Colorectal cancer consensus molecular subtypes translated to preclinical models uncover potentially targetable cancer cell dependencies. *Clin. Cancer Res.* 24:794–806

Tahara E. 1995. Molecular biology of gastric cancer. *World J. Surg.* 19:484–8

Takada K, Zhu D, Bird GH, Sukhdeo K, Zhao JJ, et al. 2012. Targeted disruption of the BCL9/β-catenin complex inhibits oncogenic Wnt signaling. *Sci. Transl. Med.* 4:148ra17

Tammela T, Sanchez-Rivera FJ, Cetinbas NM, Wu K, Joshi NS, et al. 2017. A Wnt-producing niche drives proliferative potential and progression in lung adenocarcinoma. *Nature* 545:355–59

Tan X, Apte U, Micsenyi A, Kotsagrelos E, Luo JH, et al. 2005. Epidermal growth factor receptor: a novel target of the Wnt/β-catenin pathway in liver. *Gastroenterology* 129:285–302

Tauriello DVF, Palomo-Ponce S, Stork D, Berenguer-Llergo A, Badia-Ramentol J, et al. 2018. TGFβ drives immune evasion in genetically reconstituted colon cancer metastasis. *Nature* 554:538–43

Topalian SL, Taube JM, Anders RA, Pardoll DM. 2016. Mechanism-driven biomarkers to guide immune checkpoint blockade in cancer therapy. *Nat. Rev. Cancer* 16:275–87

Townsley FM, Cliffe A, Bienz M. 2004. Pygopus and Legless target Armadillo/β-catenin to the nucleus to enable its transcriptional co-activator function. *Nat. Cell Biol.* 6:626–33

van Amerongen R, Bowman AN, Nusse R. 2012. Developmental stage and time dictate the fate of Wnt/β-catenin-responsive stem cells in the mammary gland. *Cell Stem Cell* 11:387–400

Vermeulen L, de Sousa e Melo F, van der Heijden M, Cameron K, de Jong JH, et al. 2010. Wnt activity defines colon cancer stem cells and is regulated by the microenvironment. *Nat. Cell Biol.* 12:468–76

Vermeulen L, Morrissey E, van der Heijden M, Nicholson AM, Sottoriva A, et al. 2013. Defining stem cell dynamics in models of intestinal tumor initiation. *Science* 342:995–98

Wang B, Tian T, Kalland KH, Ke X, Qu Y. 2018. Targeting Wnt/β-catenin signaling for cancer immunotherapy. *Trends Pharmacol. Sci.* 39:648–58

Wang B, Zhao L, Fish M, Logan CY, Nusse R. 2015. Self-renewing diploid Axin2^{+} cells fuel homeostatic renewal of the liver. *Nature* 524:180–85

Wang K, Yuen ST, Xu J, Lee SP, Yan HH, et al. 2014. Whole-genome sequencing and comprehensive molecular profiling identify new driver mutations in gastric cancer. *Nat. Genet.* 46:573–82

Wei SC, Duffy CR, Allison JP. 2018. Fundamental mechanisms of immune checkpoint blockade therapy. *Cancer Discov.* 8:1069–86

Winston JT, Strack P, Beer-Romero P, Chu CY, Elledge SJ, Harper JW. 1999. The SCF$^{\beta\text{-TRCP}}$-ubiquitin ligase complex associates specifically with phosphorylated destruction motifs in IκBα and β-catenin and stimulates IκBα ubiquitination in vitro. *Genes Dev.* 13:270–83

Xiao Q, Wu J, Wang WJ, Chen S, Zheng Y, et al. 2018. DKK2 imparts tumor immunity evasion through β-catenin-independent suppression of cytotoxic immune-cell activation. *Nat. Med.* 24:262–70

Xue G, Romano E, Massi D, Mandala M. 2016. Wnt/β-catenin signaling in melanoma: preclinical rationale and novel therapeutic insights. *Cancer Treat. Rev.* 49:1–12

Yaeger R, Chatila WK, Lipsyc MD, Hechtman JF, Cercek A, et al. 2018. Clinical sequencing defines the genomic landscape of metastatic colorectal cancer. *Cancer Cell* 33:125–36.e3

Yan KS, Janda CY, Chang J, Zheng GXY, Larkin KA, et al. 2017. Non-equivalence of Wnt and R-spondin ligands during Lgr5^{+} intestinal stem-cell self-renewal. *Nature* 545:238–42

Yi N, Liao QP, Li ZH, Xie BJ, Hu YH, et al. 2013. RNA interference-mediated targeting of DKK1 gene expression in Ishikawa endometrial carcinoma cells causes increased tumor cell invasion and migration. *Oncol. Lett.* 6:756–62

Yong X, Tang B, Xiao YF, Xie R, Qin Y, et al. 2016. *Helicobacter pylori* upregulates Nanog and Oct4 via Wnt/β-catenin signaling pathway to promote cancer stem cell-like properties in human gastric cancer. *Cancer Lett.* 374:292–303

Yuan G, Regel I, Lian F, Friedrich T, Hitkova I, et al. 2013. WNT6 is a novel target gene of caveolin-1 promoting chemoresistance to epirubicin in human gastric cancer cells. *Oncogene* 32:375–87

Zhang Z, Cheng L, Li J, Farah E, Atallah NM, et al. 2018. Inhibition of the Wnt/β-catenin pathway overcomes resistance to enzalutamide in castration-resistant prostate cancer. *Cancer Res.* 78:3147–62

Zhong Y, Katavolos P, Nguyen T, Lau T, Boggs J, et al. 2016. Tankyrase inhibition causes reversible intestinal toxicity in mice with a therapeutic index <1. *Toxicol. Pathol.* 44:267–78

Zysman M, Saka A, Millar A, Knight J, Chapman W, Bapat B. 2002. Methylation of *Adenomatous Polyposis Coli* in endometrial cancer occurs more frequently in tumors with microsatellite instability phenotype. *Cancer Res.* 62:3663–66