


Review

Shifting the Focus of Signaling Abnormalities in Colon Cancer

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Simple Summary: The major signaling pathways in colon cancer are WNT, RAS, and TGF- β . Components of these pathways are mutated in the majority of colon cancers, resulting in aberrantly high or low activity of the pathway. The functional consequences of the mutations reflect the behavior of these signaling pathways in intestinal stem cells. To better understand the roles of each pathway, we cover the basic function as well as points of intersection between the different pathways, to describe how they function individually, as well as together, to regulate cell proliferation.

Abstract: Colon cancer tumorigenesis occurs incrementally. The process involves the acquisition of mutations which typically follow an established pattern: activation of WNT signaling, activation of RAS signaling, and inhibition of TGF- β signaling. This arrangement recapitulates, to some degree, the stem cell niche of the intestinal epithelium, which maintains WNT and EGF activity while suppressing TGF- β . The resemblance between the intestinal stem cell environment and colon cancer suggests that the concerted activity of these pathways generates and maintains a potent growth-inducing stimulus. However, each pathway has a myriad of downstream targets, making it difficult to identify which aspects of these pathways are drivers. To address this, we utilize the cell cycle, the ultimate regulator of cell proliferation, as a foundation for cross-pathway integration. We attempt to generate an overview of colon cancer signaling patterns by integrating the major colon cancer signaling pathways in the context of cell replication, specifically, the entrance from G₁ into S-phase.

Keywords: WNT signaling; RAS signaling; TGF- β signaling; β -catenin; K-RAS; SMAD4; MYC



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1. Introduction

Cancer is a disease in which cells of the body reject the commands of their tissue. This disobedience is a result of the disruption of the signaling pathways, which are no longer able to balance the activities of the cell nor effectively communicate with the extracellular environment. Signaling pathways are disrupted through a myriad of causes, though the most common is the mutation of a gene which produces a critical regulatory protein in the signaling cascade. The mutation severs the connection between the stimulus and its downstream response, because one of the proteins required to relay the appropriate signal is unable to do so. Thereby, the cell loses control over a specific aspect of its behavior and the output of the pathway becomes relatively constant, as opposed to dynamic. This influences the resting state of the cell as well as any dynamic cellular responses which require input of this (now defunct) pathway. As the mutant cell multiplies, more mutations can occur, providing the basis for selection, which results in disease progression as the cancer cell further deviates from normal cell function. Eventually, the cancer cell accumulates a sufficient number of driver mutations, approximately 5–7 in colon cancer, in various pathways, which render it ignorant of its surrounding environment. This forms the basis for metastasis, as the cancer cell is able to traverse the surrounding tissues, withstand secreted growth inhibitory signals, as well as grow in a remote site of the body, independent of the signaling pathway requirements of its tissue of origin.

1.1. Signaling in the Healthy Intestines

In the healthy intestinal epithelium, the WNT, EGF, Notch, and BMP pathways maintain homeostasis [1]. The intestinal stem cells are maintained by high levels of WNT, EGF, and Notch, as well as low levels of BMP signaling [2–5]. The activity of these pathways is maintained by signals from the mesenchyme surrounding the stem cell compartment, as well as other cells (such as Paneth or cKit+/Reg4+) which are intermingled with the stem cells [6–8]. As the stem cell progeny leave the compartment and ascend the crypt, the WNT, EGF, and Notch levels decrease, while the activity of BMP increases [9–11]. The changing levels of these signaling pathways results in the differentiation of the stem cells, now transit amplifying cells, into the various cell types necessary to perform the intestinal functions. Cell type is, therefore, an emergent property determined by the output of several key signaling pathways (given the context of the cell type and its epigenetic status).

1.2. Signaling in Colon Cancer

In colon cancer, the activities of the previously mentioned pathways are altered by mutation and closely mirror the signaling pathway arrangement found within the intestinal stem cell niche, i.e., high WNT, high EGF, and low BMP. However, Notch signaling does not appear to be heavily targeted by mutation in colon cancer [12]. Given the stochastic nature of mutation, higher mutation prevalence for specific genes is indicative of increased cellular fitness of the mutation-harboring cell compared to the surrounding normal intestinal cells, i.e., selection. Recapitulation of the stem cell state by the cancer cell indicates that the cooperativity of these pathways results in an intestinal cell with indefinite replicative potential. However, even the increased activity of a single pathway (WNT or EGF) through mutation is sufficient to result in aberrant growth and adenoma formation [13–15], though more faithful recapitulation of the stem cell state, i.e., increased WNT and EGF, and low BMP signaling, results in a cancer cell with malignant potential [13,16,17]. It is, therefore, the combined influence of these signaling pathways, rather than the actions of any one pathway in particular, that is necessary for the transition to malignant disease. However, cooperation between pathways does not imply synergism.

2. WNT Signaling

The WNT signaling pathway is an ancient mechanism necessary for guiding development and maintaining tissue homeostasis throughout multicellular life [18]. In the intestinal epithelium, canonical WNT signaling is crucial for stem cell maintenance, and thereby, homeostasis, while WNT signaling loss abrogates the intestinal proliferative compartment [2,19]. It is no coincidence that the signaling pathway most critical for maintaining the intestinal stem cell state is also the most frequently mutated in colon cancer.

2.1. WNT Production

WNT signaling begins with the production of a small (~40 kDa), cysteine-rich protein, referred to as a WNT [20,21]. Humans produce 19 different WNTs, which perform various developmental functions with limited functional redundancy [22–27]. As part of their maturation, WNTs are lipid-modified by the addition of palmitoleic acid, which is appended by the O-acyltransferase enzyme, Porcupine, within the endoplasmic reticulum (ER) [28–34]. WNT acylation is required for binding to WLS (Wntless/Evi), an essential component of the WNT secretory pathway, while inhibition of WNT acylation results in WNT accumulation within the ER [34–40]. Following the binding of WNT to WLS, the WNT is transported from the Golgi to the plasma membrane for secretion and transfer to its target cells via WNT-binding proteins, exosomes, or cytonemes [41–45]. WNT acylation renders it hydrophobic and limits diffusion through the extracellular space [30].

2.2. WNT Binding

Once at the target cell membrane, the WNT ligand binds to a Frizzled (FZD) receptor as well as a Low-Density Lipoprotein Receptor-Related Protein (LRP) to form a FZD-LRP

coreceptor, thus initiating canonical WNT signaling [46–51] (Figure 1, left). Humans have 10 FZDs, to which the 19 WNTs bind promiscuously [52–54]. The LRPs associated with WNT signaling are LRP5 and LRP6 [47–49]. Importantly, the palmitoleic acid appended to the WNT is also required for FZD and LRP binding [50,51,55]. Lipid modification is, therefore, necessary for WNT secretion, as well as receptor binding (which leads to active WNT signaling). Taken together, the hydrophobicity of the acylated WNT severely limits its diffusion in the extracellular space, enforcing strict control over which cells receive a productive WNT ligand [30,34,56]. However, additional regulatory mechanisms exist to modulate WNT–FZD–LRP binding. These include NOTUM, which functions by WNT deacylation; DKK, which functions by binding LRP and blocking WNT binding; or sFRP, which sequesters the WNT itself [55,57–59]. Additionally, FZD may be ubiquitinated and internalized by the transmembrane E3 ubiquitin ligases, RNF43 and ZNRF3, which block WNT signaling activity [60,61]. However, RNF43 and ZNRF3 activity can be inhibited by the intestinal stem cell marker, LGR5, in the presence of its ligand, RSPO [19,60–62]. RSPO and LGR5, therefore, permit WNT signal transduction by maintaining FZD at the plasma membrane for WNT binding through inhibition of RNF43 and ZNRF3.

2.3. WNT Signal Transduction

Upon successful WNT–FZD–LRP binding, the cytoplasmic domains of FZD and LRP become associated, which results in the recruitment of DVL and AXIN and the phosphorylation of LRP by CK1 γ and GSK3, in an incompletely understood mechanism [63–67]. AXIN is a core component of the Destruction Complex (DC), and thus, sequestration of AXIN at FZD–LRP results in sequestration of the DC [68]. The DC is comprised of AXIN, APC, CK1 α , GSK3, β -catenin, and β -TrCP [69–74]. DC sequestration inhibits β -catenin ubiquitination, but not phosphorylation, halting β -catenin turnover and saturating the DCs [68]. Since β -catenin is consistently produced, DC saturation results in β -catenin accumulation in the cytoplasm, which then migrates to the nucleus [68]. Nuclear migration of β -catenin may be facilitated by RAPGEF5 [75]. Once within the nucleus, β -catenin competes with the transcriptionally repressive TLE factors to bind with the TCF/LEF family of transcription factors, which are the canonical WNT transcription factors [76–79]. Association of the potent transcriptional activation domain of β -catenin with the TCF/LEF transcription factors results in the activation of WNT target gene expression [78,79] (Figure 1, left). The role of each TCF/LEF factor is largely distinct, though there remains limited functional redundancy, such as between TCF1 and LEF1 in the lymphoid lineage [80–88]. In the colon, TCF4, encoded by *TCF7L2*, is necessary for intestinal development in mouse embryos as well as intestinal maintenance in adult mice [2,83,84]. While it is difficult to comprehensively identify all crucial downstream TCF4 targets in the intestine; *MYC*, *CCND1*, and *AXIN2* are generally considered to be key transcriptional targets of an active WNT signal and serve as WNT reporter genes [89–91].

2.4. Inactive WNT Signaling

In the WNT OFF state, the FZD and LRP receptors remain unbound by WNT, and therefore, remain unassociated in the cytoplasm. FZD receptors are internalized by ZNRF3 and RNF43, blocking accessibility to WNT [60,61]. The DC remains, unrestricted, in the cytoplasm. APC facilitates β -catenin retrieval from the nucleus, as APC contains nuclear import and export domains, as well as incorporation of β -catenin into the DC via the 15- and 20-amino acid domains (β -catenin binding), and SAMP domains (AXIN binding) of APC [69,70,92–96]. Once within the DC, CK1 α phosphorylates β -catenin at S45, which primes it for subsequent phosphorylation by GSK3 at T41, S37, and S33 [71,97]. The phosphorylated residues form a motif recognized by β -TrCP, which ubiquitinates β -catenin, targeting it for destruction by the proteasomal machinery [71,72,98]. β -catenin is rapidly degraded and does not accumulate in the cytoplasm, nor does it migrate to the nucleus. The TCF/LEF factors, therefore, remain bound by the TLE family of transcriptional repressors and inhibit WNT target gene expression [99,100] (Figure 1, center).

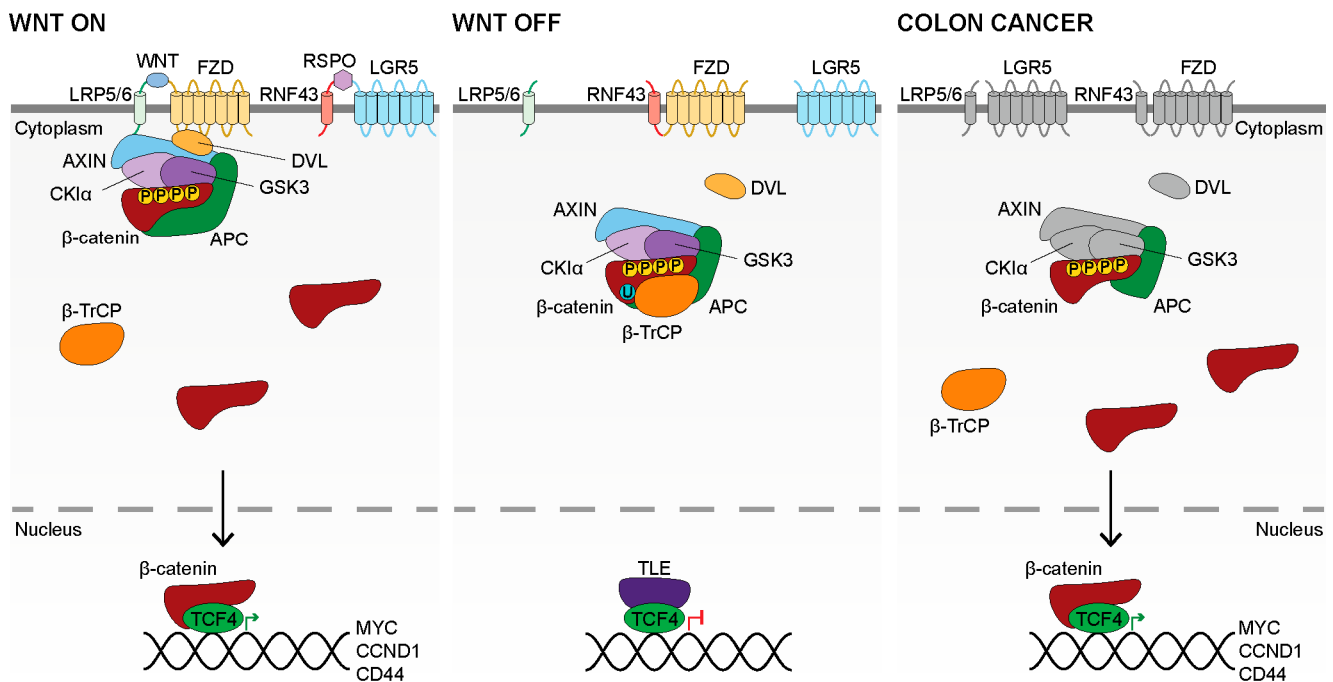


Figure 1. The WNT Signaling Pathway. In the **WNT ON** state (**left**), an R-spondin ligand (RSPO) binds LGR5 and RNF43/ZNRF3, preventing the inhibition of FZD. FZD is, therefore, able to bind a WNT ligand and LRP5/6 to form a co-receptor complex. DVL and AXIN, as well as the rest of the DC, is recruited to WNT–FZD–LRP at the plasma membrane. β-catenin may be phosphorylated within the sequestered DC, however, it cannot be ubiquitinated by β-TrCP, and is not degraded by the proteasome. The DCs become saturated with β-catenin, and newly synthesized β-catenin accumulates in the cytoplasm. β-catenin then migrates to the nucleus where it binds the TCF/LEF family of transcription factors (TCF4 in the intestines). The potent transcriptional activation domain of β-catenin, complexed with TCF4, results in the expression of WNT signaling target genes such as *MYC*, *CCND1*, and *CD44*. In the **WNT OFF** state (**center**), no WNT or RSPO ligands are present, and FZD is inhibited by RNF43/ZNRF3. The DC remains in the cytoplasm where it actively phosphorylates β-catenin. Phosphorylated β-catenin is recognized by β-TrCP and ubiquitinated, resulting in degradation of β-catenin (not diagrammed). β-catenin is unable to accumulate in the cytoplasm. The TCF/LEF factors are bound by the inhibitory TLE factors and repress target gene expression. In **Colon Cancer** (**right**), APC is truncated due to mutation, which compromises the ability of the DC to target β-catenin for degradation. The upstream components of the pathway are in grey as their functional role, to regulate β-catenin, has been compromised by APC truncation. β-catenin accumulates in the cytoplasm, after which it migrates to the nucleus. β-catenin binds the TCF/LEF transcription factors and constitutively activates the expression of WNT signaling target genes which leads to proliferation and the maintenance of stemness.

2.5. WNT Signaling in Colon Cancer

The majority (93%) of colon cancers harbor mutations in the WNT signaling pathway, which result in altered WNT signaling activity [12]. The majority of these mutations arise in *APC*, resulting in a truncated APC protein which can not efficiently facilitate the degradation of β-catenin, resulting in constitutively active WNT signaling [101–104] (Figure 1, right). *APC* appears to follow Knudsen’s two-hit theory, as both alleles have been found to be mutated in the majority of adenomas from patients with familial adenomatous polyposis (FAP) [105–109]. Stabilizing mutations in β-catenin also occur, rendering it refractory to phosphorylation, and therefore degradation, resulting in constitutive WNT signaling [103]. Together, *APC* and *CTNNB1* account for the majority of WNT mutations in colon cancer [12]. These mutations sever the dependence of the cell upon WNT ligands for activated WNT signaling activity, and mutant cells no longer require signals from the

mesenchyme or Paneth cells to maintain active WNT. These mutations, therefore, result in the protracted maintenance of a WNT signaling phenotype outside of the intestinal stem cell niche. The *APC* and *CTNNB1* mutations are mutually exclusive, indicating that they serve equivalent roles in WNT signaling activation [110]. Unfortunately, β -catenin accesses a wide array of target genes through its association with the TCF/LEF factors, complicating our understanding of the downstream consequences of high levels of nuclear β -catenin. However, *MYC* appears to be the single most important target, as *MYC* deletion rescues the consequences of *APC* loss, despite high levels of β -catenin [111].

3. RAS Signaling

RAS signaling is crucial for mediating cellular growth, differentiation, and survival [112]. As a result of their growth and anti-apoptotic functions, members of the RAS signaling pathway, most notably *K-RAS*, are frequently mutated in cancer [113]. Approximately 20% of all cancers and 50% of all colon cancers harbor an activating mutation in *RAS* [112,113]. In colon cancer these mutations typically occur following *APC* mutation during tumorigenesis, though this order is not invariant [113].

3.1. RAS Signaling Receptors and Ligands

RAS signaling relies on the actions of the Epidermal Growth Factor Receptors (EGFR), which are Receptor Tyrosine Kinases (RTKs), for signal transduction through the plasma membrane. The EGFR family consists of four members: ERBB1 (EGFR), ERBB2 (HER2), ERBB3 (HER3), and ERBB4 (HER4) [114–117]. EGFR is capable of binding a variety of ligands, including Epidermal Growth Factor (EGF), Transforming Growth Factor- α (TGF- α), and heparin-binding EGF-like growth factor (HB-EGF), as well as several others [114,118–122]. ERBB2 has no known ligands and ERBB3 and ERBB4 bind to the neuregulins (NRG) [123,124]. The major RAS ligands in the intestinal epithelium are EGF and TGF- α [6]. EGF–EGFR binding results in EGFR dimerization into homo or heterodimers with other members of the EGFR family, such as ERBB2 [125–127] (Figure 2, left). The ability to form receptor heterodimers allows for a wider variety of ligand binding and diversifies the intracellular responses based on the participating receptors [128].

3.2. RAS Signal Transduction

Oligomerization of the EGFR family of receptors results in transphosphorylation of the dimerized, cytoplasmic receptor domains [129–132]. The phosphorylated residue Y1068 on EGFR forms a docking site for GRB2, via its SH2 domain, which recruits its binding partner, SOS, a RAS-specific Guanine nucleotide Exchange Factor (GEF) [133–137]. SOS activates the small guanosine triphosphatase (GTPase) RAS by inducing the exchange of GDP for GTP [138–140]. When activated (bound by GTP), RAS activates a variety of downstream processes involving PI3K, RALGDS, and PLC ϵ , as well as the protein serine/threonine kinase RAF [112,141–143]. This review discusses the major downstream process, the MAPK cascade, which is initiated by RAF. Once activated by RAS, RAF phosphorylates, and by doing so activates, the mitogen-activated protein kinase kinase, MEK [144–147]. Subsequently, MEK phosphorylates ERK on its activation loop [146–149]. A fraction of ERK is transported to the nucleus following activation, resulting in the phosphorylation of both cytosolic and nuclear substrates [150–153]. Within the nucleus, ERK phosphorylates a variety of transcription factors, including *MYC* and members of the ETS family, such as ELK1 [154–156]. Phosphorylation of ELK1 allows it to form a ternary complex with serum response factor (SRF) at serum response elements (SREs) [155,156]. A notable transcriptional target of ELK1 is *FOS*, a member of the AP-1 transcription factor [155,156]. Additionally, SREs are present within many immediate early response genes [157]. RAS signaling therefore results in the expression of key cell-cycle regulatory proteins which promote proliferation and survival.

3.3. Inactive RAS Signaling

In the absence of EGF or an EGF-like ligand, EGFR does not dimerize, since the dimerization interface remains sequestered until EGF binding, which induces a conformational change in the receptor [132,158]. EGFR dimerization alters the conformation of the kinase domains which permits phosphorylation [159,160]. Following EGFR autophosphorylation, the EGF–EGFR complex is rapidly internalized and subsequently degraded, limiting the amount of RAS activation [161–163]. RAS proteins contain intrinsic GTPase activity and gradually exchange GTP for GDP, leading to their own inactivation, a process greatly facilitated by the GTPase-activating proteins (GAPs) [164–166] (Figure 2, center).

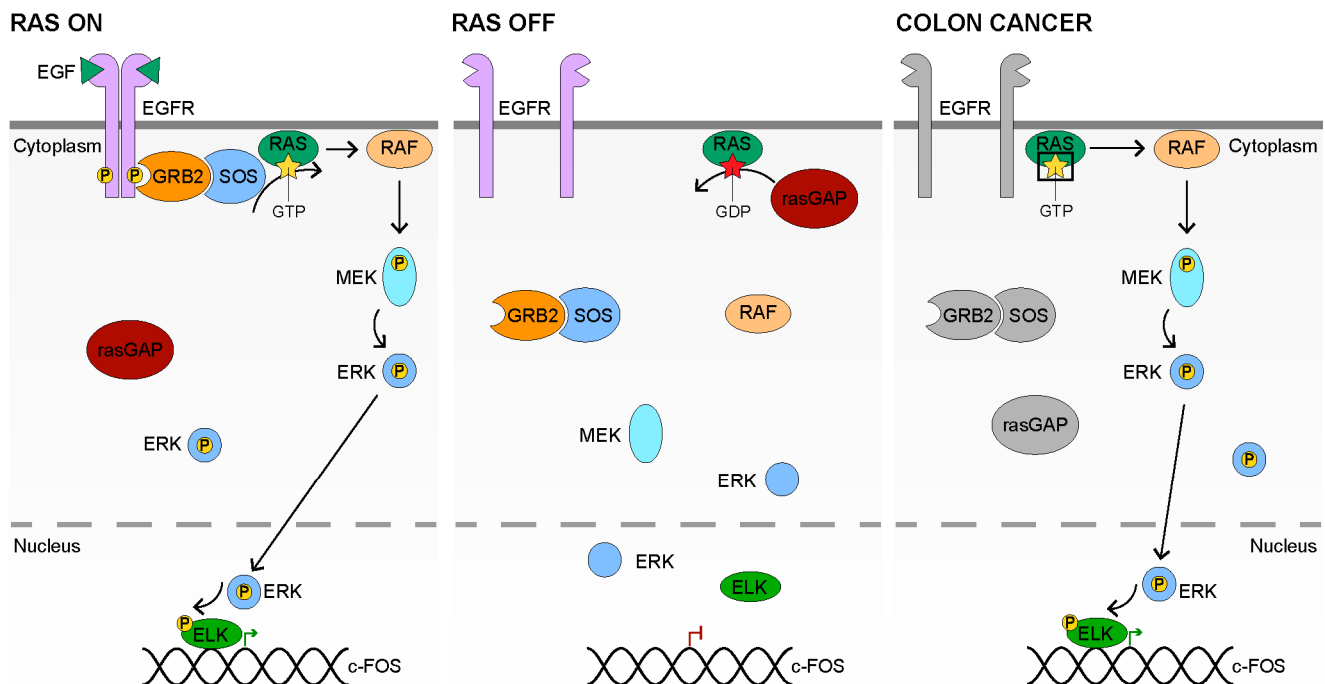


Figure 2. The RAS-MAPK Signaling Pathway. In the **RAS ON** state (**left**), EGF binds to EGFR with 2:2 stoichiometry at the plasma membrane. EGF binding results in dimerization of the receptors, and their autophosphorylation. GRB2 binds to the phosphorylated tyrosine on EGFR via its SH2 domain, and subsequently recruits the rasGEF, SOS. SOS catalyzes the exchange of GDP for GTP on RAS, activating it. Activated RAS then activates RAF, which phosphorylates MEK, which, in turn, phosphorylates ERK. Phosphorylated ERK travels to the nucleus, though some remains in the cytoplasm, resulting in the phosphorylation of cytoplasmic and nuclear targets. ERK phosphorylates the transcription factor ELK, which allows it to form a ternary complex with serum response factor (SRF) at serum response elements (SREs). The complex then drives the expression of growth factor response genes, such as *FOS*, a component of the AP-1 transcription factor. In the **RAS OFF** state (**center**), no EGF or EGF-like ligand is present and EGFR remains unbound, its dimerization inhibited by its conformation. GTP bound to RAS is hydrolyzed to GDP by rasGAP, inactivating it. RAS which is bound to GDP, remains so. GRB2 and SOS remain unbound to EGFR, and RAF is inactive. MEK and ERK remain unphosphorylated, with some ERK remaining in the nucleus. ELK is unphosphorylated and is unable to form a ternary complex with SRF at the SRE. In **Colon Cancer** (**right**), *K-RAS* is mutated, at codon 12, which renders it refractory to GTP–GDP exchange by rasGAP, and rasGAP is, therefore, in grey. The actions of the upstream components EGFR, GRB2, and SOS are also in grey as their purpose, promoting the GTP bound state of RAS, has been achieved. RAF–MEK–ERK is active, inducing the activity of ELK and others.

3.4. RAS Signaling in Colon Cancer

In colon cancer, *K-RAS* mutations, which occur almost exclusively in codons 12, 13, or 61, are found in approximately 50% of tumors [12,113]. These mutations alter the residues

surrounding the GTP–GDP exchange site on RAS, inhibiting the ability of rasGAPs to hydrolyze GTP and return K-RAS to the GDP-bound state [167,168] (Figure 2, right). RAS signaling, therefore, remains persistently active in K-RAS mutant cells and significantly reduces the reliance of the cell upon EGF and EGFR for an active RAS growth signal. Mutations in K-RAS are mutually exclusive with mutations in N-RAS or B-RAF, implying similar regulatory functions [12,169]. Consistent activation of RAS signaling results in the phosphorylation of ERK targets, such as the ETS transcription factors, which are necessary for the transforming effects of RAS [155,156]. ELK1 phosphorylation leads to ternary complex formation and the expression of FOS [155,156]. The other major component of the AP-1 transcription factor, JUN is also mediated by RAS signaling; however, it relies upon the JNK cascade [170–172]. Tumors containing an activated RAS pathway display increased proliferation through the actions of the ETS factors and AP-1 [112].

4. TGF- β Signaling

TGF- β signaling influences a diverse array of cellular processes throughout the lifespan of the organism, including proliferation, differentiation, morphogenesis, and regeneration [173]. In the colonic epithelium, finely regulated gradients of TGF- β , alongside WNT, mediate differentiation of the intestinal stem cells and transit-amplifying cells [1]. In colon cancer, mutations which influence TGF- β are typically the last perturbation preceding malignant transformation [174].

4.1. TGF- β Ligands and Receptors

The TGF- β family of ligands constitutes the largest family of secreted morphogens (33 members) and can be divided into the TGF β –Activin–Nodal and BMP subfamilies [175]. These ligands are produced by cleavage of a pro-domain, which releases the mature domain of the ligand [176,177]. The mature ligand dimerizes, which is essential for receptor activation [178] (Figure 3, left). These dimeric ligands bind to the serine/threonine kinase family of receptors [179–181]. Humans utilize seven Type I and five Type II receptors, which are dedicated to TGF- β signaling [182,183]. In the intestines, the major TGF- β ligands are BMP2 and BMP4, which bind the Type I receptors BMPRIA and BMPRIB as well as the Type II receptor, BMPRII; however, the TGF- β subfamily is active as well [184,185].

4.2. TGF- β Signal Transduction

The intracellular workhorses of the TGF- β signaling pathway are the SMAD proteins. There are three functional classes of SMADs: receptor-regulated (R-SMAD), co-mediator (Co-SMAD), and inhibitory (I-SMAD) [186]. The R-SMADs include SMAD1, 2, 3, 5, and 8, and the Co-SMAD is SMAD4, while the I-SMADs are SMAD6 and 7 [182,186]. SMAD2 and 3 transduce the TGF- β ligand message, while SMAD1, 5, and 8 transmit the BMP message. BMP ligand binding cooperatively brings together two Type I and Type II BMP receptors, forming a hetero-tetramer [186]. Complex formation allows the Type II receptor to phosphorylate the Type I receptor's GS domain, facilitating the exchange of the inhibitory FKBP12 for an R-SMAD(1, 5, 8) [187–190]. R-SMAD localization to the receptor is facilitated by the protein SARA [191]. Upon R-SMAD binding, the Type I kinase domain phosphorylates the R-SMAD, after which it dissociates from the receptor [187,192,193]. Phosphorylated R-SMADs then form a hetero-trimeric complex with SMAD4, the Co-SMAD [194,195]. The SMAD complex then translocates to the nucleus, where it regulates target gene transcription alongside various nuclear cofactors [187,193,196]. These nuclear co-factors bind the R-SMAD, ensuring the specificity of the transcriptional response [182]. TGF- β target genes include p15^{Ink4b} and p21^{Cip1}, the cyclin-dependent kinase inhibitors which mediate the growth-repressive actions of TGF- β [197–199].

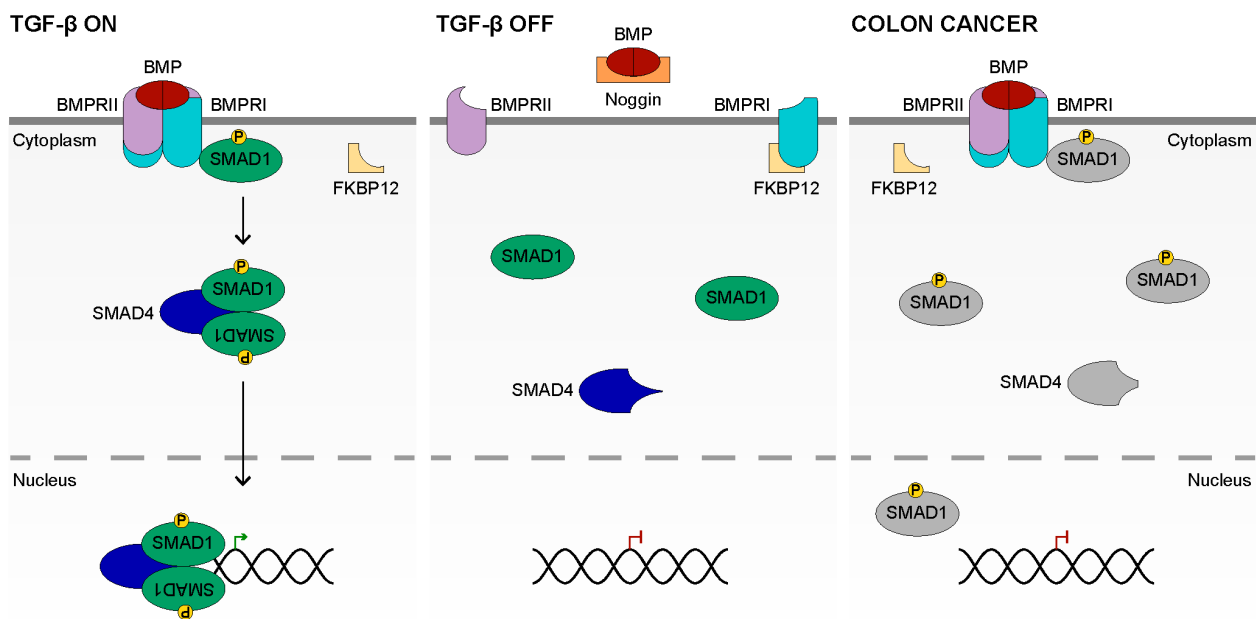


Figure 3. The TGF- β Signaling Pathway. In the **TGF- β ON** state (left), a BMP dimer binds to two BMPRI and two BMPRII to form a hetero-tetrameric complex. The Type II receptor phosphorylates the Type I receptor (not shown), which removes the inhibitory FKBP12 protein. An R-SMAD, such as SMAD1, binds the Type I receptor and is phosphorylated. The phosphorylated R-SMAD then forms a hetero-trimeric complex with SMAD4, the Co-SMAD and another SMAD1. The complex migrates to the nucleus where it binds the DNA and influences target gene expression. In the **TGF- β OFF** state (center), the BMP dimer is bound by Noggin which blocks binding to the Type II or Type I BMP receptors. The receptors remain unassociated, which prevents the phosphorylation of BMPRI. Therefore, BMPRI remains bound by FKBP12, which inhibits the phosphorylation of the R-SMAD. SMAD4 does not form a complex with the R-SMAD. In **Colon Cancer** (right), with a mutated *SMAD4*, the BMP ligand remains capable of inducing hetero-tetramer formation and phosphorylation of the R-SMAD. However, the mutation inhibits formation of the Co-SMAD::R-SMAD complex and the R-SMAD and Co-SMAD is therefore in grey. SMAD1 may migrate to the nucleus alone, however the intended function of the Co-SMAD::R-SMAD complex is not performed.

4.3. Inactive TGF- β Signaling

TGF- β signaling is regulated at several levels. At the ligand level, Noggin and Chordin, are both capable of binding to the BMP ligands, which prevents their association with the BMP receptors [200–202] (Figure 3, center). When the receptors remain unassociated, either through a lack of BMP ligand or the presence of Noggin or Chordin, FKBP12 binds to, and inhibits, the phosphorylation of the Type I receptors [203]. The R-SMAD is unable to bind to the Type I receptor and the Co-SMAD::R-SMAD complex is not formed. The I-SMADs also negatively regulate TGF- β signaling by competing with R-SMADs for receptor binding or with SMAD4 complex formation [204,205]. SMAD6 inhibits BMP signaling, while SMAD7 can inhibit either TGF- β or BMP signaling.

4.4. TGF- β Signaling in Colon Cancer

TGF- β signaling negatively regulates cell proliferation in the colon and is often mutated in colon cancer [13,206]. These mutations, which occur in *TGFBR2*, *SMAD2*, or *SMAD4* inactivate the protein and abrogate the ability of TGF- β to regulate proliferation [206–209] (Figure 3, right). Mutations in *SMAD4* (10%) are more common than mutations in *SMAD2* (6%); however, in hypermutated tumors, mutations in *TGFBR2* were observed in 51% of tumors [12]. The *SMAD4* mutations may occur within the MH2 domain, which inhibits the ability of SMAD4 to bind the R-SMAD and transfer to the nucleus, or may occur within the MH1 domain, which results in autoinhibition of the SMAD, which

also inhibits SMAD complex formation [210,211]. Ultimately, biallelic mutation of the TGF- β signaling pathway components results in decreased regulation of cell growth and is typically the last mutation to occur prior to the transition to malignancy [212].

5. The Cell Cycle in Cancer

The cell cycle is initiated by progression of the cells past the restriction point in G₁ [213,214]. Once this point has been passed, no additional external stimuli are required for progression through the cell cycle [213,214]. The restriction point represents the shift from a reliance on cyclin D-CDK4/6 for progression to S-phase, to a reliance on cyclin E-CDK2 [214–216]. The goal of the cancer cell is, therefore, to progress through the restriction point by activating cyclin E, which depends upon cyclin D.

6. WNT, RAS and TGF- β

The WNT, RAS, and TGF- β signaling pathways are frequently mutated in sporadic colon cancer [12,13]. WNT signaling is necessary for intestinal homeostasis throughout life, as loss of WNT signaling activity results in the loss of the proliferative compartment and breakdown of the intestinal epithelium in neonatal and adult mice [2,84,217]. However, when WNT signaling is aberrantly activated, the intestinal crypts exhibit exacerbated proliferation [218–220]. A similar, yet less drastic, effect occurs when EGF signaling is inhibited in the intestines, which induces stem cell quiescence, while EGF activation results in aberrant proliferation [221–223]. The role of TGF- β is to negatively regulate proliferation in the intestine, striking a balance with the proliferative actions of WNT and EGF. Loss of TGF- β regulation also results in aberrant proliferation [5,11]. Taken together, disruption of the WNT–EGF–TGF β interaction, such as by an *APC*, *K-RAS*, or *SMAD4* mutation, results in an imbalance of the signaling pathways and confers a proliferative advantage upon the mutation-harboring cell.

The aberrant activation of WNT signaling leads to the constitutive formation of β -catenin/TCF4 transcriptional complexes, which enforce a crypt progenitor phenotype [103,104,224]. The downstream target genes of TCF4 include *MYC* and *CCND1*, which both facilitate progression into S-phase [89,90]. However, *MYC* appears to be the major target of WNT signaling, as *MYC* deletion rescues *APC* loss, despite high levels of β -catenin [111]. *MYC* lies at the core of cell cycle progression and facilitates the expression of genes associated with nucleotide biosynthesis, ribosome biogenesis, RNA processing, cell cycle progression, and DNA replication [225,226]. Additionally, *MYC* also represses the expression of key cell cycle inhibitors such as *CDKN1A* (p21) and *CDKN2B* (p15) [227,228]. The ability of TGF- β to counteract the WNT signal is centered on *MYC*, as *MYC* expression is repressed by SMAD3, which is necessary for TGF- β -induced G₁ arrest [229,230]. Furthermore, TGF- β expresses *CDKN1A* and *CDKN2B*, the genes which are inhibited by *MYC* [197,198]. WNT and TGF- β , therefore, compete to regulate cell cycle progression at the *MYC* level, with TGF- β dominance resulting in p21 and p15 production and cell cycle arrest, while WNT dominance results in *CDKN1A* and *CDKN2B* repression. The *APC* mutation appears to give WNT a slight advantage. The increased levels of *MYC*, induced by activating WNT mutations, are complemented by activated RAS signaling via K-RAS [231]. Phosphorylated ELK1 leads to expression of *FOS*, a member of the AP-1 transcription factor [155,156]. The remaining component of AP-1, JUN, is phosphorylated by the JNK cascade of RAS signaling, which is also active in colon cancer [231]. Once formed, AP-1 leads to proliferation and survival by expressing genes such as *CCND1* and repressing *TP53* [232–235]. Therefore, WNT and RAS cooperate to express *CCND1*, by the concerted efforts of β -catenin/TCF4 and AP-1. It has been noted that WNT alone does not appear sufficient to fully express *CCND1* [236]. Additionally, RAS signaling, acting via ERK, leads to the phosphorylation of *MYC* and *FOS*, enhancing their transcriptional output [150,154]. Furthermore, ERK phosphorylates the SMAD proteins, which inhibits their nuclear accumulation and, therefore, their signaling capabilities [237,238]. Oncogenic RAS, therefore, aids WNT signaling in *CCND1* expression via AP-1 and *MYC* phosphorylation, helps to weaken TGF- β signaling

by SMAD phosphorylation via ERK, and represses *TP53* via AP-1. The high levels of cyclin D, from WNT and RAS, bind to CDK4, a *MYC* target gene, allowing for the formation of cyclin D and CDK4 complexes [239]. CyclinD/CDK4 phosphorylates pRb, releasing the E2F factors [240,241]. *MYC* then functions alongside the E2F factors to facilitate binding at the *E2F* gene promoters, facilitating the activation of the E2F transcriptional program [242]. E2F target genes are necessary for DNA replication and the transition from G₁ to S, such as *CCNE1* [243]. Cyclin E binds to CDK2, which further phosphorylates pRb, leading to the complete release of the E2F factors, and transition to the growth factor-independent stages of the cell cycle [214,244]. The concerted actions of WNT and RAS, therefore, generate a potent proliferative signal through the combined function of βcat/TCF4, *MYC*, and RAS/AP-1. Despite the combination of WNT and RAS, TGF-β appears to retain some proliferation inhibitory functions, as demonstrated by selection of TGF-β mutations. Its ability to repress *MYC* expression, as well as express *CDKN1A* and *CDKN2B*, may be the last checks on proliferation, which upon mutation of *SMAD4* or one of the TGF-β receptors, results in the complete release of *MYC* and unregulated proliferation of cancerous cells (Figure 4).

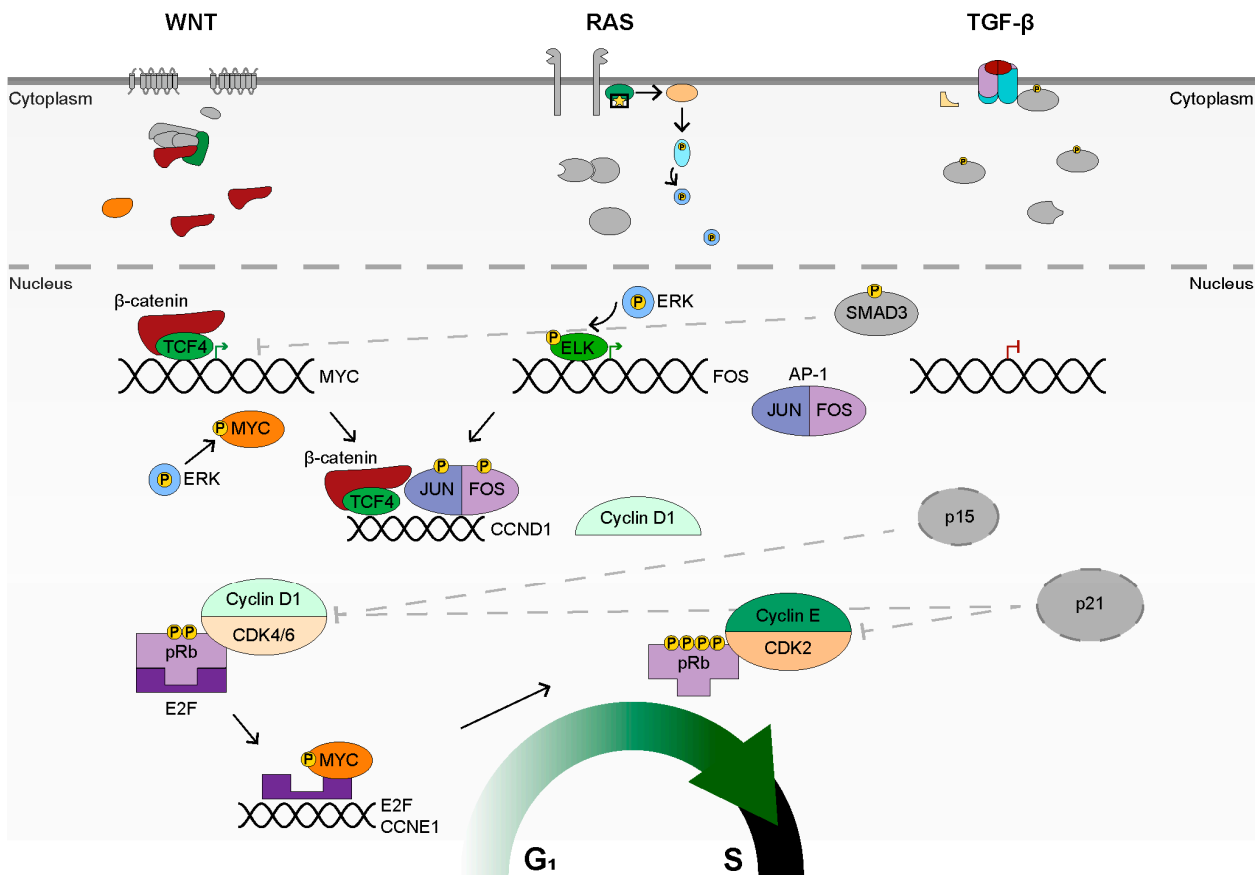


Figure 4. Signaling Overview in Sporadic Colon Cancer. Activated WNT signaling, via mutations in *APC* or *CTNNB1*, results in constitutive formation of βcat/TCF4 complexes, which drive WNT target gene expression, such as *MYC* and *CCND1*. Activated RAS signaling, via mutations in *K-RAS*, result in activation of ERK, which phosphorylates *MYC*, as well as the R-SMADs, inhibiting SMAD migration to the nucleus (not shown). ERK also phosphorylates ELK, which results in expression of *FOS*. JUN is regulated by the JNK cascade (not shown), and binds to *FOS*, forming the AP-1 transcription factor. AP-1 and βcat/TCF4 drive expression of *CCND1*. Cyclin D1 binds to CDK4 or CDK6 and phosphorylates pRb, causing the release of E2F. E2F, with *MYC*, drives the expression of other E2F genes and *CCNE1*. Cyclin E binds to CDK2, which fully phosphorylates pRb, leading to progression into S-phase. The TGF-β pathway is inactivated due to mutation, though low levels of phosphorylated R-SMAD may be found in the nucleus. The cell cycle inhibitors, p15 and p21, which would be expressed given active TGF-β, cannot inhibit CDK4/6 or CDK2, and are in grey.

7. The Intestinal Microbiota

The microorganisms which reside in the colon are often overlooked in regard to their influence on colorectal cancer tumorigenesis. These organisms can generate a profound impact on the gut by the alteration of chemical species, interaction with intestinal cells, or by the release of toxins [245–247]. Given that intestinal polyps extrude into the lumen of the colon, an interaction between cells within the polyps and gut-resident microbes is expected. The pathogen, *C. difficile* secretes toxins which bind the FZD receptors, inhibiting their ability to bind a WNT ligand [248,249]. This inhibition of WNT signaling may result in extended wound-healing and inflammation. However, the actions of bacteria are not solely deleterious. Probiotic strains can compete against virulent bacterial species, protecting the intestines from inflammation and damage [250]. Evidently, much work is to be done to understand the capability of bacteria to influence intestinal health.

8. Conclusions

In colon cancer, the mutations of the WNT, RAS, and TGF- β signaling pathways recapitulate the signaling arrangement present in the intestinal stem cells. This stochastic recapitulation of a similar functional program indicates that the coordinated activities of these pathways results in a potent growth-inducing stimulus. These efforts are, at times, overlapping, such as the expression of *CCND1* through β cat/TCF4 and AP-1, whereas other efforts are complementary, such as *MYC* expression by WNT and *MYC* phosphorylation by RAS. TGF- β antagonizes the influences of WNT and RAS on the cell cycle by down-regulating *MYC* and up-regulating the cell cycle regulators *CDKN1A* and *CDKN2B*. The ability of TGF- β to regulate the cell cycle relies on the SMAD transcription factors, which are phosphorylated by the activated RAS pathway to inhibit their nuclear translocation. As colon cancer develops, mutations in the WNT pathway lead to an increase of *MYC*, which results in small adenoma formation. The coupling of oncogenic RAS to deregulated WNT leads to the formation of a large adenoma, as β cat/TCF4 and *MYC* are complemented by AP-1 of RAS. Loss of TGF- β , the developmentally designated gatekeeper, allows for the unrestrictive action of *MYC* and RAS. Taken together, the WNT, RAS, and TGF- β mutations function cooperatively to form a malignant cell.

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References

1. Gehart, H.; Clevers, H. Tales from the crypt: New insights into intestinal stem cells. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 19–34. [[CrossRef](#)] [[PubMed](#)]
2. Korinek, V.; Barker, N.; Moerer, P.; Van Donselaar, E.; Huls, G.; Peters, P.J.; Clevers, H. Depletion of epithelial stem-cell compartments in the small intestine of mice lacking Tcf-4. *Nat. Genet.* **1998**, *19*, 379–383. [[CrossRef](#)] [[PubMed](#)]
3. Sato, T.; Vries, R.G.; Snippert, H.J.; Van De Wetering, M.; Barker, N.; Stange, D.E.; Van Es, J.H.; Abo, A.; Kujala, P.; Peters, P.J.; et al. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. *Nature* **2009**, *459*, 262–265. [[CrossRef](#)] [[PubMed](#)]
4. van Es, J.H.; van Gijn, M.E.; Riccio, O. Notch/ γ -secretase inhibition turns proliferative cells in intestinal crypts and adenomas into goblet cells. *Nature* **2005**, *435*, 959–963. [[CrossRef](#)]
5. Haramis, A.-P.G.; Begthel, H.; Born, M.; van Es, J.; Jonkheer, S.; Offerhaus, G.J.A.; Clevers, H. De Novo Crypt Formation and Juvenile Polyposis on BMP Inhibition in Mouse Intestine. *Science* **2004**, *303*, 1684–1686. [[CrossRef](#)] [[PubMed](#)]
6. Sato, T.; Van Es, J.H.; Snippert, H.J.; Stange, D.E.; Vries, R.G.; van den Born, M.; Barker, N.; Shroyer, N.F.; Van De Wetering, M.; Clevers, H. Paneth cells constitute the niche for Lgr5 stem cells in intestinal crypts. *Nature* **2011**, *469*, 415–418. [[CrossRef](#)]
7. Rothenberg, M.E.; Nusse, Y.; Kalisky, T.; Lee, J.J.; Dalerba, P.; Scheeren, F.; Lobo, N.; Kulkarni, S.; Sim, S.; Qian, D.; et al. Identification of a cKit+ Colonic Crypt Base Secretory Cell That Supports Lgr5+ Stem Cells in Mice. *Gastroenterology* **2012**, *142*, 1195–1205.e6. [[CrossRef](#)] [[PubMed](#)]

8. Sasaki, N.; Sachs, N.; Wiebrands, K.; Ellenbroek, S.; Fumagalli, A.; Lyubimova, A.; Begthel, H.; Born, M.V.D.; van Es, J.H.; Karthaus, W.R.; et al. Reg4+ deep crypt secretory cells function as epithelial niche for Lgr5+ stem cells in colon. *Proc. Natl. Acad. Sci. USA* **2016**, *113*, E5399–E5407. [[CrossRef](#)]
9. Farin, H.F.; Jordens, I.; Mosa, H.F.F.M.H.; Basak, O.; Korving, J.; Tauriello, D.V.F.; De Punder, K.; Angers, S.; Peters, K.D.P.P.J.; Maurice, M.; et al. Visualization of a short-range Wnt gradient in the intestinal stem-cell niche. *Nature* **2016**, *530*, 340–343. [[CrossRef](#)]
10. Tian, H.; Biehs, B.; Chiu, C.; Siebel, C.W.; Wu, Y.; Costa, M.; de Sauvage, F.J.; Klein, O.D. Opposing Activities of Notch and Wnt Signaling Regulate Intestinal Stem Cells and Gut Homeostasis. *Cell Rep.* **2015**, *11*, 33–42. [[CrossRef](#)]
11. He, X.C.; Zhang, J.; Tong, W.G. BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat. Genet.* **2004**, *36*, 1117–1121. [[CrossRef](#)]
12. Cancer Genome Atlas Network. Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **2012**, *487*, 330–337. [[CrossRef](#)] [[PubMed](#)]
13. Vogelstein, B.; Fearon, E.R.; Hamilton, S.R.; Kern, S.E.; Preisinger, A.C.; Leppert, M.; Smits, A.M.; Bos, J.L. Genetic Alterations during Colorectal-Tumor Development. *N. Engl. J. Med.* **1988**, *319*, 525–532. [[CrossRef](#)] [[PubMed](#)]
14. Powell, S.M.; Zilz, N.; Beazer-Barclay, Y.; Bryan, T.M.; Hamilton, S.R.; Thibodeau, S.N.; Vogelstein, B.; Kinzler, K.W. APC mutations occur early during colorectal tumorigenesis. *Nature* **1992**, *359*, 235–237. [[CrossRef](#)] [[PubMed](#)]
15. Smith, A.J.; Stern, H.S.; Penner, M.; Hay, K.; Mitri, A.; Bapat, B.V.; Gallinger, S. Somatic APC and K-ras codon 12 mutations in aberrant crypt foci from human colons. *Cancer Res.* **1994**, *54*, 5527–5530.
16. Matano, M.; Date, S.; Shimokawa, M.; Takano, A.; Fujii, M.; Ohta, Y.; Watanabe, T.; Kanai, T.; Sato, T. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nat. Med.* **2015**, *21*, 256–262. [[CrossRef](#)]
17. Drost, J.; Van Jaarsveld, R.H.; Ponsioen, B.; Zimmerlin, C.; Van Boxtel, R.; Buijs, A.; Sachs, N.; Overmeer, R.M.; Offerhaus, G.J.; Begthel, H.; et al. Sequential cancer mutations in cultured human intestinal stem cells. *Nature* **2015**, *521*, 43–47. [[CrossRef](#)]
18. Kusserow, A.; Pang, K.; Sturm, C.; Hroudá, M.; Lentfer, J.; Schmidt, H.; Technau, U.; Von Haeseler, A.; Hobmayer, B.; Martindale, M.Q.; et al. Unexpected complexity of the Wnt gene family in a sea anemone. *Nature* **2005**, *433*, 156–160. [[CrossRef](#)]
19. Barker, N.; Van Es, J.H.; Kuipers, J.; Kujala, P.; Van Den Born, M.; Cozijnsen, M.; Haegerbarth, A.; Korving, J.; Begthel, H.; Peters, P.J.; et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* **2007**, *449*, 1003–1007. [[CrossRef](#)]
20. Nusse, R.; Varmus, H.E. Many tumors induced by the mouse mammary tumor virus contain a provirus integrated in the same region of the host genome. *Cell* **1982**, *31*, 99–109. [[CrossRef](#)]
21. Nusse, R.; Brown, A.; Papkoff, J.; Scambler, P.; Shackelford, G.; McMahon, A.; Moon, R.; Varmus, H. A new nomenclature for int-1 and related genes: The Wnt gene family. *Cell* **1991**, *64*, 231. [[CrossRef](#)]
22. Miller, J.R. The Wnts. *Genome Biol.* **2002**, *3*, 3001.
23. Stark, K.; Vainio, S.; Vassileva, G.; McMahon, A.P. Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature* **1994**, *372*, 679–683. [[CrossRef](#)] [[PubMed](#)]
24. Monkley, S.J.; Delaney, S.J.; Pennisi, D.J.; Christiansen, J.H.; Wainwright, B.J. Targeted disruption of the Wnt2 gene results in placentation defects. *Development* **1996**, *122*, 3343–3353. [[CrossRef](#)]
25. Ikeya, M.; Lee, S.M.K.; Johnson, J.E.; McMahon, A.P.; Takada, S. Wnt signalling required for expansion of neural crest and CNS progenitors. *Nature* **1997**, *389*, 966–970. [[CrossRef](#)]
26. Millar, S.E.; Willert, K.; Salinas, P.C.; Roelink, H.; Nusse, R.; Sussman, D.J.; Barsh, G.S. WNT Signaling in the Control of Hair Growth and Structure. *Dev. Biol.* **1999**, *207*, 133–149. [[CrossRef](#)]
27. Yamaguchi, T.P.; Bradley, A.; McMahon, A.P.; Jones, S. A Wnt5a pathway underlies outgrowth of multiple structures in the vertebrate embryo. *Development* **1999**, *126*, 1211–1223. [[CrossRef](#)]
28. van den Heuvel, M.; Harryman-Samos, C.; Klingensmith, J. Mutations in the segment polarity genes wingless and porcupine impair secretion of the wingless protein. *EMBO J.* **1993**, *12*, 5293–5302. [[CrossRef](#)] [[PubMed](#)]
29. Kadowaki, T.; Wilder, E.; Klingensmith, J.; Zachary, K.; Perrimon, N. The segment polarity gene porcupine encodes a putative multitransmembrane protein involved in Wingless processing. *Genes Dev.* **1996**, *10*, 3116–3128. [[CrossRef](#)]
30. Willert, K.; Brown, J.D.; Danenberg, E.; Duncan, A.W.; Weissman, I.L.; Reya, T.; Yates, J.R.; Nusse, R. Wnt proteins are lipid-modified and can act as stem cell growth factors. *Nature* **2003**, *423*, 448–452. [[CrossRef](#)] [[PubMed](#)]
31. Rios-Esteves, J.; Haugen, B.; Resh, M.D. Identification of Key Residues and Regions Important for Porcupine-mediated Wnt Acylation. *J. Biol. Chem.* **2014**, *289*, 17009–17019. [[CrossRef](#)] [[PubMed](#)]
32. Biechele, S.; Cox, B.J.; Rossant, J. Porcupine homolog is required for canonical Wnt signaling and gastrulation in mouse embryos. *Dev. Biol.* **2011**, *355*, 275–285. [[CrossRef](#)]
33. Barrott, J.J.; Cash, G.M.; Smith, A.P.; Barrow, J.R.; Murtaugh, L.C. Deletion of mouse Porcn blocks Wnt ligand secretion and reveals an ectodermal etiology of human focal dermal hypoplasia/Goltz syndrome. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 12752–12757. [[CrossRef](#)] [[PubMed](#)]
34. Takada, R.; Satomi, Y.; Kurata, T.; Ueno, N.; Norioka, S.; Kondoh, H.; Takao, T.; Takada, S. Monounsaturated Fatty Acid Modification of Wnt Protein: Its Role in Wnt Secretion. *Dev. Cell* **2006**, *11*, 791–801. [[CrossRef](#)]
35. Bänziger, C.; Soldini, D.; Schütt, C.; Zipperlen, P.; Hausmann, G.; Basler, K. Wntless, a Conserved Membrane Protein Dedicated to the Secretion of Wnt Proteins from Signaling Cells. *Cell* **2006**, *125*, 509–522. [[CrossRef](#)] [[PubMed](#)]

36. Bartscherer, K.; Pelte, N.; Ingelfinger, D.; Boutros, M. Secretion of Wnt Ligands Requires Evi, a Conserved Transmembrane Protein. *Cell* **2006**, *125*, 523–533. [[CrossRef](#)]
37. Goodman, R.M.; Thombre, S.; Firtina, Z.; Gray, D.; Betts, D.; Roebuck, J.; Spana, E.P.; Selva, E.M. Sprinter: A novel transmembrane protein required for Wg secretion and signaling. *Development* **2006**, *133*, 4901–4911. [[CrossRef](#)]
38. Coombs, G.S.; Yu, J.; Canning, C.A.; Veltri, C.A.; Covey, T.M.; Cheong, J.K.; Utomo, V.; Banerjee, N.; Zhang, Z.H.; Jadulco, R.C.; et al. WLS-dependent secretion of WNT3A requires Ser209 acylation and vacuolar acidification. *J. Cell Sci.* **2010**, *123*, 3357–3367. [[CrossRef](#)]
39. Herr, P.; Basler, K. Porcupine-mediated lipidation is required for Wnt recognition by Wls. *Dev. Biol.* **2012**, *361*, 392–402. [[CrossRef](#)]
40. Nygaard, R.; Yu, J.; Kim, J.; Ross, D.R.; Parisi, G.; Clarke, O.B.; Virshup, D.M.; Mancina, F. Structural Basis of WLS/Evi-Mediated Wnt Transport and Secretion. *Cell* **2021**, *184*, 194–206.e14. [[CrossRef](#)]
41. Baeg, G.; Lin, X.; Khare, N.; Baumgartner, S.; Perrimon, N. Heparan sulfate proteoglycans are critical for the organization of the extracellular distribution of Wingless. *Development* **2001**, *128*, 87–94. [[CrossRef](#)]
42. Korkut, C.; Ataman, B.; Ramachandran, P.; Ashley, J.; Barria, R.; Gherbesi, N.; Budnik, V. Trans-Synaptic Transmission of Vesicular Wnt Signals through Evi/Wntless. *Cell* **2009**, *139*, 393–404. [[CrossRef](#)] [[PubMed](#)]
43. Gross, J.C.; Chaudhary, V.; Bartscherer, K.; Boutros, M. Active Wnt proteins are secreted on exosomes. *Nat. Cell Biol.* **2012**, *14*, 1036–1045. [[CrossRef](#)] [[PubMed](#)]
44. Alexandre, C.; Baena-Lopez, A.; Vincent, J.-P. Patterning and growth control by membrane-tethered Wingless. *Nature* **2014**, *505*, 180–185. [[CrossRef](#)]
45. Stanganello, E.; Hagemann, A.; Mattes, B.; Sinner, C.; Meyen, D.; Weber, S.; Schug, A.; Raz, E.; Scholpp, S. Filopodia-based Wnt transport during vertebrate tissue patterning. *Nat. Commun.* **2015**, *6*, 5846. [[CrossRef](#)] [[PubMed](#)]
46. Bhanot, P.; Brink, M.; Samos, C.H.; Hsieh, J.-C.; Wang, Y.; Macke, J.P.; Andrew, D.; Nathans, J.; Nusse, R. A new member of the frizzled family from Drosophila functions as a Wingless receptor. *Nature* **1996**, *382*, 225–230. [[CrossRef](#)]
47. Wehrli, M.; Dougan, S.T.; Caldwell, K.; Okeefe, L.V.; Schwartz, S.; Vaizel-Ohayon, D.; Schejter, E.D.; Tomlinson, A.; DiNardo, S. Arrow encodes an LDL-receptor-related protein essential for Wingless signalling. *Nature* **2000**, *407*, 527–530. [[CrossRef](#)] [[PubMed](#)]
48. Tamai, K.; Semenov, M.; Kato, Y.; Spokony, R.; Liu, C.; Katsuyama, Y.; Hess, F.; Saint-Jeannet, J.-P.; He, X. LDL-receptor-related proteins in Wnt signal transduction. *Nature* **2000**, *407*, 530–535. [[CrossRef](#)] [[PubMed](#)]
49. Tamai, K.; Zeng, X.; Liu, C.; Zhang, X.; Harada, Y.; Chang, Z.; He, X. A Mechanism for Wnt Coreceptor Activation. *Mol. Cell* **2004**, *13*, 149–156. [[CrossRef](#)]
50. Janda, C.Y.; Waghray, D.; Levin, A.M.; Thomas, C.; Garcia, K.C. Structural Basis of Wnt Recognition by Frizzled. *Science* **2012**, *337*, 59–64. [[CrossRef](#)]
51. Janda, C.Y.; Dang, L.T.; You, C.; Chang, J.; De Lau, W.; Zhong, Z.A.; Yan, K.S.; Marecic, O.; Siepe, D.; Li, X.; et al. Surrogate Wnt agonists that phenocopy canonical Wnt and β -catenin signalling. *Nature* **2017**, *545*, 234–237. [[CrossRef](#)] [[PubMed](#)]
52. Bourhis, E.; Tam, C.; Franke, Y.; Bazan, J.F.; Ernst, J.; Hwang, J.; Costa, M.; Cochran, A.; Hannoush, R.N. Reconstitution of a Frizzled8-Wnt3a-LRP6 Signaling Complex Reveals Multiple Wnt and Dkk1 Binding Sites on LRP6. *J. Biol. Chem.* **2010**, *285*, 9172–9179. [[CrossRef](#)] [[PubMed](#)]
53. Yu, H.; Ye, X.; Guo, N.; Nathans, J. Frizzled 2 and frizzled 7 function redundantly in convergent extension and closure of the ventricular septum and palate: Evidence for a network of interacting genes. *Development* **2012**, *139*, 4383–4394. [[CrossRef](#)]
54. Dijksterhuis, J.; Baljinnyam, B.; Stanger, K.; Sercan, H.O.; Ji, Y.; Andres, O.; Rubin, J.S.; Hannoush, R.N.; Schulte, G. Systematic Mapping of WNT-FZD Protein Interactions Reveals Functional Selectivity by Distinct WNT-FZD Pairs. *J. Biol. Chem.* **2015**, *290*, 6789–6798. [[CrossRef](#)] [[PubMed](#)]
55. Kakugawa, S.; Langton, P.F.; Zebisch, M.; Howell, S.A.; Chang, T.-H.; Liu, Y.; Feizi, T.; Bineva-Todd, G.; O'Reilly, N.; Snijders, B.; et al. Notum deacylates Wnt proteins to suppress signalling activity. *Nature* **2015**, *519*, 187–192. [[CrossRef](#)] [[PubMed](#)]
56. Nusse, R.; Fuerer, C.; Ching, W.; Harnish, K.; Logan, C.; Zeng, A.; ten Berge, D.; Kalani, Y. Wnt Signaling and Stem Cell Control. *Cold Spring Harb. Symp. Quant. Biol.* **2008**, *73*, 59–66. [[CrossRef](#)]
57. Glinka, A.; Wu, W.; Delius, H.; Monaghan, A.P.; Blumenstock, C.; Niehrs, C. Dickkopf-1 is a member of a new family of secreted proteins and functions in head induction. *Nature* **1998**, *391*, 357–362. [[CrossRef](#)] [[PubMed](#)]
58. Leyns, L.; Bouwmeester, T.; Kim, S.-H.; Piccolo, S.; De Robertis, E. Frzb-1 Is a Secreted Antagonist of Wnt Signaling Expressed in the Spemann Organizer. *Cell* **1997**, *88*, 747–756. [[CrossRef](#)]
59. Cruciati, C.-M.; Niehrs, C. Secreted and Transmembrane Wnt Inhibitors and Activators. *Cold Spring Harb. Perspect. Biol.* **2013**, *5*, a015081. [[CrossRef](#)]
60. Hao, H.-X.; Xie, Y.; Zhang, Y.; Charlat, O.; Oster, E.; Avello, M.; Lei, H.; Mickanin, C.; Liu, D.; Ruffner, H.; et al. ZNRF3 promotes Wnt receptor turnover in an R-spondin-sensitive manner. *Nature* **2012**, *485*, 195–200. [[CrossRef](#)]
61. Koo, B.-K.; Spit, M.; Jordens, I.; Low, T.Y.; Stange, D.; Van De Wetering, M.; Van Es, J.H.; Mohammed, S.; Heck, A.; Maurice, M.; et al. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. *Nature* **2012**, *488*, 665–669. [[CrossRef](#)] [[PubMed](#)]
62. Carmona, K.S.; Gong, X.; Lin, Q. R-spondins function as ligands of the orphan receptors LGR4 and LGR5 to regulate Wnt/ β -catenin signaling. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 11452–11457. [[CrossRef](#)] [[PubMed](#)]
63. Cliffe, A.; Hamada, F.; Bienz, M. A Role of Dishevelled in Relocating Axin to the Plasma Membrane during Wingless Signaling. *Curr. Biol.* **2003**, *13*, 960–966. [[CrossRef](#)]

64. Bilić, J.; Huang, Y.-L.; Davidson, G.; Zimmermann, T.; Cruciat, C.-M.; Bienz, M.; Niehrs, C. Wnt Induces LRP6 Signalosomes and Promotes Dishevelled-Dependent LRP6 Phosphorylation. *Science* **2007**, *316*, 1619–1622. [[CrossRef](#)] [[PubMed](#)]
65. Zeng, X.; Huang, H.; Tamai, K.; Zhang, X.; Harada, Y.; Yokota, C.; Almeida, K.; Wang, J.; Doble, B.; Woodgett, J.; et al. Initiation of Wnt signaling: Control of Wnt coreceptor Lrp6 phosphorylation/activation via frizzled, dishevelled and axin functions. *Development* **2008**, *135*, 367–375. [[CrossRef](#)]
66. Davidson, G.; Wu, W.; Shen, J. Casein kinase 1 gamma couples Wnt receptor activation to cytoplasmic signal transduction. *Nature* **2005**, *438*, 867–872. [[CrossRef](#)]
67. Zeng, X.; Tamai, K.; Doble, B.; Li, S.; Huang, H.; Habas, R.; Okamura, H.; Woodgett, J.; He, X. A dual-kinase mechanism for Wnt co-receptor phosphorylation and activation. *Nature* **2005**, *438*, 873–877. [[CrossRef](#)]
68. Li, V.; Ng, S.S.; Boersema, P.J.; Low, T.Y.; Karthaus, W.R.; Gerlach, J.; Mohammed, S.; Heck, A.; Maurice, M.; Mahmoudi, T.; et al. Wnt Signaling through Inhibition of β -Catenin Degradation in an Intact Axin1 Complex. *Cell* **2012**, *149*, 1245–1256. [[CrossRef](#)]
69. Su, L.-K.; Vogelstein, B.; Kinzler, K.W. Association of the APC Tumor Suppressor Protein with Catenins. *Science* **1993**, *262*, 1734–1737. [[CrossRef](#)]
70. Behrens, J.; Jerchow, B.-A.; Würtele, M.; Grimm, J.; Asbrand, C.; Wirtz, R.; Kühl, M.; Wedlich, D.; Birchmeier, W. Functional Interaction of an Axin Homolog, Conductin, with β -Catenin, APC, and GSK3 β . *Science* **1998**, *280*, 596–599. [[CrossRef](#)]
71. Liu, C.; Li, Y.; Semenov, M. Control of beta-catenin phosphorylation/degradation by a dual-kinase mechanism. *Cell* **2002**, *108*, 837–847. [[CrossRef](#)]
72. Kitagawa, M.; Hatakeyama, S.; Shirane, M. An F-box protein, FWD1, mediates ubiquitin-dependent proteolysis of beta-catenin. *EMBO J.* **1999**, *18*, 2401–2410. [[CrossRef](#)] [[PubMed](#)]
73. Kishida, S.; Yamamoto, H.; Ikeda, S. Axin, a negative regulator of the wnt signaling pathway, directly interacts with adenomatous polyposis coli and regulates the stabilization of beta-catenin. *J. Biol. Chem.* **1998**, *273*, 10823–10826. [[CrossRef](#)] [[PubMed](#)]
74. Ikeda, S.; Kishida, S.; Yamamoto, H.; Murai, H.; Koyama, S.; Kikuchi, A. Axin, a negative regulator of the Wnt signaling pathway, forms a complex with GSK-3 β and beta-catenin and promotes GSK-3 β -dependent phosphorylation of beta-catenin. *EMBO J.* **1998**, *17*, 1371–1384. [[CrossRef](#)]
75. Griffin, J.; del Viso, F.; Duncan, A.R.; Robson, A.; Hwang, W.; Kulkarni, S.; Liu, K.; Khokha, M.K. RAPGEF5 Regulates Nuclear Translocation of β -Catenin. *Dev. Cell* **2018**, *44*, 248–260.e4. [[CrossRef](#)] [[PubMed](#)]
76. Daniels, D.L.; Weis, W.I. Beta-catenin directly displaces Groucho/TLE repressors from Tcf/Lef in Wnt-mediated transcription activation. *Nat. Struct. Mol. Biol.* **2005**, *12*, 364–371. [[CrossRef](#)] [[PubMed](#)]
77. Arce, L.; Pate, K.T.; Waterman, M.L. Groucho binds two conserved regions of LEF-1 for HDAC-dependent repression. *BMC Cancer* **2009**, *9*, 159. [[CrossRef](#)]
78. Behrens, J.; von Kries, J.P.; Kühl, M. Functional interaction of beta-catenin with the transcription factor LEF-1. *Nature* **1996**, *38*, 638–642. [[CrossRef](#)]
79. Molenaar, M.; van de Wetering, M.; Oosterwegel, M. XTcf-3 transcription factor mediates beta-catenin-induced axis formation in *Xenopus* embryos. *Cell* **1996**, *86*, 391–399. [[CrossRef](#)]
80. van de Wetering, M.; Oosterwegel, M.; Dooijes, D. Identification and cloning of TCF-1, a T lymphocyte-specific transcription factor containing a sequence-specific HMG box. *EMBO J.* **1991**, *10*, 123–132. [[CrossRef](#)]
81. Verbeek, S.; Izon, D.; Hofhuis, F.; Robanus-Maandag, E.; Riele, H.T.; Van De Wetering, M.; Oosterwegel, M.; Wilson, A.; Macdonald, H.R.; Clevers, H. An HMG-box-containing T-cell factor required for thymocyte differentiation. *Nature* **1995**, *374*, 70–74. [[CrossRef](#)] [[PubMed](#)]
82. Nguyen, H.; Rendl, M.; Fuchs, E. Tcf3 Governs Stem Cell Features and Represses Cell Fate Determination in Skin. *Cell* **2006**, *127*, 171–183. [[CrossRef](#)] [[PubMed](#)]
83. Angus-Hill, M.L.; Elbert, K.M.; Hidalgo, J.; Capecchi, M.R. T-cell factor 4 functions as a tumor suppressor whose disruption modulates colon cell proliferation and tumorigenesis. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4914–4919. [[CrossRef](#)] [[PubMed](#)]
84. van Es, J.H.; Haegerbarth, A.; Kujala, P. A critical role for the Wnt effector Tcf4 in adult intestinal homeostatic self-renewal. *Mol. Cell Biol.* **2014**, *32*, 1918–1927. [[CrossRef](#)] [[PubMed](#)]
85. van Genderen, C.; Okamura, R.M.; Fariñas, I. Development of several organs that require inductive epithelial-mesenchymal interactions is impaired in LEF-1-deficient mice. *Genes. Dev.* **1994**, *8*, 2691–2703. [[CrossRef](#)] [[PubMed](#)]
86. Okamura, R.M.; Sigvardsson, M.; Galceran, J. Redundant regulation of T cell differentiation and TCR α gene expression by the transcription factors LEF-1 and TCF-1. *Immunity* **1998**, *8*, 11–20. [[CrossRef](#)]
87. Oosterwegel, M.; Van De Wetering, M.; Timmerman, J.; Kruisbeek, A.; Destree, O.; Meijlink, F.; Clevers, H. Differential expression of the HMG box factors TCF-1 and LEF-1 during murine embryogenesis. *Development* **1993**, *118*, 439–448. [[CrossRef](#)]
88. Van de Wetering, M.; Castrop, J.; Korinek, V. Extensive alternative splicing and dual promoter usage generate Tcf-1 protein isoforms with differential transcription control properties. *Mol. Cell Biol.* **1996**, *16*, 745–752. [[CrossRef](#)]
89. He, T.-C.; Sparks, A.B.; Rago, C.; Hermeking, H.; Zawel, L.; da Costa, L.T.; Morin, P.J.; Vogelstein, B.; Kinzler, K.W. Identification of c-MYC as a Target of the APC Pathway. *Science* **1998**, *281*, 1509–1512. [[CrossRef](#)]
90. Shtutman, M.; Zhurinsky, J.; Simcha, I. The cyclin D1 gene is a target of the beta-catenin/LEF-1 pathway. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 5522–5527. [[CrossRef](#)]
91. Yan, D.; Wiesmann, M.; Rohan, M. Elevated expression of axin2 and hnk4 mRNA provides evidence that Wnt/beta-catenin signaling is activated in human colon tumors. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 14973–14978. [[CrossRef](#)]

92. Munemitsu, S.; Albert, I.; Souza, B.; Rubinfeld, B.; Polakis, P. Regulation of intracellular beta-catenin levels by the adenomatous polyposis coli (APC) tumor-suppressor protein. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 3046–3050. [[CrossRef](#)] [[PubMed](#)]
93. Rosin-Arbesfeld, R.; Townsley, F.; Bienz, M. The APC tumour suppressor has a nuclear export function. *Nature* **2000**, *406*, 1009–1012. [[CrossRef](#)]
94. Rosin-Arbesfeld, R.; Cliffe, A.; Brabletz, T.; Bienz, M. Nuclear export of the APC tumour suppressor controls beta-catenin function in transcription. *EMBO J.* **2003**, *22*, 1101–1113. [[CrossRef](#)] [[PubMed](#)]
95. Groden, J.; Thliveris, A.; Samowitz, W.; Carlson, M.; Gelbert, L.; Albertsen, H.; Joslyn, G.; Stevens, J.; Spirio, L.; Robertson, M.; et al. Identification and characterization of the familial adenomatous polyposis coli gene. *Cell* **1991**, *66*, 589–600. [[CrossRef](#)]
96. Morishita, E.C.; Murayama, K.; Kato-Murayama, M. Crystal structures of the armadillo repeat domain of adenomatous polyposis coli and its complex with the tyrosine-rich domain of Sam68. *Structure* **2011**, *19*, 1496–1508. [[CrossRef](#)] [[PubMed](#)]
97. Yost, C.; Torres, M.; Miller, J.R.; Huang, E.; Kimelman, D.; Moon, R.T. The axis-inducing activity, stability, and subcellular distribution of beta-catenin is regulated in *Xenopus* embryos by glycogen synthase kinase 3. *Genes Dev.* **1996**, *10*, 1443–1454. [[CrossRef](#)] [[PubMed](#)]
98. Aberle, H.; Bauer, A.; Stappert, J. β -catenin is a target for the ubiquitin-proteasome pathway. *EMBO J.* **1997**, *16*, 3797–3804. [[CrossRef](#)] [[PubMed](#)]
99. Cavallo, R.A.; Cox, R.T.; Moline, M.M.; Roose, J.; Polevoy, G.A.; Clevers, H.; Peifer, M.; Bejsovec, A. Drosophila Tcf and Groucho interact to repress Wingless signalling activity. *Nature* **1998**, *395*, 604–608. [[CrossRef](#)]
100. Levanon, D.; Goldstein, R.E.; Bernstein, Y.; Tang, H.; Goldenberg, D.; Stifani, S.; Paroush, Z.; Groner, Y. Transcriptional repression by AML1 and LEF-1 is mediated by the TLE/Groucho corepressors. *Proc. Natl. Acad. Sci. USA* **1998**, *95*, 11590–11595. [[CrossRef](#)]
101. Smith, K.J.; Johnson, K.A.; Bryan, T.M.; Hill, D.E.; Markowitz, S.; Willson, J.K.; Paraskeva, C.; Petersen, G.M.; Hamilton, S.R.; Vogelstein, B. The APC gene product in normal and tumor cells. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2846–2850. [[CrossRef](#)] [[PubMed](#)]
102. Kinzler, K.W.; Vogelstein, B. Lessons from Hereditary Colorectal Cancer. *Cell* **1996**, *87*, 159–170. [[CrossRef](#)]
103. Morin, P.J.; Sparks, A.B.; Korinek, V. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* **1997**, *275*, 1787–1790. [[CrossRef](#)]
104. Korinek, V.; Barker, N.; Morin, P.J. Constitutive transcriptional activation by a beta-catenin-Tcf complex in APC^{-/-} colon carcinoma. *Science* **1997**, *275*, 1784–1787. [[CrossRef](#)]
105. Levy, D.B.; Smith, K.J.; Beazer-Barclay, Y.; Hamilton, S.R.; Vogelstein, B.; Kinzler, K.W. Inactivation of both APC alleles in human and mouse tumors. *Cancer Res.* **1994**, *54*, 5953–5958.
106. Luongo, C.; Moser, A.R.; Gledhill, S.; Dove, W.F. Loss of Apc⁺ in intestinal adenomas from Min mice. *Cancer Res.* **1994**, *54*, 5947–5952.
107. Miyaki, M.; Seki, M.; Okamoto, M.; Yamanaka, A.; Maeda, Y.; Tanaka, K.; Kikuchi, R.; Iwama, T.; Ikeuchi, T.; Tonomura, A. Genetic changes and histopathological types in colorectal tumors from patients with familial adenomatous polyposis. *Cancer Res.* **1990**, *50*, 7166–7173. [[PubMed](#)]
108. Miyaki, M.; Tanaka, K.; Kikuchi-Yanoshita, R.; Muraoka, M.; Konishi, M. Familial polyposis: Recent advances. *Crit. Rev. Oncol.* **1995**, *19*, 1–31. [[CrossRef](#)]
109. Knudson, A.G. Antioncogenes and human cancer. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 10914–10921. [[CrossRef](#)]
110. Sparks, A.B.; Morin, P.J.; Vogelstein, B.; Kinzler, K.W. Mutational analysis of the APC/beta-catenin/Tcf pathway in colorectal cancer. *Cancer Res.* **1998**, *58*, 1130–1134.
111. Sansom, O.J.; Meniel, V.S.; Muncan, V.; Phesse, T.J.; Wilkins, J.A.; Reed, K.R.; Vass, J.K.; Athineos, D.; Clevers, H.; Clarke, A.R. Myc deletion rescues Apc deficiency in the small intestine. *Nature* **2007**, *446*, 676–679. [[CrossRef](#)] [[PubMed](#)]
112. Downward, J. Targeting RAS signalling pathways in cancer therapy. *Nat. Cancer* **2003**, *3*, 11–22. [[CrossRef](#)] [[PubMed](#)]
113. Bos, J.L. Ras oncogenes in human cancer: A review. *Cancer Res.* **1989**, *49*, 4682–4689.
114. Cohen, S.; Carpenter, G.; King, L. Epidermal growth factor-receptor-protein kinase interactions. Co-purification of receptor and epidermal growth factor-enhanced phosphorylation activity. *J. Biol. Chem.* **1980**, *255*, 4834–4842. [[CrossRef](#)]
115. Schechter, A.L.; Stern, D.F.; Vaidyanathan, L.; Decker, S.J.; Drebin, J.A.; Greene, M.I.; Weinberg, R.A. The neu oncogene: An erb-B-related gene encoding a 185,000-Mr tumour antigen. *Nature* **1984**, *312*, 513–516. [[CrossRef](#)]
116. Kraus, M.H.; Issing, W.; Miki, T.; Popescu, N.C.; Aaronson, S.A. Isolation and characterization of ERBB3, a third member of the ERBB/epidermal growth factor receptor family: Evidence for overexpression in a subset of human mammary tumors. *Proc. Natl. Acad. Sci. USA* **1989**, *86*, 9193–9197. [[CrossRef](#)] [[PubMed](#)]
117. Plowman, G.D.; Culouscou, J.M.; Whitney, G.S.; Green, J.M.; Carlton, G.W.; Foy, L.; Neubauer, M.G.; Shoyab, M. Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 1746–1750. [[CrossRef](#)]
118. Cohen, S.; Carpenter, G. Human epidermal growth factor: Isolation and chemical and biological properties. *Proc. Natl. Acad. Sci. USA* **1975**, *72*, 1317–1321. [[CrossRef](#)]
119. Cohen, S.; Ushiro, H.; Stoscheck, C.; Chinkers, M. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. *J. Biol. Chem.* **1982**, *257*, 1523–1531. [[CrossRef](#)]
120. Massagué, J. Transforming growth factor- α . A model for membrane-anchored growth factors. *J. Biol. Chem.* **1990**, *15*, 21393–21396. [[CrossRef](#)]

121. Higashiyama, S.; Abraham, J.A.; Miller, J.; Fiddes, J.C.; Klagsbrun, M. A Heparin-Binding Growth Factor Secreted by Macrophage-Like Cells That Is Related to EGF. *Science* **1991**, *251*, 936–939. [[CrossRef](#)] [[PubMed](#)]
122. Harris, R.C.; Chung, E.; Coffey, R.J. EGF receptor ligands. *Exp. Cell Res.* **2003**, *284*, 2–13. [[CrossRef](#)]
123. Riethmacher, D.; Sonnenberg-Riethmacher, E.; Brinkmann, V.; Yamaai, T.; Lewin, G.R.; Birchmeier, C. Severe neuropathies in mice with targeted mutations in the ErbB3 receptor. *Nature* **1997**, *389*, 725–730. [[CrossRef](#)] [[PubMed](#)]
124. Gassmann, M.; Casagrande, F.; Orioli, D.; Simon, H.; Lai, C.; Klein, R.; Lemke, G. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. *Nature* **1995**, *378*, 390–394. [[CrossRef](#)] [[PubMed](#)]
125. Stern, D.F.; Kamps, M.P. EGF-stimulated tyrosine phosphorylation of p185neu: A potential model for receptor interactions. *EMBO J.* **1988**, *7*, 995–1001. [[CrossRef](#)]
126. King, C.R.; Borrello, I.; Bellot, F.; Comoglio, P.; Schlessinger, J. Egf binding to its receptor triggers a rapid tyrosine phosphorylation of the erbB-2 protein in the mammary tumor cell line SK-BR-3. *EMBO J.* **1988**, *7*, 1647–1651. [[CrossRef](#)]
127. Yarden, Y.; Sliwkowski, M.X. Untangling the ErbB signalling network. *Nat. Rev. Mol. Cell Biol.* **2001**, *2*, 127–137. [[CrossRef](#)]
128. Lemmon, M.; Schlessinger, J. Regulation of signal transduction and signal diversity by receptor oligomerization. *Trends Biochem. Sci.* **1994**, *19*, 459–463. [[CrossRef](#)]
129. Yarden, Y.; Schlessinger, J. Epidermal growth factor induces rapid, reversible aggregation of the purified epidermal growth factor receptor. *Biochemistry* **1987**, *26*, 1443–1451. [[CrossRef](#)]
130. Yarden, Y.; Schlessinger, J. Self-phosphorylation of epidermal growth factor receptor: Evidence for a model of intermolecular allosteric activation. *Biochemistry* **1987**, *26*, 1434–1442. [[CrossRef](#)]
131. Ferguson, K.M.; Berger, M.; Mendrola, J.M.; Cho, H.-S.; Leahy, D.J.; A Lemmon, M. EGF Activates Its Receptor by Removing Interactions that Autoinhibit Ectodomain Dimerization. *Mol. Cell* **2003**, *11*, 507–517. [[CrossRef](#)]
132. Burgess, A.W.; Cho, H.-S.; Eigenbrot, C.; Ferguson, K.M.; Garrett, T.P.J.; Leahy, D.J.; Lemmon, M.A.; Sliwkowski, M.X.; Ward, C.W.; Yokoyama, S. An Open-and-Shut Case? Recent Insights into the Activation of EGF/ErbB Receptors. *Mol. Cell* **2003**, *12*, 541–552. [[CrossRef](#)]
133. Lowenstein, E.; Daly, R.; Batzer, A.; Li, W.; Margolis, B.; Lammers, R.; Ullrich, A.; Skolnik, E.; Bar-Sagi, D.; Schlessinger, J. The SH2 and SH3 domain-containing protein GRB2 links receptor tyrosine kinases to ras signaling. *Cell* **1992**, *70*, 431–442. [[CrossRef](#)]
134. Buday, L.; Downward, J. Epidermal growth factor regulates p21ras through the formation of a complex of receptor, Grb2 adapter protein, and Sos nucleotide exchange factor. *Cell* **1993**, *73*, 611–620. [[CrossRef](#)]
135. Gale, N.W.; Kaplan, S.; Lowenstein, E.J.; Schlessinger, J.; Bar-Sagi, D. Grb2 mediates the EGF-dependent activation of guanine nucleotide exchange on Ras. *Nature* **1993**, *363*, 88–92. [[CrossRef](#)] [[PubMed](#)]
136. Chardin, P.; Camonis, J.H.; Gale, N.W.; Van Aelst, L.; Schlessinger, J.; Wigler, M.H.; Bar-Sagi, D. Human Sos1: A guanine nucleotide exchange factor for Ras that binds to GRB2. *Science* **1993**, *260*, 1338–1343. [[CrossRef](#)]
137. Schlessinger, J. SH2/SH3 signaling proteins. *Curr. Opin. Genet. Dev.* **1994**, *4*, 25–30. [[CrossRef](#)]
138. Boriack-Sjodin, P.A.; Margarit, S.M.; Bar-Sagi, D.; Kuriyan, J. The structural basis of the activation of Ras by Sos. *Nature* **1998**, *394*, 337–343. [[CrossRef](#)]
139. Medema, R.H.; de Vries-Smits, A.M.; van der Zon, G.C.; A Maassen, J.; Bos, J.L. Ras activation by insulin and epidermal growth factor through enhanced exchange of guanine nucleotides on p21ras. *Mol. Cell. Biol.* **1993**, *13*, 155–162. [[CrossRef](#)]
140. Bar-Sagi, D. The Sos (Son of sevenless) protein. *Trends Endocrinol. Metab.* **1994**, *5*, 165–169. [[CrossRef](#)]
141. Moodie, S.A.; Wolfman, A. The 3Rs of life: Ras, Raf and growth regulation. *Trends Genet.* **1994**, *10*, 44–48. [[CrossRef](#)]
142. Bruder, J.T.; Heidecker, G.; Rapp, U.R. Serum-, TPA-, and Ras-induced expression from Ap-1/Ets-driven promoters requires Raf-1 kinase. *Genes Dev.* **1992**, *6*, 545–556. [[CrossRef](#)] [[PubMed](#)]
143. Brtva, T.R.; Drugan, J.K.; Ghosh, S.; Terrell, R.S.; Campbell-Burk, S.; Bell, R.M.; Der, C.J. Two Distinct Raf Domains Mediate Interaction with Ras. *J. Biol. Chem.* **1995**, *270*, 9809–9812. [[CrossRef](#)] [[PubMed](#)]
144. Dent, P.; Haser, W.; Haystead, T.A.J.; Vincent, L.A.; Roberts, T.M.; Sturgill, T.W. Activation of Mitogen-Activated Protein Kinase Kinase by v-Raf in NIH 3T3 Cells and in Vitro. *Science* **1992**, *257*, 1404–1407. [[CrossRef](#)]
145. Kyriakis, J.M.; App, H.; Zhang, X.-F.; Banerjee, P.; Brautigan, D.L.; Rapp, U.R.; Avruch, J. Raf-1 activates MAP kinase-kinase. *Nature* **1992**, *358*, 417–421. [[CrossRef](#)]
146. Crews, C.M.; Erikson, R.L. Extracellular signals and reversible protein phosphorylation: What to Mek of it all. *Cell* **1993**, *74*, 215–217. [[CrossRef](#)]
147. Howe, L.; Leever, S.J.; Gómez, N.; Nakielnny, S.; Cohen, P.; Marshall, C.J. Activation of the MAP kinase pathway by the protein kinase raf. *Cell* **1992**, *71*, 335–342. [[CrossRef](#)]
148. Crews, C.M.; Alessandrini, A.; Erikson, R.L. Erks: Their fifteen minutes has arrived. *Cell Growth Differ. Mol. Biol. J. Am. Assoc. Cancer Res.* **1992**, *3*, 135–142.
149. Payne, D.M.; Rossomando, A.J.; Martino, P.; Erickson, A.K.; Her, J.H.; Shabanowitz, J.; Hunt, D.F.; Weber, M.J.; Sturgill, T.W. Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase). *EMBO J.* **1991**, *10*, 885–892. [[CrossRef](#)]
150. Chen, R.H.; Sarnecki, C.; Blenis, J. Nuclear localization and regulation of erk- and rsk-encoded protein kinases. *Mol. Cell. Biol.* **1992**, *12*, 915–927. [[CrossRef](#)]
151. Gonzalez, A.F.; Seth, A.; Raden, D.L.; Bowman, D.S.; Fay, F.S.; Davis, R.J. Serum-induced translocation of mitogen-activated protein kinase to the cell surface ruffling membrane and the nucleus. *J. Cell Biol.* **1993**, *122*, 1089–1101. [[CrossRef](#)] [[PubMed](#)]

152. Karin, M.; Hunter, T. Transcriptional control by protein phosphorylation: Signal transmission from the cell surface to the nucleus. *Curr. Biol.* **1995**, *5*, 747–757. [[CrossRef](#)]
153. Hunter, T. Signaling-2000 and beyond. *Cell* **2000**, *100*, 113–127. [[CrossRef](#)]
154. Seth, A.; A Gonzalez, F.; Gupta, S.; Raden, D.L.; Davis, R.J. Signal transduction within the nucleus by mitogen-activated protein kinase. *J. Biol. Chem.* **1992**, *267*, 24796–24804. [[CrossRef](#)]
155. Gille, H.; Kortenjann, M.; Thomae, O.; Moomaw, C.; Slaughter, C.; Cobb, M.H.; E Shaw, P. ERK phosphorylation potentiates Elk-1-mediated ternary complex formation and transactivation. *EMBO J.* **1995**, *14*, 951–962. [[CrossRef](#)]
156. Kortenjann, M.; Thomae, O.; E Shaw, P. Inhibition of v-raf-dependent c-fos expression and transformation by a kinase-defective mutant of the mitogen-activated protein kinase Erk2. *Mol. Cell. Biol.* **1994**, *14*, 4815–4824. [[CrossRef](#)] [[PubMed](#)]
157. Yordy, J.S.; Muise-Helmericks, R.C. Signal transduction and the Ets family of transcription factors. *Oncogene* **2000**, *19*, 6503–6513. [[CrossRef](#)]
158. Schlessinger, J. Ligand-Induced, Receptor-Mediated Dimerization and Activation of EGF Receptor. *Cell* **2002**, *110*, 669–672. [[CrossRef](#)]
159. Zhang, X.; Gureasko, J.; Shen, K.; Cole, P.A.; Kuriyan, J. An Allosteric Mechanism for Activation of the Kinase Domain of Epidermal Growth Factor Receptor. *Cell* **2006**, *125*, 1137–1149. [[CrossRef](#)]
160. Jura, N.; Endres, N.F.; Engel, K.; Deindl, S.; Das, R.; Lamers, M.H.; Wemmer, D.E.; Zhang, X.; Kuriyan, J. Mechanism for Activation of the EGF Receptor Catalytic Domain by the Juxtamembrane Segment. *Cell* **2009**, *137*, 1293–1307. [[CrossRef](#)]
161. Haigler, H.T.; A McKanna, J.; Cohen, S. Rapid stimulation of pinocytosis in human carcinoma cells A-431 by epidermal growth factor. *J. Cell Biol.* **1979**, *83*, 82–90. [[CrossRef](#)]
162. Beguinot, L.; Lyall, R.M.; Willingham, M.C.; Pastan, I. Down-regulation of the epidermal growth factor receptor in KB cells is due to receptor internalization and subsequent degradation in lysosomes. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 2384–2388. [[CrossRef](#)] [[PubMed](#)]
163. Miller, K.; Beardmore, J.; Kanety, H.; Schlessinger, J.; Hopkins, C.R. Localization of the epidermal growth factor (EGF) receptor within the endosome of EGF-stimulated epidermoid carcinoma (A431) cells. *J. Cell Biol.* **1986**, *102*, 500–509. [[CrossRef](#)] [[PubMed](#)]
164. Boguski, M.S.; McCormick, F. Proteins regulating Ras and its relatives. *Nature* **1993**, *366*, 643–654. [[CrossRef](#)]
165. Trahey, M.; McCormick, F. A cytoplasmic protein stimulates normal N-ras p21 GTPase, but does not affect oncogenic mutants. *Science* **1987**, *238*, 542–545. [[CrossRef](#)] [[PubMed](#)]
166. Martin, G.A.; Yatani, A.; Clark, R.; Conroy, L.; Polakis, P.; Brown, A.M.; McCormick, F. Gap Domains Responsible for Ras P21-Dependent Inhibition of Muscarinic Atrial K + Channel Currents. *Science* **1992**, *255*, 192–194. [[CrossRef](#)] [[PubMed](#)]
167. Hunter, J.C.; Manandhar, A.; Carrasco, M.A.; Gurbani, D.; Gondi, S.; Westover, K.D. Biochemical and Structural Analysis of Common Cancer-Associated KRAS Mutations. *Mol. Cancer Res.* **2015**, *13*, 1325–1335. [[CrossRef](#)]
168. Smith, M.J.; Neel, B.G.; Ikura, M. NMR-based functional profiling of RASopathies and oncogenic RAS mutations. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 4574–4579. [[CrossRef](#)]
169. Rajagopalan, H.; Bardelli, A.; Lengauer, C.; Kinzler, K.W.; Vogelstein, B.; Velculescu, V.E. Tumorigenesis: RAF/RAS oncogenes and mismatch-repair status. *Nature* **2002**, *418*, 934. [[CrossRef](#)]
170. Minden, A.; Lin, A.; Smeal, T.; Dérijard, B.; Cobb, M.; Davis, R.; Karin, M. c-Jun N-terminal phosphorylation correlates with activation of the JNK subgroup but not the ERK subgroup of mitogen-activated protein kinases. *Mol. Cell. Biol.* **1994**, *14*, 6683–6688. [[CrossRef](#)]
171. Minden, A.; Lin, A.; McMahon, M.; Lange-Carter, C.; Dérijard, B.; Davis, R.J.; Johnson, G.L.; Karin, M. Differential Activation of ERK and JNK Mitogen-Activated Protein Kinases by Raf-1 and MEKK. *Science* **1994**, *266*, 1719–1723. [[CrossRef](#)]
172. Davis, R.J. Signal transduction by the JNK group of MAP kinases. *Cell* **2000**, *103*, 239–252. [[CrossRef](#)]
173. Massagué, J. TGF β signalling in context. *Nat. Rev. Mol. Cell Biol.* **2012**, *13*, 616–630. [[CrossRef](#)]
174. Vogelstein, B.; Papadopoulos, N.; Velculescu, V.E.; Zhou, S.; Diaz, L.A., Jr.; Kinzler, K.W. Cancer Genome Landscapes. *Science* **2013**, *339*, 1546–1558. [[CrossRef](#)] [[PubMed](#)]
175. Moses, H.L.; Roberts, A.B.; Derynck, R. The Discovery and Early Days of TGF-beta: A Historical Perspective. *Cold Spring Harb. Perspect. Biol.* **2016**, *8*, a021865. [[CrossRef](#)] [[PubMed](#)]
176. Derynck, R.; Jarrett, J.A.; Chen, E.Y.; Eaton, D.H.; Bell, J.R.; Assoian, R.K.; Roberts, A.B.; Sporn, M.B.; Goeddel, D.V. Human transforming growth factor-beta complementary DNA sequence and expression in normal and transformed cells. *Nature* **1985**, *316*, 701–705. [[CrossRef](#)] [[PubMed](#)]
177. Gentry, E.L.; Lioubin, M.N.; Purchio, A.F.; Marquardt, H. Molecular events in the processing of recombinant type 1 pre-pro-transforming growth factor beta to the mature polypeptide. *Mol. Cell. Biol.* **1988**, *8*, 4162–4168. [[CrossRef](#)]
178. Kirsch, T.; Sebald, W.; Dreyer, M.K. Crystal structure of the BMP-2-BRIA ectodomain complex. *Nat. Genet.* **2000**, *7*, 492–496. [[CrossRef](#)]
179. Massagué, J. Receptors for the TGF-beta family. *Cell* **1992**, *69*, 1067–1070. [[CrossRef](#)]
180. Lin, H.Y.; Lodish, H.F. Receptors for the TGF-beta superfamily: Multiple polypeptides and serine/threonine kinases. *Trends Cell Biol.* **1993**, *3*, 14–19. [[CrossRef](#)]
181. Kingsley, D.M. The TGF-beta superfamily: New members, new receptors, and new genetic tests of function in different organisms. *Genes Dev.* **1994**, *8*, 133–146. [[CrossRef](#)]
182. Shi, Y.; Massagué, J. Mechanisms of TGF-beta signaling from cell membrane to the nucleus. *Cell* **2003**, *113*, 685–700. [[CrossRef](#)]

183. Wrana, J.L. Signaling by the TGF β superfamily. *Cold Spring Harb Perspect Biol.* **2013**, *5*, a011197. [[CrossRef](#)]
184. Hardwick, J.C.; Van Den Brink, G.R.; Bleuming, S.A. Bone morphogenetic protein 2 is expressed by, and acts upon, mature epithelial cells in the colon. *Gastroenterology* **2004**, *126*, 111–121. [[CrossRef](#)] [[PubMed](#)]
185. Liu, F.; Ventura, F.; Doody, J.; Massagué, J. Human type II receptor for bone morphogenetic proteins (BMPs): Extension of the two-kinase receptor model to the BMPs. *Mol. Cell. Biol.* **1995**, *15*, 3479–3486. [[CrossRef](#)] [[PubMed](#)]
186. Massagué, J. TGF-beta signal transduction. *Annu. Rev. Biochem.* **1998**, *67*, 753–791. [[CrossRef](#)]
187. Kretschmar, M.; Liu, F.; Hata, A.; Doody, J.; Massagué, J. The TGF-beta family mediator Smad1 is phosphorylated directly and activated functionally by the BMP receptor kinase. *Genes Dev.* **1997**, *11*, 984–995. [[CrossRef](#)]
188. Wrana, J.L.; Attisano, L.; Wieser, R. Mechanism of activation of the TGF-beta receptor. *Nature* **1994**, *370*, 341–377. [[CrossRef](#)]
189. Wieser, R.; Wrana, J.L.; Massagué, J. GS domain mutations that constitutively activate T beta R-I, the downstream signaling component in the TGF-beta receptor complex. *EMBO J.* **1995**, *14*, 2199–2208. [[CrossRef](#)]
190. Huse, M.; Muir, T.W.; Xu, L.; Chen, Y.-G.; Kuriyan, J.; Massagué, J. The TGF beta receptor activation process: An inhibitor- to substrate-binding switch. *Mol. Cell* **2001**, *8*, 671–682. [[CrossRef](#)]
191. Attisano, L.; Wrana, J.L. Signal transduction by the TGF-beta superfamily. *Science* **2002**, *296*, 1646–1647. [[CrossRef](#)]
192. Macías-Silva, M.; Abdollah, S.; Hoodless, P.A. MADR2 is a substrate of the TGFbeta receptor and its phosphorylation is required for nuclear accumulation and signaling. *Cell* **1996**, *87*, 1215–1224. [[CrossRef](#)]
193. Liu, F.; Hata, A.; Baker, J.C.; Doody, J.; Cárcamo, J.; Harland, R.M.; Massague, J. A human Mad protein acting as a BMP-regulated transcriptional activator. *Nature* **1996**, *381*, 620–623. [[CrossRef](#)] [[PubMed](#)]
194. Zhang, Y.; Feng, X.; We, R. Receptor-associated Mad homologues synergize as effectors of the TGF-beta response. *Nature* **1996**, *383*, 168–172. [[CrossRef](#)]
195. Lagna, G.; Hata, A.; Hemmati-Brivanlou, A. Partnership between DPC4 and SMAD proteins in TGF-beta signalling pathways. *Nature* **1996**, *383*, 832–836. [[CrossRef](#)]
196. Liu, F.; Poupponnot, C.; Massagué, J. Dual role of the Smad4/DPC4 tumor suppressor in TGFbeta-inducible transcriptional complexes. *Genes. Dev.* **1997**, *11*, 3157–3167. [[CrossRef](#)]
197. Hannon, G.J.; Beach, D. p15INK4B is a potential effector of TGF-beta-induced cell cycle arrest. *Nature* **1994**, *371*, 257–361. [[CrossRef](#)]
198. Datto, M.B.; Li, Y.; Panus, J.F.; Howe, D.J.; Xiong, Y.; Wang, X.F. Transforming growth factor beta induces the cyclin-dependent kinase inhibitor p21 through a p53-independent mechanism. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 5545–5549. [[CrossRef](#)] [[PubMed](#)]
199. Reynisdóttir, I.; Polyak, K.; Iavarone, A.; Massagué, J. Kip/Cip and Ink4 Cdk inhibitors cooperate to induce cell cycle arrest in response to TGF-beta. *Genes Dev.* **1995**, *9*, 1831–1845. [[CrossRef](#)]
200. Zimmerman, L.B.; De Jesús-Escobar, J.M.; Harland, R.M. The Spemann Organizer Signal noggin Binds and Inactivates Bone Morphogenetic Protein 4. *Cell* **1996**, *86*, 599–606. [[CrossRef](#)]
201. Groppe, J.; Greenwald, J.; Wiater, E.; Leon, J.M.R.; Economides, A.; Kwiatkowski, W.; Affolter, M.; Vale, W.W.; Belmonte, J.C.I.; Choe, S. Structural basis of BMP signalling inhibition by the cystine knot protein Noggin. *Nature* **2002**, *420*, 636–642. [[CrossRef](#)] [[PubMed](#)]
202. Piccolo, S.; Sasai, Y.; Lu, B.; De Robertis, E. Dorsal-ventral Patterning in Xenopus: Inhibition of Ventral Signals by Direct Binding of Chordin to BMP-4. *Cell* **1996**, *86*, 589–598. [[CrossRef](#)]
203. Chen, Y.G.; Liu, F.; Massague, J. Mechanism of TGFbeta receptor inhibition by FKBP12. *EMBO J.* **1997**, *16*, 3866–3876. [[CrossRef](#)]
204. Imamura, T.; Takase, M.; Nishihara, A. Smad6 inhibits signalling by the TGF-beta superfamily. *Nature* **1997**, *389*, 622–626. [[CrossRef](#)]
205. Nakao, A.; Afrakhte, M.; Morén, A. Identification of Smad7, a TGFbeta-inducible antagonist of TGF-beta signalling. *Nature* **1997**, *389*, 631–635. [[CrossRef](#)] [[PubMed](#)]
206. MacGrogan, D.; Pegram, M.; Slamon, D.; Bookstein, R. Comparative mutational analysis of DPC4 (Smad4) in prostatic and colorectal carcinomas. *Oncogene* **1997**, *15*, 1111–1114. [[CrossRef](#)]
207. Markowitz, S.; Wang, J.; Myeroff, L. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* **1995**, *268*, 1336–1338. [[CrossRef](#)]
208. Parsons, R.; Myeroff, L.L.; Liu, B.; Willson, J.K.; Markowitz, S.D.; Kinzler, K.W.; Vogelstein, B. Microsatellite instability and mutations of the transforming growth factor beta type II receptor gene in colorectal cancer. *Cancer Res.* **1995**, *55*, 5548–5550.
209. Riggins, G.J.; Thiagalingam, S.; Rozenblum, E.; Weinstein, C.L.; Kern, S.E.; Hamilton, S.R.; Willson, J.K.; Markowitz, S.D.; Kinzler, K.W.; Vogelstein, B. Mad-related genes in the human. *Nat. Genet.* **1996**, *13*, 347–349. [[CrossRef](#)]
210. Shi, Y.; Hata, A.; Lo, R.S.; Massague, J.; Pavletich, N.P. A structural basis for mutational inactivation of the tumour suppressor Smad4. *Nature* **1997**, *388*, 87–93. [[CrossRef](#)]
211. Hata, A.; Lo, R.S.; Wotton, D.; Lagna, G.; Massague, J. Mutations increasing autoinhibition inactivate tumour suppressors Smad2 and Smad4. *Nature* **1997**, *388*, 82–87. [[CrossRef](#)]
212. Batlle, E.; Massagué, J. Transforming Growth Factor- β Signaling in Immunity and Cancer. *Immunity* **2019**, *50*, 924–940. [[CrossRef](#)]
213. Pardee, A.B. G 1 Events and Regulation of Cell Proliferation. *Science* **1989**, *246*, 603–608. [[CrossRef](#)] [[PubMed](#)]
214. Blagosklonny, M.V.; Pardee, A.B. The Restriction Point of the Cell Cycle. *Cell Cycle* **2002**, *1*, 102–109. [[CrossRef](#)]
215. Gong, J.; Traganos, F.; Darzynkiewicz, Z. Threshold expression of cyclin E but not D type cyclins characterizes normal and tumour cells entering S phase. *Cell Prolif.* **1995**, *28*, 337–346. [[CrossRef](#)]

216. Ekholm, S.V.; Zickert, P.; Reed, S.I.; Zetterberg, A. Accumulation of Cyclin E Is Not a Prerequisite for Passage through the Restriction Point. *Mol. Cell. Biol.* **2001**, *21*, 3256–3265. [[CrossRef](#)] [[PubMed](#)]
217. Kuhnert, F.; Davis, C.R.; Wang, H.-T.; Chu, P.; Lee, M.; Yuan, J.; Nusse, R.; Kuo, C.J. Essential requirement for Wnt signaling in proliferation of adult small intestine and colon revealed by adenoviral expression of Dickkopf-1. *Proc. Natl. Acad. Sci. USA* **2004**, *101*, 266–271. [[CrossRef](#)] [[PubMed](#)]
218. Barker, N.; Ridgway, R.A.; Van Es, J.H.; Van De Wetering, M.; Begthel, H.; van den Born, M.; Danenberg, E.; Clarke, A.R.; Sansom, O.J.; Clevers, H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* **2009**, *457*, 608–611. [[CrossRef](#)]
219. Moser, A.; Luongo, C.; Gould, K.; McNeley, M.; Shoemaker, A.; Dove, W. ApcMin: A mouse model for intestinal and mammary tumorigenesis. *Eur. J. Cancer* **1995**, *31*, 1061–1064. [[CrossRef](#)]
220. Dow, L.E.; O'Rourke, K.P.; Simon, J.; Tschaharganeh, D.F.; van Es, J.H.; Clevers, H.; Lowe, S.W. Apc Restoration Promotes Cellular Differentiation and Reestablishes Crypt Homeostasis in Colorectal Cancer. *Cell* **2015**, *161*, 1539–1552. [[CrossRef](#)]
221. Basak, O.; Beumer, J.; Wiebrands, K.; Seno, H.; van Oudenaarden, A.; Clevers, H. Induced Quiescence of Lgr5+ Stem Cells in Intestinal Organoids Enables Differentiation of Hormone-Producing Enteroendocrine Cells. *Cell Stem Cell* **2017**, *20*, 177–190.e4. [[CrossRef](#)] [[PubMed](#)]
222. Wong, V.W.Y.; Stange, D.E.; Page, M.E.; Buczacki, S.; Wabik, A.; Itami, S.; van de Wetering, M.; Poulsom, R.; Wright, N.A.; Trotter, M.W.B.; et al. Lrig1 controls intestinal stem-cell homeostasis by negative regulation of ErbB signalling. *Nature* **2012**, *14*, 401–408. [[CrossRef](#)]
223. Snippert, H.J.; Schepers, A.G.; Van Es, J.H.; Simons, B.; Clevers, H. Biased competition between Lgr5 intestinal stem cells driven by oncogenic mutation induces clonal expansion. *EMBO Rep.* **2014**, *15*, 62–69. [[CrossRef](#)]
224. van de Wetering, M.; Sancho, E.; Verweij, C. The beta-catenin/TCF-4 complex imposes a crypt progenitor phenotype on colorectal cancer cells. *Cell* **2002**, *111*, 241–250. [[CrossRef](#)]
225. Meyer, N.; Penn, L.Z. Reflecting on 25 years with MYC. *Nat. Cancer* **2008**, *8*, 976–990. [[CrossRef](#)] [[PubMed](#)]
226. Dang, C.V. MYC on the Path to Cancer. *Cell* **2012**, *149*, 22–35. [[CrossRef](#)]
227. Claassen, G.F.; Hann, S.R. A role for transcriptional repression of p21CIP1 by c-Myc in overcoming transforming growth factor beta -induced cell-cycle arrest. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 9498–9503. [[CrossRef](#)] [[PubMed](#)]
228. Staller, P.; Peukert, K.; Kiermaier, A.; Seoane, J.; Lukas, J.; Karsunky, H.; Möröy, T.; Bartek, J.; Massague, J.; Hänel, F.; et al. Repression of p15INK4b expression by Myc through association with Miz-1. *Nat. Cell Biol.* **2001**, *3*, 392–399. [[CrossRef](#)]
229. Chen, C.-R.; Kang, Y.; Siegel, P.M.; Massagué, J. E2F4/5 and p107 as Smad cofactors linking the TGFbeta receptor to c-myc repression. *Cell* **2002**, *110*, 19–32. [[CrossRef](#)]
230. Warner, B.J.; Blain, S.W.; Seoane, J. Myc downregulation by transforming growth factor beta required for activation of the p15(Ink4b) G(1) arrest pathway. *Mol. Cell Biol.* **1999**, *19*, 5913–5922. [[CrossRef](#)]
231. Nateri, A.S.; Spencer-Dene, B.; Behrens, A. Interaction of phosphorylated c-Jun with TCF4 regulates intestinal cancer development. *Nature* **2005**, *437*, 281–285. [[CrossRef](#)]
232. Albanese, C.; Johnson, J.; Watanabe, G. Transforming p21ras mutants and c-Ets-2 activate the cyclin D1 promoter through distinguishable regions. *J. Biol. Chem.* **1995**, *270*, 23589–23597. [[CrossRef](#)]
233. Bakiri, L.; Lallemand, D.; Bossy-Wetzel, E.; Yaniv, M. Cell cycle-dependent variations in c-Jun and JunB phosphorylation: A role in the control of cyclin D1 expression. *EMBO J.* **2000**, *19*, 2056–2068. [[CrossRef](#)] [[PubMed](#)]
234. Schreiber, M.; Kolbus, A.; Piu, F.; Szabowski, A.; Möhle-Steinlein, U.; Tian, J.; Karin, M.; Angel, P.; Wagner, E.F. Control of cell cycle progression by c-Jun is p53 dependent. *Genes Dev.* **1999**, *13*, 607–619. [[CrossRef](#)] [[PubMed](#)]
235. Shaulian, E.; Karin, M. AP-1 in cell proliferation and survival. *Oncogene* **2001**, *20*, 2390–2400. [[CrossRef](#)] [[PubMed](#)]
236. Sansom, O.J.; Reed, K.R.; van de Wetering, M. Cyclin D1 is not an immediate target of beta-catenin following Apc loss in the intestine. *J. Biol. Chem.* **2005**, *280*, 28463–28467. [[CrossRef](#)]
237. Kretzschmar, M.; Doody, J.; Massagué, J. Opposing BMP and EGF signalling pathways converge on the TGF-beta family mediator Smad1. *Nature* **1997**, *389*, 618–622. [[CrossRef](#)]
238. Kretzschmar, M.; Doody, J.; Timokhina, I.; Massague, J. A mechanism of repression of TGFbeta / Smad signaling by oncogenic Ras. *Genes Dev.* **1999**, *13*, 804–816. [[CrossRef](#)]
239. Hermeking, H.; Rago, C.; Schuhmacher, M.; Li, Q.; Barrett, J.F.; Obaya-Gonzalez, A.J.; O'Connell, B.C.; Mateyak, M.; Tam, W.; Kohlhuber, F.; et al. Identification of CDK4 as a target of c-MYC. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 2229–2234. [[CrossRef](#)]
240. Hinds, P.W.; Mittnacht, S.; Dulic, V.; Arnold, A.; Reed, S.I.; Weinberg, R.A. Regulation of retinoblastoma protein functions by ectopic expression of human cyclins. *Cell* **1992**, *70*, 993–1006. [[CrossRef](#)]
241. Harbour, J.W.; Dean, D.C. The Rb/E2F pathway: Expanding roles and emerging paradigms. *Genes Dev.* **2000**, *14*, 2393–2409. [[CrossRef](#)] [[PubMed](#)]
242. Leung, J.Y.; Ehmann, G.L.; Giangrande, P.H.; Nevins, J.R. A role for Myc in facilitating transcription activation by E2F1. *Oncogene* **2008**, *27*, 4172–4179. [[CrossRef](#)] [[PubMed](#)]
243. Ohtani, K.; DeGregori, J.; Nevins, J.R. Regulation of the cyclin E gene by transcription factor E2F1. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 12146–12150. [[CrossRef](#)]
244. Bartek, J.; Bartkova, J.; Lukas, J. The retinoblastoma protein pathway and the restriction point. *Curr. Opin. Cell Biol.* **1996**, *8*, 805–814. [[CrossRef](#)]

245. Reddy, B.S.; Narisawa, T.; Wright, P.; Vukusich, D.; Weisburger, J.H.; Wynder, E.L. Colon carcinogenesis with azoxymethane and dimethylhydrazine in germ-free rats. *Cancer Res.* **1975**, *35*, 287–290. [[PubMed](#)]
246. Li, L.; Li, X.; Sun, Y.; Jiang, K.; Wang, B.; Cao, H. 626-Gut Microbiota from Colorectal Cancer Patients Enhances the Progression of Intestinal Adenoma in Apc Min/+Mice. *Gastroenterology* **2019**, *156*, 301–315. [[CrossRef](#)]
247. Wu, S.; Rhee, K.-J.; Albesiano, E.; Rabizadeh, S.; Wu, X.; Yen, H.-R.; Huso, D.L.; Brancati, F.L.; Wick, E.; McAllister, F.; et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. *Nat. Med.* **2009**, *15*, 1016–1022. [[CrossRef](#)]
248. Bezerra, L.B.; Faria, F.B.; da Graça, A.N. Clostridium difficile toxin A attenuates Wnt/ β -catenin signaling in intestinal epithelial cells. *Infect Immun.* **2014**, *82*, 2680–2687. [[CrossRef](#)]
249. Tao, L.; Zhang, J.; Meraner, P.; Tovaglieri, A.; Wu, X.; Gerhard, R.; Zhang, X.; Stallcup, W.B.; Miao, J.; He, X.Z.X.; et al. Frizzled proteins are colonic epithelial receptors for C. difficile toxin B. *Nature* **2016**, *538*, 350–355. [[CrossRef](#)] [[PubMed](#)]
250. Derwa, Y.; Gracie, D.J.; Hamlin, P.J.; Ford, A.C. Systematic review with meta-analysis: The efficacy of probiotics in inflammatory bowel disease. *Aliment. Pharmacol. Ther.* **2017**, *46*, 389–400. [[CrossRef](#)]