

## Review article

# The interplay between intrinsic and extrinsic Wnt signaling in controlling intestinal transformation



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## ABSTRACT

The intestinal epithelial layer is the fastest renewing tissue in the human body. Due to its incredible turnover rate, the intestine is especially prone to develop cancer, in particular in the colon. Colorectal cancer (CRC) development is characterized by the stepwise accumulation of mutations over time, of which mutations in the tumor suppressor *APC* are often very early to occur. Generally, mutations in this gene lead to truncated *APC* proteins that cannot bind to  $\beta$ -catenin to promote its degradation, resulting in a constant overstimulation of the Wnt pathway. The level of intrinsic Wnt activation is dependent on the number of functional  $\beta$ -catenin binding sites remaining within the *APC* proteins, and the right amount of Wnt signaling is rate-limiting in the formation of polyps. In addition, the intestinal niche provides an extensive spectrum of Wnt ligands, amplifiers and antagonists that locally regulate basal Wnt levels and consequently influence polyp formation propensity. Here we will discuss the crosstalk between transforming epithelial cells and their regional niche in the development of intestinal cancer.

## 1. Introduction

The epithelial monolayer of the gastrointestinal (GI) tract is one of the fastest regenerating tissues of the human body, replacing the entire intestinal lining every week. This replacement is initiated by asymmetric division of intestinal stem cells (ISCs) residing at the bottom of crypt-like invaginations. The ISCs give rise to a pool of highly proliferative progenitors that can differentiate into all intestinal lineages including enterocytes, goblet cells, Paneth cells and neuroendocrine cells. While undergoing differentiation, cells migrate from the crypt bottom towards the lumen where they will eventually shed at the tip of the villi (Barker et al., 2007; Vermeulen and Snippert, 2014). In contrast, Paneth cells, which exert a supporting role for the ISCs, descent back into the crypt bottom where they contribute to the stem cell niche (Sato et al., 2011).

In addition to Paneth cells, the ISC niche comprises several non-epithelial cell types such as fibroblasts, pericytes, endothelial cells, smooth muscle cells, nerve cells and immune cells which all secrete various growth factors and matrix components impacting ISCs (Meran et al., 2017). To efficiently regulate the ISC niche and maintain a functioning crypt/villus axis, several pathways involved in self-renewal, proliferation and differentiation are tightly regulated. The master regulator of the ISC niche is the Wnt pathway, an evolutionary conserved pathway essential during embryogenesis where it aids cell fate determination, cell polarity and organogenesis (Nusse and Clevers, 2017). In intestinal homeostasis, it is responsible for the maintenance of

the stem cell pool via Wnt/ $\beta$ -catenin dependent canonical signaling. Other key signaling pathways that regulate ISC fate and differentiation include the Notch and Hedgehog pathways but these are beyond the scope of this review.

Wnt ligands are ~40 kDa glycoproteins that are rich in cysteines. During synthesis, Wnt ligands are modified in the endoplasmic reticulum (ER) by addition of a palmitoleic acid chain by porcupine (PORCN), which prepares the Wnt ligands for secretion (Willert et al., 2003; Takada et al., 2006). The lipid modification is recognized by Wntless (WLS) that transports the Wnts to the membrane where they are secreted (Bänziger et al., 2006). Although it remains elusive how the Wnts are transferred to target cells, it has been suggested that they are partially secreted in vesicles and function as short-distance morphogens that can activate Wnt signaling in distant cells by binding a receptor complex of Frizzled (Fzd) and LRP5/6 (Korkut et al., 2009; Gross et al., 2012). In the absence of Wnt ligands, a multi-protein destruction complex containing Axin, adenomatosis polyposis coli (APC), protein phosphatase 2A (PP2a), glycogen synthase kinase 3 (GSK3 $\beta$ ) and casein kinase 1 $\alpha$  (CK1 $\alpha$ ) is active in the cytoplasm and is responsible for degradation of  $\beta$ -catenin. Phosphorylation of  $\beta$ -catenin by the destruction complex targets the protein for ubiquitination and proteolytic degradation by the proteasome (Stamos and Weis, 2013). In the presence of Wnt ligands, the Fzds and LRP5/6 receptor complex dimerizes and sequesters the destruction complex to the membrane, thereby inhibiting its function resulting in the translocation of  $\beta$ -catenin from the cytoplasm to the nucleus and transcription of stem cell and

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proliferative genes via binding to the TCF/LEF family of transcription factors.

In the intestine, the Wnt ligands form a gradient of which the highest concentration is present at the crypt bottom to maintain the stem cell niche. The Wnt ligands are provided by Paneth cells and the stroma (Sato et al., 2011; Gregorieff et al., 2005). The Wnt gradient is counteracted by BMP ligands that are highly expressed near the lumen and are responsible for regulating growth, differentiation and apoptosis. The ISC niche prevents BMP signaling in the crypt bottom by expression of BMP antagonists such as Noggin, Gremlin1 and Gremlin2 (Kosinski et al., 2007; Reynolds et al., 2014). The balance between the Wnt active, stem cell rich crypt bottoms and the BMP high, differentiated cells near the lumen is delicate, and slight alterations can lead to severe malformation of the intestinal tissue structure and eventually result in disease. Loss of Wnt signaling or increase in BMP signaling results in terminal differentiation of stem cells and subsequent loss of crypt/villus organization (Fevr et al., 2007; van Es et al., 2012). Conversely, an increase in Wnt signaling or loss of BMP signaling leads to multiplication of the stem cell pool and excessive proliferation, a phenomenon that often proclaims transformation of healthy crypts into pre-cancerous polyps (Batts et al., 2006; Haramis et al., 2004; Krausova and Korinek, 2014).

Overactivation of the Wnt pathway and the formation of adenomas is often the first step in colorectal cancer (CRC) development. As 90% of all CRCs show acquired mutations in the tumor suppressor and Wnt regulator *APC*, and this mutations is also most frequently detected in early precursor lesions (adenomatous polyps) this mutation is often referred to as the ‘gatekeeper’ of CRC (Fearon and Vogelstein, 1990; Fodde, 2002; Powell et al., 1992). Since the development of CRC usually takes years, it is assumed that the mutations are acquired by the long-lived ISCs residing in the crypt bottoms (Merlos-Suárez et al., 2011). Indeed, targeting *Apc* mutations towards differentiated cells or ISCs in a mouse model, only resulted in effective adenoma formation in the latter case (Barker et al., 2009). These mutant stem cells disturb the neutral homeostasis within a crypt (Lopez-Garcia et al., 2010; Snippert et al., 2010), and gradually replace all healthy stem cell with mutant ones thereby fixating the mutation within the crypt and priming the crypt for transformation (Vermeulen et al., 2013; Snippert et al., 2014). Patients with familial adenomatous polyposis (FAP) have a germline mutation in the *APC* gene and develop hundreds to thousands of polyps at young age since they only need to acquire a second *APC* mutation (Ficari et al., 2000). Similar to FAP patients, conditional loss of *Apc* in an *in vivo* mouse model leads to growth of multiple polyps throughout the intestine (Young et al., 2013). However, restoration of the *Apc* gene function results in rapid regression of the polyps and the reoccurrence of healthy intestinal architecture, thereby not only corroborating *Apc*'s gatekeeper function but also suggesting that transformation is a dynamic process that can only be maintained at an optimal level of Wnt signaling (Dow et al., 2015). In this review we will discuss how Wnt signaling is regulated both intrinsically and extrinsically during malignant transformation.

## 2. Intrinsic regulation of Wnt signaling during transformation

The human *APC* gene spans 58 kb and contains 15 exons that upon translation form a 310 kDa protein (Fig. 1). The APC protein has three 15 amino acids (AA) repeats that can bind  $\beta$ -catenin and seven 20AA repeats that regulate the destruction of  $\beta$ -catenin (Su et al., 1993). In addition, APC has three SAMP repeats (comprising the sequence serine-alanine-methionine-proline) that bind Conductin or Axin and ensure optimal regulation of  $\beta$ -catenin (Behrens et al., 1998). The majority of mutations in the *APC* gene occur between codons 1286 and 1513, which is dubbed the mutation cluster region (MCR) (Kohler et al., 2008). Mutations in the MCR result in the synthesis of truncated proteins that have lost their C-terminal tail including all SAMP repeats and leaves a variable number of 20AA repeats intact. The amount of

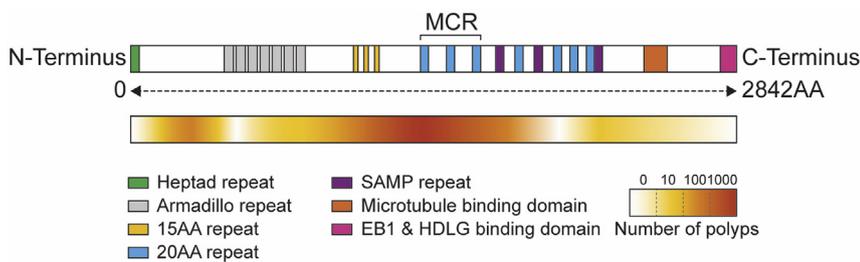
remaining 20AA repeats is inversely correlated to the amount of stabilized  $\beta$ -catenin that can translocate to the nucleus and activate Wnt responsive genes (Kwong and Dove, 2009).

As *APC* functions as a tumor suppressor, two individual mutational events must occur in order to constitutively activate the Wnt pathway at the right level. Unexpectedly, the acquisition of the second hit does not occur randomly as suggested by the classical Knudson's two-hit hypothesis for inactivation of a tumor suppressor gene, but rather selects for the mutation that provides the most favorable level of Wnt signaling by either having more or less 20AA repeats than the first hit. This was shown in a population of familial adenomatous polyposis (FAP) patients where the combination of the initial germline *APC* mutations and the spectrum of second somatic *APC* mutations in multiple polyps was studied (Albuquerque et al., 2002; Crabtree et al., 2003). Two theories have emerged concerning this phenomenon; the ‘just right’ and ‘loose fit’ models. Albuquerque et al., studied multiple polyps from the same FAP patients and discovered that whenever the germline mutation results in proteins that have lost all seven 20AA repeats, the second mutations will be selected for having favorably one functional 20AA repeat, and vice versa, suggesting that retaining some ‘just right’  $\beta$ -catenin binding activity is required for effective transformation and excessive Wnt might interfere with polyp development (Albuquerque et al., 2002). In agreement with this, Crabtree et al., also detect the association between the first and second mutational event, however did find striking differences in disease severity between different combinations of mutations and even discovered polyps with two functional 20AAs are more common than one 20AA. Rather than suggesting an ultimate optimum Wnt level, they describe this non-random selection as a ‘loose fit’ of Wnt signaling (Crabtree et al., 2003). Either way, both models agree that ‘too much’ or ‘too little’ Wnt signaling are rate-limiting steps in formation of polyps. Furthermore, another group of FAP patients has been described that exert a milder course of disease, where 10–100 polyps can be detected that arise at a later time of onset. This population, termed attenuated familial adenomatous polyposis (AFAP), has mutations in either the first five exons of the *APC* gene, in exon 9 or in the distal 3' end of the gene (Knudsen et al., 2003). In case of AFAP, germline mutations either retained or lost all 20AA repeats, further supporting the hypothesis of a certain Wnt threshold for polyp formation.

Although transformation is principally the result of intrinsic Wnt activation by truncating *APC* mutations, the disruption of crypt architecture and the formation of polyps is also dependent on the location within the intestine (Leedham et al., 2013). FAP patients who develop multiple polyps, grow polyps at preferred locations instead of all over the intestinal lining, suggesting that communication with the local niche is of essential importance in transformation towards a polyp (Will et al., 2010). In addition, regional differences in the niche cell types and functions also explain why GI cancers often develop in the stomach and the large intestine, but hardly in the small intestine (Lamprecht and Fich, 2016). Next, we will discuss how extrinsic niche factors can influence transformation of healthy intestinal tissue into pre-malignant polyps.

## 3. Extrinsic regulation of Wnt signaling during transformation

The intestine can be divided into several compartments such as the duodenum, jejunum and ileum that comprise the small intestine, the caecum, the left and right colon, and the rectum, all with their own biological functions. The small intestine is mainly involved in the absorption of vitamins and nutrients, whilst the colon exchange of water and salts across its membrane. Due to the diversity in functions, the architecture of the epithelial layer varies between the intestinal compartments. Consequently the basal Wnt signaling levels vary between the regions. In humans, basal Wnt levels are higher in the small intestine than in the colon, with highest levels detected in the ileum (Leedham et al., 2013).



therefore dubbed the mutation cluster region (MCR) (Kohler et al., 2008). Heritable mutations within the MCR will lead to the most severe FAP phenotype, where up to thousands of polyps may arise within the intestine. However, mutations in other regions have also been described to facilitate the development of several polyps. In this case, we speak of attenuated familial adenomatous polyposis (AFAP), where 10–100 polyps can arise with a delayed time of onset (Knudsen et al., 2003).

It is hypothesized that formation of polyps occurs under perfectly balanced Wnt conditions, where Wnt signaling is increased and reached a certain polyp initiating threshold, but is not excessively activated (for an excellent comprehensive review see Albuquerque et al. (2011)). Considering the different basal levels of Wnt in each GI compartment, a certain truncated mutation in the APC gene may provide the right amount of Wnt signaling in one compartment, whilst excessively overstimulating the Wnt pathway in another. For example, mutations in the APC gene that retain a few  $\beta$ -catenin 20AA repeats and thus modestly elevate intrinsic Wnt levels are favorably forming polyps in the small intestine where higher basal Wnt levels are reported. Mutations that leave no  $\beta$ -catenin 20AA repeats result in a highly activate Wnt pathway, and cross a certain barrier where excessive Wnt leads to apoptosis within the crypts instead of polyp formation. Therefore, polyps that have high Wnt activity are mostly found in the colon and rectum, where the basal levels of Wnt are lower than the small intestine (Jaeger et al., 2012).

### 3.1. Wnt ligands

The differences in basal Wnt levels can in part be explained by the absence of WNT3-producing Paneth cells in the colon. However, it has become clear that Paneth cells are not the only Wnt producing cells in the ISC niche, as ablation of Paneth cells or Wnt3 by conditional knockout of *Math1* or *Wnt3* respectively, did not alter crypt architecture, whilst in an *in vitro* organoid setting a loss of stem cells can be observed (Durand et al., 2012; Farin et al., 2012). In addition, inhibition of secretion of Wnt ligands in epithelial cells by conditional inactivation of *Porcn1* does not influence intestinal homeostasis (Kabiri et al., 2014). This suggests that, at least *in vivo*, other cells can be a source of Wnt that maintain the ISCs niche. Study of the expression patterns in the adult intestine show that epithelial cells provide a source of Wnt3a, Wnt6 and Wnt9b, whereas non-epithelial cells secrete Wnt2b, Wnt4, Wnt5a and Wnt5b (Gregorieff et al., 2005). It is important to realize that the binding of each of the nineteen Wnt ligands to one of the ten Frizzled (Fzd) receptors can drastically influence the choice of either canonical or noncanonical Wnt signaling pathways in the mammalian body (Dijksterhuis et al., 2014). Specifically, Fzd7 upregulation has been associated with cancers in various tissues (Vincan and Barker, 2008). In the intestine, Fzd7 is expressed in stem cell populations and conditional loss or inhibition of Fzd7 leads to depletion of the stem cell pool (Flanagan et al., 2015; Nile et al., 2018). Besides the secretion of Wnt ligands by the niche and their subsequent binding to Fzd receptors, also other molecules are found to locally influence Wnt signaling and aid transformation, which will be discussed below.

### 3.2. Wnt amplifiers

R-spondins (RSPO1-RSPO4) are secreted polypeptides that sensitize cells to Wnt ligands. R-spondins have two furin domains that bind to cell membrane proteins Lgr4-5-6 and to ubiquitin ligases RNF43 and ZNRF3 (de Lau et al., 2012). Binding of R-spondins to the ubiquitin

ligases prevents degradation of Wnt receptors Frizzled and LRP6, and results in an increase in Wnt signaling due to increased availability of Wnt receptors (Wang et al., 2013; Hao et al., 2012). It has long been assumed that RSPO-LGR binding was necessary for inhibition of the ubiquitin ligases, however, a recent study by Szenker-Ravi et al. suggests that RSPO2 can also directly inhibit RNF43 and ZNRF3 in cells containing a triple knockout of Lgr4-5-6 (Szenker-Ravi et al., 2018). Irrespective of the signaling cascade, all four R-spondins have been shown to activate the Wnt pathway (Kim et al., 2006), where RSPO2-3 are considered to be the most potent activators, followed by RSPO1<sup>56</sup>. RSPO1 is produced by the intestinal epithelium itself, although at relatively low levels, whereas RSPO2-3 are produced by mesenchymal cells residing in the local microenvironment (Greicius et al., 2018). Administration of RSPO1 has been reported to cause hyperplasia (Kim et al., 2005), however, considering the low binding affinity of RSPO1 to Lgr5 it is not the most potent candidate for initiation of tumorigenesis (Kim et al., 2008; Warner et al., 2015). In contrast, RSPO2/3 bind Lgr5 with more affinity and overexpression of these two have been shown to drive the development of several cancers including CRC (Warner et al., 2015; Rudin et al., 2012; Hilken et al., 2017; Seshagiri et al., 2012).

Differences in Wnt amplification potency between RSPO1 and RSPO2/3 become clear when culturing mouse intestinal organoid *ex vivo*, in the absence of a local microenvironment. Firstly, crypt cultures cannot be sustained in the absence of additional administered recombinant Rspodin1, suggesting that the epithelial produced RSPO1 is insufficient to maintain the required high Wnt levels in the ISCs (Ootani et al., 2009). In addition, a recent study shows that intestinal sub-epithelial myofibroblasts (ISEMFs) provide a source of Rspo3 and allow intestinal organoids to grow in the absence of Rspo1 when co-cultured with ISEMFs (Kabiri et al., 2014; Greicius et al., 2018), thereby emphasizing the importance of microenvironmental regulation of the ISC niche.

BMP antagonists Gremlin1, Gremlin2 and Noggin can drastically influence the ISC niche. CD34<sup>+</sup> mesenchymal cells within the ISC niche have recently been reported to provide a source of Gremlin1, Wnt2b and Rspodin (Stzepourginski et al., 2017). Furthermore, Foxl1 + mesenchymal cells provided a source of Wnt2b, Wnt5a, Rspo3, Gremlin1 and Gremlin2. Aberrant expression of Gremlin1 leads to hyperproliferation and transformation of epithelial cells outside of the ISC niche (Davis et al., 2015).

### 3.3. Wnt antagonists

As mentioned, overstimulation of the Wnt pathway can lead to hyperproliferation and transformation. To keep levels of Wnt signaling in check, a spectrum of Wnt antagonists are secreted. Wnt inhibiting factor 1 (WIF1) is a secreted antagonist that directly binds to Wnt ligands and prevents binding to their receptors. In addition, Dickkopf proteins DKK1-4 prevent Wnt signaling by isolating the Wnt co-receptors LRP5 and 6 (Niehrs, 2006). WIF1 and DKK genes are often epigenetically silenced in many cancers leading to enhanced Wnt signaling (Sato et al., 2007; Delmas et al., 2011). Adenoviral expression of

Dkk1 leads to dramatic loss of ISCs and disruption of the structural organization of the intestine (Kuhnert et al., 2004). Another class of antagonists are Secreted Frizzled related proteins SFRP1-5 that directly bind Wnt ligands and neutralize them (Bovolenta et al., 2008). *Sfrp2* expression was more than 130-fold more present in colonic crypts than in the small intestinal crypts of the mouse intestine, which provides an additional explanation why the basal Wnt gradient within the colon is lower (Leedham et al., 2013). Furthermore, Notum is a secreted hydrolase enzyme that can acetylate Wnt ligands resulting in their inactivation, thus functioning as a further negative feedback regulator of the Wnt pathway (Kakugawa et al., 2015).

Recently, *Sfrp1* secretion by fibroblasts has been reported to be implicated in influencing transformation. A distinct group of sub-epithelial fibroblasts express *Foxf1* and *Foxf2* and provide the connection between epithelial and mesenchymal cells in the gut during embryonic development (Ormestad, 2006; Wang et al., 2003; Stankiewicz et al., 2009). Loss or decrease in expression of these genes results in decreased Wnt signaling and serious malformations of the gut leading to perinatal death in both mouse and human. Recent work has pointed out that *Foxf1* and *Foxf2* expressing fibroblasts also regulate stem cells in the adult mouse intestine by influencing *Lgr5*<sup>+</sup> stem cell numbers via secretion of Wnt antagonist *Sfrp1*<sup>75</sup>. In addition, loss of *Foxf* proteins leads to an increase of adenomas in a *Foxf1/2*<sup>-/+</sup> *Apc*<sup>min/+</sup> mutant mice (Nik et al., 2013). Recently, another group of myofibroblast has also been described to influence ISC number by secretion of angiopoietin-like protein 2 (ANGPTL2) (Horiguchi et al., 2017). Loss of *Angptl2* is associated with a decrease in  $\beta$ -catenin signaling and an increase in *Bmp2* and *Bmp7* transcription resulting in impaired regeneration after injury in *Angptl2*<sup>-/-</sup> mice. This suggests that increase in BMP signaling by the niche also has an antagonistic effect on Wnt signaling (Qi et al., 2017).

As mentioned, there are many cells residing in the ISC niche that can influence the level of Wnt signaling in the crypt. Ultimately, the sum of all these factors determines the stem cell number and renewal rate in each crypt. Nevertheless, it remains hard to study the exact contributions of the 19 mammalian Wnt ligands, R-spondins, Wnt antagonists and Fzd receptors, as redundancy is a common phenomenon. However, a recent paper has shown that Wnt ligands and R-spondin1 have some non-interchangeable effects in ISC regeneration, and that both should be present to ensure proper ISC function (Yan et al., 2017a). In this study, only a slight reduction in the binding domains of R-spondin1 leads to a reduced number of ISCs. Although normal tissue homeostasis remains unaffected, the number of ISCs in each crypt is of importance in transformation as it partially influences the time it takes for a mutation to reach fixation in the crypt (Vermeulen et al., 2013). This suggests *Apc* mutations in crypts with fewer ISCs are prone to fixation, as fewer healthy stem cells have to be replaced. Indeed, in a recent elegant study by Huels and colleagues, reduction of Wnt secretion by Porcupine inhibitors resulted in more rapid fixation of the mutation in the crypt and as a result more efficient polyp formation *in vivo* (Huels et al., 2018).

In conclusion, transformation of healthy intestinal tissue into pre-malignant polyps is a process involving many intrinsic and extrinsic factors that modify Wnt signaling activity (Fig. 2). The amount of intrinsic Wnt activation relies on the location of mutations within the *APC* gene and the subsequent amount of  $\beta$ -catenin binding sites left on the truncated APC proteins. Whenever an ISC has acquired two *APC* mutations, it might outcompete the healthy ISCs present in the crypt and fixates the mutation within this crypt. The number of healthy ISCs that have to be replaced before fixation can be reached is heavily dependent on extrinsic factors from the ISC niche. The ISC niche provides the ISCs with various Wnt influencing factors that together constitute a basal Wnt level that varies across the different regions of the GI tract. The combination of basal Wnt levels in the niche and intrinsic Wnt activation in ISCs that determines whether a certain Wnt threshold is reached to initiate polyp formation. Thus, a delicate cross-talk between niche

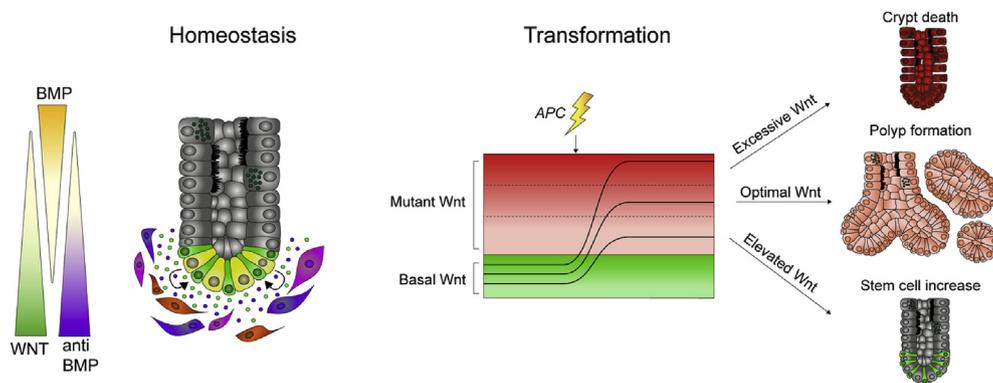
cells and ISCs is required to maintain homeostasis and prevent CRC formation.

#### 4. Outlook

As described in this review, various cell types in the ISC niche provide different Wnt ligands, amplifiers and antagonists, to support and regulate stem cell renewal and prevent depletion of the ISC compartment. However, the secretion of these factors is not as straightforward as it may seem, as many different cell populations can provide similar growth factors upon different stimuli. This sparks the debate about which cell types are redundant in the ISCs niche, and which are essential (San Roman et al., 2014; Valenta et al., 2016; Degirmenci et al., 2018). For example, San Roman et al. shows that conditional knockout of porcupine in epithelial cells, in *Myh11* + myofibroblasts, or in both compartments simultaneously did not alter crypt architecture. In contrast, it was observed that inducible knockout of *Wingless* (*Wls*), a protein required for the secretion of Wnt ligands, in the whole organism results in ISC loss and crypt destruction, followed by death 10 days after induction (Valenta et al., 2016). Supporting this, Degirmenci et al. demonstrate that *GLI1*-expressing mesenchymal cells, produce Wnt ligands and are indispensable in the mouse colon. Inhibiting expression of *Wls* in *GLI1*-positive mesenchymal cells resulted in rapid crypt loss in the colon, but not in the small intestine. However, conditional knockout of *Wls* in both *GLI1* + mesenchymal cells and epithelial cells did result in loss of ISCs in the small intestine, emphasizing the variation in Wnt dependency in the local niche (Degirmenci et al., 2018). Of note, there are probably a lot of rare cell types that remain to be discovered within the adult gut that can also influence local Wnt levels. Recent advances in single cell RNA sequencing techniques enable the discovery of new populations within the intestinal crypt and these techniques can be used to identify novel niche-supporting cell types (Grün et al., 2015; Herring et al., 2018).

In addition, cell plasticity can be observed in both niche cell types as well as the epithelial compartment, further intruding the complex between ISCs and their niche. For example, upon inflammation, sub-populations of mesenchymal cells secrete prostaglandins that inhibit *GSK3 $\beta$*  in epithelial cells while others secrete *RSPO1*, thereby activating the Wnt pathway (Stzepourginski et al., 2017; Castellone et al., 2005). Furthermore, circulating bone marrow derived monocytes can rescue intestinal homeostasis after radiation induced tissue damage by secreting Wnt ligands (Tata et al., 2013). It has also been suggested that the composition of the gut microbiome can influence Wnt levels, and mice lacking commensal bacteria have decreased expression of *Wnt5a* and *Wnt11* (Neumann et al., 2014). It has also become increasingly clear that intestinal cell types are not a static entity and that bidirectional interconversion between differentiated epithelial cells and ISCs may occur (van Neerven et al., 2016). Upon tissue damage, differentiated cell types such as enterocytes, enteroendocrine cells and secretory precursors can dedifferentiate into stem-like cells alter the niche from a Wnt deprived, ISC low environment into a Wnt high, ISC promoting environment, or vice versa (Tetteh et al., 2016; Yan et al., 2017b; Jadhav et al., 2017).

The balance between the Wnt-high ISCs and the Wnt-low differentiated cells is extremely sensitive, which makes the GI tract exceptionally prone to transform and develop polyps with hyperactivated Wnt signaling. This transformation is again a combination of intrinsic mutations and extrinsic regulation of the Wnt signaling pathway by the niche. Mutations in the *APC* gene that lead to truncated APC proteins are the most common mutations, however, mutations in other Wnt pathway members such as  $\beta$ -catenin, *RSPO2-3*, *MUTYH* and *BMPRI1A* (Howe et al., 1998, 2001; Segditsas and Tomlinson, 2006) have also been reported. As mutations that affect the Wnt pathway have been reported in 90% of all CRCs, which sparks the debate whether therapeutic strategies should be designed to target the Wnt pathway. Even though transformation is the result of aberrant Wnt signaling, targeting



**Fig. 2. Sources of extrinsic driven Wnt signaling in the intestine.** During homeostasis, several cell types provide Wnt ligands and other factors such as Wnt and BMP agonists/antagonists. The epithelial Paneth cells that reside in the crypt near the ISCs produce Wnt3a, Wnt6 and Wnt9b, whereas cells in the intestines' micro-environment secrete Wnt2b, Wnt4, Wnt5a and Wnt5b. Together, all these cells determine the basal Wnt level of the tissue. This basal Wnt level is essential in aiding transformation into pre-malignant polyps, as the sum of the basal Wnt level and the amount of residual APC protein function determine the resulting level of Wnt sig-

naling. For the development of polyps, a precise amount of Wnt activation should be present. Too much Wnt signaling results in crypt death (red), whilst sometimes a mutation does not induce enough Wnt signaling to pass a certain “polyp initiation threshold”. In this case, an increase in healthy stem cells can be observed (green) (Leedham et al., 2013).

of the Wnt pathway remains a challenge due to the requirement of Wnt in normal tissue maintenance (Anastas and Moon, 2012).

In this review, we have described how the local niche can influence transformation of healthy epithelium. However, it is important to realize that transformed epithelial cells in their turn can again influence their niche. In CRCs, the transformation of fibroblasts into cancer associated fibroblasts (CAFs) by the cancer cells has already been thoroughly described (Madar et al., 2013; Tao et al., 2017; Kalluri and Zeisberg, 2006). These CAFs subsequently provide a favorable niche for the cancer cells and can stimulate growth, metastasis and protect against therapies (Lenos et al., 2018; Lotti et al., 2013; Junttila and de Sauvage, 2013; Karagiannis et al., 2012). Recently, Maywald et al. described a similar mechanism of niche stimulation by adenomatous polyps by secretion of IL-33 (Maywald et al., 2015). In both mouse and human adenomas and carcinomas an increase in IL-33 expression has been observed, which stimulates secretion of growth factors by IL-33 receptor (IL1RL1) carrying fibroblast, which in their turn secrete factors such as to stimulate their new cancerous niche. Blocking of IL-33 signaling lead to a significant decrease in polyp number and size in *Apc*<sup>min/+</sup> mice, suggesting that the interaction between the intestinal epithelium and its stroma is indeed bidirectional.

As polyps are considered to be the first step in the development of CRC, it is essential to design (chemo)preventive strategies that prevent polyp formation, especially for individuals that grow multiple polyps at early age, e.g. FAP patients. Since polyp formation is characterized by mutation in the *APC* gene leading to overactivation of the Wnt pathway, and restoration of *Apc* function in mice reverses polyposis inhibition of Wnt might seem a logical therapeutic approach (Dow et al., 2015). Currently, many Wnt influencing drugs such as Porcupine inhibitors,  $\beta$ -catenin/TCF antagonists, Tankyrase inhibitors and Fzd blockers are being evaluated. Nevertheless, despite substantial effort, none of the current strategies provides sensitivity to CRC without influencing normal homeostasis. Therefore, drugs that do not directly influence Wnt signaling are also being evaluated, such as nonsteroidal anti-inflammatory drugs (NSAIDs). NSAIDs are inhibitors of the cyclooxygenase enzymes COX1 and COX2 that are responsible for the conversion of arachidonic acid into prostaglandins. Elevated levels of prostaglandin PGE<sub>2</sub> have been observed in polyps and CRC and are suggested to promote adenoma development (Castellone et al., 2005; Pugh and Thomas, 1994; Sonoshita et al., 2001). Indeed, deletion of *Cox2* or prostaglandin receptors results in a decrease in polyp formation in various mouse models, and long-term use of NSAIDs is associated with a lower risk in CRC development (Oshima et al., 1996; Chulada et al., 2000). Another therapeutic strategy might be to prevent fixation of the *APC* mutation within the crypt by reducing the competition advantage of mutant cells (Vermeulen et al., 2013; Snippert et al., 2014). Although the mechanisms of this biased competition have not

yet been unraveled, diminishing the advantage of *APC* mutant cells might lead to increased loss of mutant ISCs, thereby reducing cancer incidence.

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