CRITICAL COMMENTARY

Morphogen regulation of stem cell plasticity in intestinal regeneration and carcinogenesis

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Abstract
The intestinal epithelium is a tissue with high cell turnover, supported by adult intestinal stem cells. Intestinal homeostasis is underpinned by crypt basal columnar stem cells, marked by expression of the LGR5 gene. However, recent research has demonstrated considerable stem cell plasticity following injury, with dedifferentiation of a range of other intestinal cell populations, induced by a permissive microenvironment in the regenerating mucosa. The regulation of this profound adaptive cell reprogramming response is the subject of current research. There is a demonstrable contribution from disruption of key homeostatic signaling pathways such as wingless-related integration site and bone morphogenetic protein, and an emerging signaling hub role for the mechano-receptor transducers Yes-associated protein 1/transcriptional coactivator with PDZ-binding motif, negatively regulated by the Hippo pathway. However, a number of outstanding questions remain, including a need to understand how tissues sense damage, and how pathways intersect to mediate dynamic changes in the stem cell population. Better understanding of these pathways, associated functional redundancies, and how they may be both enhanced for recovery of inflammatory diseases, and co-opted in neoplasia development, may have significant clinical implications, and could lead to development of more targeted molecular therapies which target individual stem or stem-like cell populations.

KEYWORDS
colorectal cancer, intestinal plasticity, intestinal regeneration, intestinal stem cell

1 | INTRODUCTION

The intestinal mucosa is an attractive tissue for research into the fundamentals of epithelial cell fate, as it has discrete and stereotypical architecture. The epithelium is a single layer of columnar cells organized into flask shaped invaginations called crypts, with multiple crypts contributing streams of cells to finger-like projections called villi in the small intestine.1 Rapid epithelial cell turnover is supported by intestinal stem cells, located in a protected niche at the bottom of the crypt.1 Intestinal epithelial cell-fate determination is controlled by a balance of secreted signaling pathways as cells move along the intestinal crypt-villus axis. There is additional key contribution from numerous juxtracrine signaling pathways, in particular Notch signaling, which is key for adjacent cell-fate determination via lateral inhibition.2 Differential intercompartmental expression of ligands, receptors, and
antagonists generate tightly regulated mucosal gradients, and the phenotypic response of a cell is determined by its position within these concentration gradients.\(^1\)

The intestinal epithelium is the first line of defense in a hostile environment, and is subject to frequent chemical, microbiological, and immunological mediated mucosal damage. The same signaling pathways that maintain epithelial homeostasis are involved in the physiological response to damage and underpin the regenerative capacity of the intestinal epithelium.\(^2\) In intestinal injury, epithelial ulceration skews homeostatic epithelial-stromal cross-talk, and barrier breach induces localized innate immune responses, temporarily dysregulating the mucosal cell-signaling balance. This transient perturbation of cell-signaling promotes epithelial stem cell expansion, cell proliferation, and migration. This is part of the physiological response to injury and is required to effect epithelial restitution. However, failure of restoration of homeostatic control in chronic inflammation, or pathological disruption of signaling pathways can result in neoplasia initiation/progression.\(^4\) It has been argued that cancer is a legacy of the evolution of tissue and cell-signaling plasticity—an infrequent, but statistically inevitable price-to-pay for the developed ability of tissues to dynamically respond to environmental pressures. The interplay between inflammation, regeneration, and cancer is established in the intestinal mucosa, with chronic inflammatory bowel disease a risk for the development of colorectal cancer.\(^5\) It has been suggested that these alternative stem cells are key for protecting and maintaining the intestinal epithelium during periods of damage. Certainly, the damage-resistant nature of these cells provides compelling evidence for this, suggesting the role of these alternative cells may be of most significance under non-homeostatic conditions.\(^8\) The signaling pathways regulating these alternative stem cells, and their role in intestinal health and disease will be a key point of discussion within this paper.

2.2 | The stem cell niche

The intestinal crypt is tightly regulated under the control of localized morphogen signaling, key for the positional determination of cell fate. The key differentiated cell fates of the intestinal crypt epithelial cells are illustrated in Figure 1.

Wnt and bone morphogenetic protein (BMP) signaling pathways have antagonistic roles on intestinal crypt cell phenotype, with Wnt signaling highest at the crypt base, promoting cell stemness, and BMP signaling in the higher levels of the crypt promoting cell differentiation.\(^9\) (Figure 2). Intercompartmental cross-talk is a key factor in maintaining this fate-determining niche, and morphogens are produced from both the epithelial cells themselves,\(^9\) and in particular the Paneth cells,\(^10,11\) and from the stromal cell compartment,\(^12\) with ablation of Wnt production from these stromal cells associated with loss of the crypt stem cell compartment.\(^13,14\)

Both the Wnt and BMP pathways are, as well as being key constituents of this imperative morphogenic gradient, also heavily implicated in our growing understanding of regulation of intestinal plasticity. In addition, there is an exciting emerging role of Yes-associated protein 1/transcriptional coactivator with PDZ-binding motif (YAP/TAZ) in cross-talk with the Wnt and BMP pathways,\(^15\) and this pathway consequently also plays a key role in regulation of the intestinal crypt, particularly in relation to the intestinal stem cell. Here, we will briefly describe each of these pathways, before discussing the potential impact of each of these three pathways on intestinal plasticity, and how these roles may translate across to both their known and emerging roles in intestinal tumorigenesis.
2.3 BMP signaling

The BMP pathway is a key developmental pathway. BMP knockout, depending on the individual BMP targeted, is either embryonically lethal or results in fatality shortly following birth.16 BMPs play many key regulatory roles, controlling bone, joint, and cartilage formation, as well as contributing to regulation of adipogenesis, cardiac septation, reproductive system development and homeostasis,16 and in the intestinal crypt, act to promote differentiation in opposition to the stem cell promoting Wnt signaling.11

BMP is a member of the transforming growth factor-β (TGF-β) superfamily of dimeric signaling molecules, which encompasses TGF-βs, nodals, and activins, in addition to the BMP subfamily.17 In humans, there are 33 ligand members of the TGF-β superfamily.17,18 The TGF-β receptor family consists of two main receptor subtypes which work synergistically, as well as a third type
III receptor which has independent function. Despite the huge number of TGF-β superfamily members, receptor diversity and specificity is surprisingly limited, and of the just seven type 1 and four type 2 TGF-βR’s identified, BMP binds to three of both the type 1 and the type 2 receptors.

Canonical BMP signaling is transduced by the Smad protein group. There are three subclasses within this group: the receptor-regulated Smads, common Smads, and the inhibitory Smads. The BMP subfamily is transduced by a complex of Smad4 with 2 out of 3 of Smads 1, 5, and 8 (Figure 3). While TGF-β/BMP signaling also has numerous noncanonical outputs, defined as signaling pathways which are not transduced by Smad proteins, these will not be of focus in this review, and we refer the interested reader to this excellent review by Zhang et al.

2.4 | Wnt signaling

Wnt is an essential developmental factor, known to be crucial for determining cell fate in embryonic development, as well as being a widely studied oncogene. β-Catenin is the key mediator of canonical Wnt signaling (Figure 4). Canonical Wnt signaling is essential for maintenance of the proliferative cell populations within the crypt, with loss of Wnt signaling, achieved via targeted β-catenin ablation, resulting in a loss of the transit amplifying cell (TAC) compartment, and a general disruption of normal crypt structure.

Wnt additionally has numerous noncanonical signaling roles, again via the Fzd and Ror1/Ror2/Ryk receptors, activating planar cell polarity, receptor tyrosine kinase, and calcium signaling cascades. Many of these cascades and their downstream targets act synergistically with those of the canonical signaling pathway, promoting stemness, and, in cases of neoplastic disease, driving malignancy and metastatic transformation. However, there is also emerging evidence for antagonism of canonical Wnt signaling via noncanonical ligands, and the exact interplay of these pathways remains to be elucidated, although noncanonical signaling will not be a focus within this text.

2.5 | Hippo/YAP/TAZ

YAP/TAZ signaling is emerging as a key integration pathway of various damage repair signals, including mechanotransduction and morphogen signaling in the intestinal tissue, and in particular undergoes considerable cross-talk with the Wnt and BMP/TGF-β pathways as part of this mediation. The process of Hippo/YAP/TAZ signal transduction is illustrated in Figure 5.

The Hippo pathway has an established role in regulating the size of organs during embryonic development, and its dysregulation is known to be implicated in malignant transformation of cells during tumor progression. In drosophilia, disruption of the Hippo signaling pathway through overexpression of Yki (the drosophilia YAP and TAZ homolog of vertebrate YAP) leads to increased organ size and transformation.

**FIGURE 3** Canonical BMP signal transduction. Binding of a BMP ligand to the BMPR (BMP receptor) heterotetramer triggers a conformational change which increases the affinity of the receptor for 2 out of 3 of Smads 1, 5, and 8. The TGF-βR1 cytoplasmic domain kinase activity phosphorylates two Smad subunits at the Sxs site, located close to the R-Smad C-terminus in the major homology region 2 (MH2). These subsequently form a heterotrimeric complex with the common mediator, Smad4, which regulates expression of a variety of target genes within the nucleus. BMP, bone morphogenetic protein; R-Smad, receptor-regulated Smad; TGF-β, transforming growth factor-β.
TAZ homolog), leads to a notable increase in cell proliferation with associated loss of apoptosis. This is also seen when Hpo (MST1 and MST2 homolog) is deleted. This aptly demonstrates the negative regulatory effect the Hippo pathway has on YAP and TAZ signaling; Hippo activation leads to a kinase cascade involving MST1/2, LATS1/2, which ultimately leads to YAP/TAZ phosphorylation, cytoplasmic retention, and degradation. In the absence of Hippo signaling (for example, following tissue damage), the loss of this signaling cascade leads to YAP/TAZ remaining unphosphorylated, and subsequently translocating to the nucleus to activate genes involved in apoptosis and proliferation, both key elements of intestinal plasticity.

Yap1 depletion during homeostasis has no observable impact in animal models, and this is highly suggestive of a constitutive role for Hippo signaling, but not for YAP/TAZ signaling during intestinal epithelial homeostasis. Hippo signaling is induced via sensing of cell density via the Crumbs complex, established during the late stage of establishment of apical-basal polarity of epithelial cells, and consequently, at homeostasis, YAP/TAZ is largely sequestered in uninjured epithelial cells, gaining a more significant signaling role following epithelial damage.

YAP/TAZ have important roles as mechanoreceptors, and YAP/TAZ signaling may be regulated by polarity/cell-adherence factors, by contact factors from neighboring cells, as well as by other stress/mechanosensing factors. Key cell adhesion proteins PTPN14 and AMOT play an important role in sequestering YAP/TAZ to tight junctions via α-catenin-mediated sequestration. This again prevents YAP/TAZ signaling in tissues with high levels of cell adhesion, and in particular, the homeostatic epithelium.
There is a role observed for YAP/TAZ signaling stimulated via the extracellular matrix (ECM) protein peristin. Peristin is produced from stromal cells, and in DSS and ApcMin mice peristin deficiency is associated with reduced rates of neoplasia postinjury. The effect of peristin appears to be mediated via FAK-Scr mediated YAP/TAZ activation, suggesting that mediation and integration of damage and remodeling signals in cells may be a key role of the YAP/TAZ pathway.

2.6 Other pathways

Additional regulation in the intestinal crypt comes from the Indian hedgehog (IHH) and Notch signaling pathways. IHH is the primary member of the hedgehog signaling protein family responsible for regulation of the intestinal crypt. IHH is secreted from epithelial cells, and is responsible for paracrine signaling to mesenchymal cells, prompting their proliferation. IHH-induced secretion of BMP is an important negative feedback mechanism, regulating epithelial cell proliferation. Notch signaling is also an important determinant of cell fate, and Notch signaling between adjacent cells via cell-cell contact means cells in close proximity will be pushed toward opposing lineages, either absorptive or secretory. Notch additionally has regulatory effects on division of the stem cell and TAC populations.

3 RESPONSE TO INTESTINAL INJURY

There are numerous models of intestinal damage, including dextran sulfate sodium (DSS)-induced colitis, physical wounding methods such as tissue excision, radiation damage, and targeted cell depletion, such as Lgr5+ depletion via use of the Lgr5+ diphtheria toxin receptor (DTR) mouse model, which allow for wide window of insight into the mechanisms that regulate tissue recovery from damage. However, the stem cell dynamics that underpin regeneration have only been investigated in the last few years.

Following the discovery of the LGR5+ CBC homeostatic stem cell population, a number of studies looked to define their role in tissue regeneration. Unexpectedly, Tian et al observed that targeted Lgr5+ cell ablation via administration of diphtheria toxin in a Lgr5-DTR mouse model does not impact crypt structure under homeostatic conditions, and the gut is capable of recovery from doses of radiation which eliminate the proliferative compartment entirely. Furthermore, in murine models of colitis, a disease characterized by chronic inflammatory intestinal damage, Lgr5+ expression was significantly reduced.

Both these observations may initially seem at odds with the presumed essential role of the LGR5+ stem cell in maintaining a tissue with the high turnover and regenerative capacity of the intestinal epithelium. Initially, it was thought that alternative stem cell populations, including a +4 BMI1 cell population, supported the crypt as a quiescent “back-up” stem cell population following LGR5+ ablation. However, there has recently been an emerging role for increased plasticity of the colonic crypt. Many studies have suggested that dedifferentiation of epithelial progenitor cells which have left the crypt base niche may be responsible for crypt repair, and for regeneration of lost stem cell populations following tissue injury. Indeed, work has shown that the hypothesized +4 BMI1 “stem” population is in fact a population of several partially differentiated cell types (including preterminal enteroendocrine and preterminal goblet cells) which undergo selective dedifferentiation in response to injury-induced loss of the LGR5+ compartment.

The characteristics of cells with stem cell plastic potential have been an area of considerable investigation. Secretory progenitor cells which express high amounts of the Notch ligand Dll1 have been demonstrated to exhibit plastic potential following tissue damage. Specifically, lineage tracing of these cells following damage showed these cells able to recapitulate the four key secretory cell populations of the intestinal epithelium. Alpi positive enterocyte cells (the major absorptive population of the epithelium) have shown similar plastic potential, able to replace the Lgr5+ population following selective diptheria toxin-mediated Lgr5+ ablation in Lgr5-DTR mice. Significant evidence is also present for the plasticity of the Paneth cell population, which, while responsible themselves for maintenance of the stem cell niche, are also capable of reversion to a stem cell phenotype under conditions of injury.

Consequently, there is strong evidence that numerous, if not all of the partially differentiated (and potentially fully differentiated) cell populations are capable of plasticity and reversion to a stem-like phenotype when tissue injury and inflammation generate a permissive signaling environment. However, there are still many unanswered questions: what initiates this reprogramming? How do cells sense damage or the loss of homeostatic stem cell populations in models of selective LGR5+ ablation? Furthermore, once damage is sensed, what epithelial cell-intrinsic or extrinsic transcriptional changes mediate this rapid adaptive change in phenotype?

One of the key observations helping to define intestinal plasticity has come from assessment of the transcriptional repertoire of these dedifferentiated cells. When the gene signatures of highly immunogenic granulomas, caused by the parasite Heligmosomoides polygyrus, were investigated, it was found that the Lgr5+ stem cell
population was again, entirely lost. Interestingly, the expression of many genes associated with the fetal intestinal epithelium were considerably upregulated. This is now known to be a key feature of the regenerating epithelium, with regenerative stem cells characterized by a fetal gene signature, including the markers Ly6a (Sca1), Anxa1, and Clu and associated suppression of the adult stem cell signature. Understanding the pathways that trigger this change from an adult to regenerative phenotype, and how the balance between the two states is sensed and maintained, will prove key for clinical utilization of this understanding.

4 | WHICH PATHWAYS CONTROL REGENERATION?

The same signaling pathways that maintain epithelial homeostasis are involved in the physiological response to damage, and underpin the regenerative capacity of the intestinal epithelium. In intestinal injury, epithelial ulceration skews homeostatic epithelial-stromal cross-talk and barrier breach induces localized innate immune responses, temporarily dysregulating the mucosal cell-signaling balance. This stromal response promotes angiogenesis and induces a profound change in neighboring intact epithelium, characterized phenotypically by crypt budding, fission, and the generation of lateral wound channels. At a cellular level, signaling instability alters epithelial cell-fate determination, with induction of stem cell function, proliferation and migration, to effect epithelial restitution. Dysregulation of the complex interacting pathways that control homeostatic cell fate are involved in this adaptive cell reprogramming.

Much of the work to date has been to assess the adaptive role of key signaling cascades at single pathway resolution. Both Wnt and YAP/TAZ pathways have been demonstrated to enhance plasticity. DSS-induced colitis is followed by an increase in YAP expression 2-5 days after regeneration, and the proinflammatory cytokine interleukin-6 has been attributed the role of being one of the key signaling routes responsible for initiating the role of YAP in intestinal regeneration. Furthermore, factors such as the Wnt enhancing ligand RSPO and canonical Wnt ligands are both associated with promotion of the plastic phenotype, and loss of these factors leads to impaired regeneration following injury. In addition, the noncanonical Wnt ligand Wnt5a has been shown to be essential for effective regeneration of intestinal crypts, acting through TGF-β to increase crypt fission, and promoting reestablishment of crypt homeostasis following proliferation.

Conversely, in homeostasis, BMP mediates cell differentiation, a process that needs to be inhibited to induce adaptive stem cell plasticity phenotypes in the wound milieu. We have recently shown that BMP inhibition results from rapid but temporary upregulation of the secreted BMP antagonist Grem1, from a heterogenous population of stromal cells. Pathway manipulation showed that antagonist-mediated BMP attenuation was obligatory, but functionally submaximal, as regeneration was impaired or enhanced by epithelial overexpression of Bmp4 or Grem1, respectively. Mechanistically, Bmp4 abrogated regenerative stem cell reprogramming, despite a convergent impact of YAP/TAZ on cell fate in remodeled wounds.

5 | HOW DO THESE PATHWAYS INTERACT?

The questions regarding plasticity deepen further when one considers that the Wnt, Hippo, and TGF-β/BMP pathways are known to undergo a considerable amount of cross-talk (Figure 6). This is already established to some degree on the basis of the previously mentioned long distance antagonism between BMP signaling and Wnt signaling in the intestinal crypt. However, interaction also occurs on a much smaller scale, allowing for diversity of cell response to signaling under differential conditions.

When sequestered in the cytosol, YAP/TAZ signaling is able to exert effects on other signaling mediators, including those of the TGF-β and Wnt signaling pathways. When YAP/TAZ is activated in a LATS1/2 knockout, Wnt activity gradients along the crypt-villus axis flatten out, losing a degree of polarity and resulting in a flatter gradient with Wnt expression increased outside of the intestinal crypt. Conversely, it has also been observed that YAP driven reprogramming of Lgr5+ ISCs is achieved via temporary inactivation of Wnt signaling, initiating a regenerative proliferative program which is separate to the usual Wnt driven homeostatic program. All of these suggest that YAP/TAZ signaling could act as a regulator of Wnt activity during regeneration, although the exact nature of this regulation remains to be characterized.

Wnt/β-catenin signaling has, in turn, been observed to have a regulatory effect on YAP signaling. Not only is the loss of the Wnt intestinal gradient associated with increased nuclear localisation of YAP outside of the crypt stem cell niche, shRNA interference with β-catenin signaling results in a subsequent decrease in cellular YAP mRNA. Furthermore, in a collagen matrix cell culture, characterized by much of the ECM remodeling which induces YAP/TAZ signaling in vivo, Wnt ligand supplementation drives YAP/TAZ activity, inducing reprogramming of cell fate.
The effect of YAP on TGF-β/BMP/Smad signaling has been demonstrated in mouse embryonic stem cells, where YAP is required for BMP induced suppression of cell-fate commitment to the neural lineage. Similarly, in human embryonic stem cells, TAZ has been shown to cooperate with Smad2/3 signaling in order to maintain pluripotency. Linker domain phosphorylation of Smads by CDK8/9 is additionally known to be associated with YAP recruitment. However, this phosphorylation also leads to ubiquitin-mediated degradation of Smads, reducing the effectiveness of TGF-β signaling. Since YAP recruitment is associated with enhanced Smad transcription, in the presence of YAP signaling there is a more closely temporally associated relationship between TGF-β receptor activation and Smad activity.

Secreted signaling pathways are not the only mechanisms involved in the complex reprogramming seen following injury. Remodeling of the ECM is a crucial part of the damage response, and the evidence would suggest that YAP/TAZ signaling is key for sensing of mechanical tissue damage, due to its role as a tissue mechanoreceptor. Following intestinal damage, extracellular remodeling results in YAP/TAZ activation, resulting in initiation of fate change for differentiated cells, and development of the regenerative stem cell phenotype in the damaged tissue.

All this suggests two things. First, that it is difficult to study these pathways as independent regulators of intestinal plasticity, and the highly evolutionarily conserved nature of plasticity implies a great degree of redundancy is present in their roles. Understanding the key regulating pathways and the nature of these redundancies is key to maximize any therapeutic opportunities. Second, it is important to consider that different models of injury are likely to trigger different adaptive signaling pathways benefitting from the convergent mechanistic effect of variable paracrine signaling and mechanosensing cascades. For example, an injury model which is initiated by tissue and matrix damage at its start (such as inflammation and ulceration) is likely to show different pathway interactions, including direct upregulation of YAP/TAZ through mechanotransduction, than a regenerative response which is, say, initiated by radiation, with reduced influence of matrix remodeling on repair.

However, much of this remains unknown, and more research is needed. Triangulation of pathway interaction

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**FIGURE 6** Elucidating signal integration. There is increasing evidence that the YAP/TAZ, BMP, and Wnt signaling pathways all demonstrate a considerable degree of interplay. In addition to the well-established antagonism exhibited between BMP and Wnt evidence suggests that Wnt may act to promote YAP/TAZ signaling, while YAP/TAZ itself has been demonstrated to have both a positive and negative effect on Wnt signaling across studies. Questions arise when the involvement of BMP and YAP/TAZ interactions are introduced, and while there is evidence that TGF-β signaling may become more closely temporally tied to receptor activation in the presence of YAP/TAZ, investigation of the BMP subfamily of TGF-β is particularly limited. In addition, while Wnt is widely acknowledged to promote plasticity and tumorigenesis, and there is emerging evidence for an antitumorigenic role of BMP signaling, evidence regarding YAP/TAZ remains conflicted, with evidence for a protumorigenic role confounded by work suggesting the pathway may be antitumorigenic in later stage CRC. Well-established positive regulation in green, well-established negative regulation in orange. Relationships in blue are newly emerging, or require further investigation. BMP, bone morphogenetic protein; TAZ, transcriptional coactivator with PDZ-binding motif; TGF-β, transforming growth factor-β; Wnt, wingless-related integration site; YAP, Yes-associated protein 1.
in the different damage/regeneration models ranging from targeted cell ablation to radiation damage, is needed to assess the critical pathways in each type of tissue repair setting. Identifying differences across models, and most importantly, identifying functional redundancy between the pathways, will be key for harnessing of these pathways for clinical translation. Certainly, YAP/TAZ seems to act as a point of convergence for much of this plasticity signaling, apparently downstream from other pathways such as Wnt and BMP, and from mechanical factors such as ECM remodeling. However, whether there remain alternative routes for differentiated cell reprogramming outside of this signaling remains to be seen.

6 | HOW IS INJURY SENSED AND ADAPTIVE CELL REPROGRAMMING INITIATED/REGULATED?

Although we now have increased understanding of the cell types capable of dedifferentiating and undergoing adaptive cell reprogramming, how this is mechanistically regulated at an (epi)genetic and transcriptional level is not clear. It has been demonstrated that dedifferentiating epithelial cells show changes in chromatin accessibility signatures, which converts from a lineage committed cell signature to an Lgr5+ characteristic signature as dedifferentiation occurs. It has also been demonstrated that cells retain transcriptionally flexible potential as they differentiate, even as they appear to become lineage committed, and this is once more due to a surprisingly accessible chromatin structure. As previously mentioned, Notch determines whether cells enter the absorptive or secretory lineages via lateral inhibition, a process which appears to be explained by this late stage of broad chromatin accessibility. However, this can also explain the plastic potential of differentiating cells, which, while lineage directed, are not committed on an epigenetic level until a surprisingly late stage in the differentiation process. The key transcriptional hubs that define and regulate lineage plasticity require further research.

More recent work from the Shivdasani group demonstrated that regenerating cell adaptive reprogramming appeared to be dependent on Ascl2, a Wnt pathway target gene. Ascl2 was not expressed throughout the whole crypt following Lgr5+ cell depletion, but instead was limited to those lower levels where the partially differentiated TAC population is found, raising the question of whether there is a “cut-off” point of differentiation past which point cells lose plastic potential. Individual lineage progenitor cell populations may have individual mechanisms that regulate adaptive cell reprogramming. For example, for the secretory cell lineage, Atoh1 phosphorylation may be of importance in mediating dedifferentiation of crypt cells to a regenerative phenotype. When Atoh1 phosphomutant mice were studied (where the Atoh1 protein is unable to be phosphorylated at key functional sites), while there was no homeostatic impact on animals, mice became considerably more sensitive to the DSS colitis model of intestinal injury. This highlights the key role of regenerative plasticity in response to damage, and highlights Atoh1 as a crucial protein in mediating this response for the secretory cell lineage. Mechanisms of plasticity may differ across different lineages. These data suggest chromatin accessibility and epigenetic flexibility is key for mediating plasticity for cells of the secretory lineage. However, it is entirely possible that different mechanisms mediate plasticity for the absorptive population, and key mechanisms may change even as differentiation progresses. Certainly, the Atoh1 cells studied here were thought to be committed to the secretory lineage until their dedifferentiation potential was identified, and the question of “how far” differentiation can proceed until cells are irreversibly committed remains unanswered.

7 | IMPLICATIONS FOR CANCER

The concept of stem cell plasticity and the controlling regulatory pathways emerging from the tissue regeneration field have huge implications for cancer biology and possible novel approaches to treatment. The cancer stem cell hypothesis states that it is a small subset of stem cells with the capacity to recapitulate all of the subpopulations of cells within a tumor which are responsible for tumor initiation and maintenance, and Barker et al demonstrated the Lgr5+ CBC can act as a cell of origin in cancer. However more recent work has shown that Lgr5-ve cells are also capable of cancer initiation and metastasis when associated with disruption of homeostatic signaling pathways. In a landmark paper, Schwitalla et al demonstrated that Lgr5-negative villus cells also had the potential to dedifferentiate and subsequently form tumors in response to inflammatory signaling, and in particular the inflammatory transcription factor NF-kB. The initiation of neoplasia through disruption of cell fate is not restricted to an inflammation setting. BMP signaling disruption is strongly associated with colon cancer, with multiple common variants associated with the disease and germline mutations in the pathway underpinning two separate hereditary polyposis syndromes: juvenile polyposis syndrome and hereditary mixed polyposis syndrome (HMPS). HMPS is a condition in which
ectopic epithelial expression of the BMP inhibitor Grem1 results in formation of a colonic polyposis of mixed histological phenotype which is associated with increased cancer risk. Modeling of this condition in mouse models has demonstrated that BMP gradient disruption results in the formation of ectopic crypt foci, containing Lgr5-negative cells that proliferate, acquire somatic mutations, and initiate dysplastic change outside of the crypt base. Here, the permanent disruption of the polarized BMP expression gradient along the vertical axis of the intestinal mucosa allows aberrant survival of a population of Lgr5-negative stem/progenitor cells, drawing comparisons with the physiological upregulation of Grem1 seen in tissue regeneration, and illustrating the fine line between dynamic, temporary disruption of signaling networks required to physiologically adapt epithelial cell fate in tissue repair, and the pathological co-option and corruption of the same pathways in neoplasia.

Although it has been demonstrated that Apc deletion and subsequent Wnt hyperactivation is not alone sufficient to induce YAP nuclear localisation, YAP deletion results in loss of polyp formation in ApcMin mice, suggesting that YAP may additionally be exhibiting non-transcriptional inhibitory roles with regard to Wnt signaling. Finally, acute knockout of Apc (Villin-CreERT2; Apc<sup>fl/fl</sup>), which results in a crypt hyperplastic phenotype, is not affected by YAP KO. This could simply be due to limited interactions of YAP and Wnt in the wild-type, healthy tissue due to existing YAP antagonism from the Hippo pathway in these animals, or alternatively could be a product of YAP activation being driven by the stromal milieu, since Apc inactivation here is restricted solely to the epithelial tissue.

In mouse models of cancers characterized by Braf mutation and loss TGF-β signaling (as opposed to being driven by Wnt activating Apc mutation), there is observation of a fetal, regenerative phenotype characterized by Sca1, and significantly, YAP/TAZ expression, with a concurrent loss of the Lgr5+ stem cell phenotype. The tumors in this model are right-sided and highly aggressive. Importantly, it has been hypothesized that, particularly in right-sided tumors where Wnt is not dysregulated, YAP/TAZ may be a key feature in driving the regenerative phenotype in early-stage neoplasia, which in turn may contribute to tumor development. This suggests that a variable balance between the Lgr5+ stem cells and the fetal-like, regenerative cell phenotype may be a key feature of different tumor subtypes. This research also highlights that there may be key differences in the role of regenerative pathways between right and left sided tumors. This is particularly interesting as there are also many environmental differences between right and left sided tumors, with formation of bacterial biofilms in these right-hand tumors associated with numerous molecular features in the cancer epithelial cells, and most notably decreased epithelial E-cadherin expression. This suggests that, just as method of injury may influence pathway activation in damaged tissue, mutation accumulation and environmental factors may play a heavy hand in determining which pathways are most fundamental in driving neoplasia, and this could provide a potential future route for patient cancer stratification.

However, there is also conflicting evidence regarding whether YAP/TAZ drives tumorigenesis under all circumstances. In multiple models of intestinal neoplasia, YAP overexpression, achieved directly or via Hippo pathway knockout, a suppression of both growth and metastasis is observed across models with an associated loss of Wnt signaling. Instead of the previously suggested role of YAP signaling as a tumor suppressive pathway, this exciting and unexpected finding shifts focus to YAP potentially playing a bipartisan role, depending on the stage of tumorigenesis itself, posing an important focus for future research into the pathway.

### 8 CLINICAL TRANSLATION AND FUTURE DIRECTIONS

Understanding plasticity is likely to be key in development of future therapeutic avenues to treat colorectal cancer. To give some clinical context, colorectal cancer is still treated predominantly by surgical resection. While exact proportion varies globally, in an analysis of CRC treatment in Denmark, England, Sweden, and Norway, around 60%-80% of cases were treated by surgical resection, while additional neoadjuvant radiotherapy or chemotherapies are generally used only in advanced cases.

Use of molecular biomarkers to guide treatment for colorectal treatment is relatively limited. Under NICE guidance, the only molecular typing of colorectal cancer which is recommended as standard practice is testing for RAS and BRAF V600E mutations, both of which are indicative of worse prognosis and negative selection criteria for anti-epithelial growth factor receptor therapy. A seminal paper by Guinney et al in 2015 reported, through integration of a range of consensus classifications, that CRC can be divided into four distinct molecular subtypes. This has subsequently provided a novel framework for scientific, and potentially clinical, understanding of CRC. However, it is also important to note that CRC has now been demonstrated to be a shapeshifter; molecular phenotype may change within the tumor and across time, and plasticity is likely to play a heavy hand in influencing this evolution.
Consequently, characterizing a tumor based on a single biopsy at a single moment is likely to prove of limited in use in targeting tumors from a molecular approach.

Accordingly, plasticity provides a significant roadblock to molecular targeting of tumors, rendering cancers able to adapt. Notably, it has been observed that YAP and TAZ expression levels are associated with worse TMN stage and greater incidence of metastasis in colorectal cancer, to the point where the YAP/TAZ status of tumors has been proposed as a potential prognostic factor. If a cancer is more able to adapt and change its phenotype, it is considerably more likely to be resistant to therapy and recur. The cancer stem cell hypothesis led to the hypothesis that these stem cells may act as a reservoir for recurrence, and by targeting the CSC, one may target the tumor at its origin. However, if many cells within a tumor have the potential to dedifferentiate to a cancer stem-like cell, this becomes considerably more difficult. By understanding and then targeting the adaptive plasticity pathways themselves, clinicians could significantly reduce the adaptive potential of tumors, and considerably improve patient outcome, particularly of those late-stage cancers where surgery is no longer curative.

The ubiquitous nature and homeostatic importance of the TGF-β/BMP and Wnt signaling pathways is a roadblock to implementation of safe and effective molecular based therapies. However, pathways downstream of YAP/TAZ, while key for regeneration, seem to play a much less significant role in homeostasis, and are an appealing potential target when one considers the desire to avoid systemic side effects of therapy. Identifying changes in signaling, including shifts in balance between canonical and noncanonical signaling for Wnt and TGF-β/BMP, the complex interplay of YAP/TAZ signaling with these pathways, and how pathways differ under different conditions of injury may help in identifying where inhibitors may be used for a cancer-specific approach.

The future of understanding of plasticity will come from better characterization of tumors; both on the basis of mutations and nongenetic modifications which influence the adaptive potential of the cancer cells themselves, alongside consideration of the microenvironment of the cancer. Ultimately, plasticity is an evolutionary mechanism; it facilitates escape in response to selective pressures, and plasticity mechanisms significantly improve the evolutionary “fitness” of the tumor; to the benefit of the cancer, and the detriment of the patient. Introduction of measures which take into account both the diversity (and potential to diversify) of the cancer cell population, and those environmental factors and resources available to facilitate this, may help to better characterize cancers, and guide clinical decision making and therapy development. Understanding and measuring the adaptive response of tumor cell phenotype, under the influence of therapeutic selective pressures, could help guide therapies to capitalize on evolutionary trade-offs and maximize stem cell killing—so called evolutionary steering. Stem cell plasticity is currently advantageous to the tumor, but conceivably, better understanding could help overcome the difficulties posed by tumor heterogeneity and evolution, and, through development of novel therapies, become advantageous to the clinician instead.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

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