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Mesenchymal–epithelial interactions during digestive tract development and epithelial stem cell regeneration

Ludovic Le Guen[1] · Stéphane Marchal[1] · Sandrine Faure[1] · Pascal de Santa Barbara[1]

Abstract The gastrointestinal tract develops from a simple and uniform tube into a complex organ with specific differentiation patterns along the anterior–posterior and dorso-ventral axes of asymmetry. It is derived from all three germ layers and their cross-talk is important for the regulated development of fetal and adult gastrointestinal structures and organs. Signals from the adjacent mesoderm are essential for the morphogenesis of the overlying epithelium. These mesenchymal–epithelial interactions govern the development and regionalization of the different gastrointestinal epithelia and involve most of the key morphogens and signaling pathways, such as the Hedgehog, BMPs, Notch, WNT, HOX, SOX and FOXF cascades. Moreover, the mechanisms underlying mesenchyme differentiation into smooth muscle cells influence the regionalization of the gastrointestinal epithelium through interactions with the enteric nervous system. In the neonatal and adult gastrointestinal tract, mesenchymal–epithelial interactions are essential for the maintenance of the epithelial regionalization and digestive epithelial homeostasis. Disruption of these interactions is also associated with bowel dysfunction potentially leading to epithelial tumor development. In this review, we will discuss various aspects of the mesenchymal–epithelial interactions observed during digestive epithelium development and differentiation and also during epithelial stem cell regeneration.

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Keywords Mesenchymal–epithelial interactions · Anterior-posterior axis · Gastrointestinal tract · Epithelial cell · Smooth muscle cell · Enteric nervous system · Myofibroblast · Stem cell · Regeneration · Metaplasia · Colorectal cancer · Hedgehog · Homeotic HOX genes · BMP pathway · Notch pathway · SOX9 · NKX2.5 · FOXF

Abbreviations

αSMA	Alpha smooth muscle actin
AIP	Anterior intestinal portal
AP	Anterior-posterior
BMP	Bone morphogenetic protein
CDX	Caudal type homeobox
CIP	Caudal intestinal portal
CRC	Colorectal cancer
ECM	Extracellular matrix
ENS	Enteric nervous system
FGF	Fibroblast growth factor
GAS1	Growth arrest specific gene 1
GI	Gastrointestinal
GSEMF	Gastric subepithelial myofibroblast
IHH	Indian hedgehog
ISEMF	Intestinal subepithelial myofibroblast
miR	microRNA
P-SMAD1	Phosphorylated SMAD1/5/8
SHH	Sonic hedgehog
SOX9	Sry-containing box gene 9
SM22	Smooth muscle protein 22
SMC	Smooth muscle cell
TGF-β	Transforming growth factor β
vENCC	Vagal enteric neural crest cell

Introduction

The vertebrate gastrointestinal (GI) tract is a vital and specialized organ system that is located behind the body wall and is characterized by its exceptional length and its morphological and functional regionalization. The GI tract starts as a uniform tube without any difference along the anterior–posterior (AP) axis. During development, each region of the GI tract will acquire its unique mesodermal and endodermal morphology that are easily discernable by gross and microscopic examination. Specifically, this uniform tube will differentiate along the AP axis into the pharynx, esophagus, stomach (foregut), small intestine (midgut) and large intestine (hindgut).

These regional morphological and functional differences are maintained throughout life and are essential for normal GI function. Briefly, the stomach secretes acid and enzymes necessary for food digestion and possesses a hypertrophic muscular structure involved in the mechanical digestion of food. Conversely, the small intestine and colon have a thin muscular layer necessary for the transit and elimination of feces. Other functions ensured by the small intestine and colon are the absorption of nutrients and water and the immune defense. Histologically, the GI tract is composed of four functional layers (mucosa, submucosa, muscularis propria and adventitia or serosa) that present morphological features specific to each part of the GI tract. The mucosa is the innermost layer, in contact with the intestinal lumen; it is composed of epithelial cells with a supporting layer of connective tissue (the lamina propria) and a thin smooth muscle layer (the muscularis mucosae). Underneath the mucosa lays the submucosa, a sheet of loose connective tissue involved in its support. This is followed by the muscularis propria that is involved in the mechanical breakage of food intake, especially in the stomach, and is responsible for its transit along the AP axis by contracting in a phasic manner under the regulation of the autonomous enteric nervous system (ENS). Finally, the GI tract is surrounded by the adventitia or serosa (depending on its AP position) to prevent frictions between the GI tract and other tissues/organs.

The specific intrinsic epithelial molecular pathways involved in GI tract regionalization and maintenance have already been reviewed elsewhere (see [1, 2]). Over the last five decades, many studies have shown that reciprocal mesenchymal–epithelial interactions drive and control the development and regionalization of the GI tract. These patterning events are remarkably well conserved across vertebrate species [3], and patterning anomalies during development have been associated with a number of human GI diseases. Recently, new molecular and cellular players in GI tract mesenchymal–epithelial interactions have been identified and our review will summarize and discuss older and newer studies that may help understanding these mechanisms and how their interactions could provide insights into disease-associated epithelial differentiation perturbations.

Epithelial–mesenchymal interactions during early development of the digestive tract

During early embryogenesis, the GI tract develops from two endoderm invaginations at the anterior (anterior intestinal portal, AIP) and posterior (caudal intestinal portal, CIP) ends of the embryo. The AIP structure forms first and it is closely followed by the CIP. Both structures elongate mirror-wise, while the subjacent lateral plate splanchnic mesoderm, which will give rise to smooth muscle, is recruited. The AIP and CIP fuse together around the connection with the yolk sac in the middle of the embryo body, forming a straight and uniform primary tube that closely associates endoderm and visceral splanchnic mesoderm. The AIP and CIP invaginations are thought to arise through an active endoderm-specific mechanism [4]. Factors that are specifically expressed in each of these two structures could be involved in their formation. This hypothesis is supported by the finding that when AIP endoderm is grafted into the CIP area, hindgut development is impaired [5]. Several transcription factors are expressed in the early AIP and CIP endoderm and their mutant phenotypes suggest roles in endoderm specification and early patterning. For instance, *Gata4* (a member of the GATA family of transcription factors) is expressed very early in the definitive AIP endoderm. *Gata4*$^{-/-}$ mouse embryos display multiple anomalies, including malformed AIP and absence of foregut [6, 7]. *Foxa2* (a forkhead domain/winged helix transcription factor, previously called *Hnf3β*) is also expressed in the definitive endoderm [8, 9] and *Foxa2*$^{-/-}$ mouse embryos do not develop foregut and midgut endoderm [9–12]. Moreover, *Gata4* is a direct transcriptional target of FOXA2 during early endoderm specification [13]. The atypical small GTPase *RhoU*, a WNT response gene involved in cell adhesion and migration [14, 15], is also expressed in the AIP and in the foregut endoderm of E8.0 mouse embryos [16, 17]. In *RhoU*$^{-/-}$ mouse embryos, endoderm cells in the foregut lose their proper columnar epithelial organization and the gut displays a deflated shape [16, 17]. In addition, F-actin distribution is no longer strongly polarized apically and microvilli are absent at the cell apical surface. Moreover, the expression of genes that are specifically found in the foregut endoderm (*Pyy, Igfbp5, Pax9* and *Apom*) is reduced. These results demonstrated that RhoU is required for regulating epithelial morphogenesis and endoderm differentiation.

Some members of the homeobox (HOX) family gene also are expressed in the visceral endoderm [18]. For instance, *Hoxa13* is detected in the CIP and the most caudal part of the GI endoderm [5, 18] and mutations in humans and mice result in anorectal malformations [19–24]. In chick embryos, the epithelial-specific expression of *Hoxa13* is essential for anorectal patterning through its involvement in endodermal–mesenchymal interactions. *Hoxa13* expression in the tailgut and cloaca endoderm regulates probably through epithelial–mesenchymal interaction, the expression of essential mesenchymal factors, such *Fgf8* and *Hoxd13* [5]. The three vertebrate caudal type homeobox (*Cdx*) genes are expressed in the CIP and posterior part of the visceral endoderm and in the developing hindgut endoderm [25–27]. Specific inactivation of *Cdx2* in the endoderm leads to blunt ending of the GI tract at the cecum, demonstrating its function in the expansion of the posterior endoderm [28]. In addition, *Cdx2* mutant mice that harbor anorectal and cloacal malformations show early expression of *Hoxa13, Hoxb13, Hoxc13* and *Hoxd13* genes [29], a finding suggestive of aberrant gut endoderm formation.

Sonic hedgehog (SHH) is a signaling morphogen involved in the patterning of different organs and tissues, and its binding to the Patched receptor induces expression of target genes, such as the GLI1-3 transcription factors. *Shh* is initially expressed in the AIP [30] and CIP [18] endoderm and then in the whole endoderm after AIP/CIP fusion. $Shh^{-/-}$ mouse embryos exhibit multiple GI tract malformations, such as anorectal and duodenal atresia and also midgut malrotation [31, 32]. Interestingly, the complexity of these abnormalities point towards mesenchymal defects and can be explained by the mesenchymal expression of the receptor Patched and of the transcriptional activator GLI1 [32]. Early ectopic activation of *Shh* in the visceral mesoderm generates an anterior shift of *Hoxd11* and *Hoxd13* expression in the mesoderm, supporting SHH function in the regulation of the early *Hox* expression domains that define the GI morphologic boundaries [18]. Altogether, these examples highlight the importance of Hedgehog signaling essentially through epithelial–mesenchymal interactions, and demonstrate the tight collaborations between these two adjacent tissue layers.

Mesenchymal–epithelial interactions during the AP regionalization of GI epithelia

After AIP and CIP fusion, the visceral endoderm is uniform along its AP axis. However, as differentiation takes place, morphological differences appear, leading to the formation of region-specific epithelium types. These mechanisms involve reciprocal (bi-directional) signals between epithelial cells and the adjacent mesoderm that are required for normal homeostasis. Many studies have demonstrated the importance of the interactions between endoderm and mesoderm during GI tract development/regionalization [33–35]. For instance, when skin fibroblasts or somitic mesoderm are co-cultured with gut endoderm, they differentiate into smooth muscle rather than fibroblasts or skeletal muscle [36] through the induction of the expression of visceral mesodermal proteins, such as tenascin [37] and alpha-smooth muscle actin (αSMA) [38]. Reciprocally, in vitro experiments have demonstrated that the primitive foregut endoderm needs to be co-cultured with mesodermal tissues to differentiate [39]. Interestingly, while heterologous splanchnic mesenchyme generally allows the survival and correct differentiation along the AP axis, somitic or cephalic mesoderm and limb buds are relatively inefficient [33–35]. This finding supports the idea that the actors governing the regionalization of digestive epithelia are specifically found in the splanchnic mesenchyme.

Moreover, there is a developmental window after which the primitive gut endoderm, although still morphologically uniform and undifferentiated, is committed to differentiate into its regional-specific epithelial types even if cultured with a variety of heterotypic mesodermal tissues [40–43]. Conversely, before this commitment, the primitive undifferentiated endoderm is flexible and its future shape and function rely on signals sent from the adjacent mesenchyme, and thus on its location along the AP axis. Many studies reported evidence that in the gut, the mesoderm dictates the ultimate epithelial pattern [44–46]. For instance, in chicken embryos, early gizzard endoderm co-cultured with proventricular mesoderm differentiates into proventricular epithelium [39]. However, there is one exception to the endoderm morphological/cytological plasticity upon exposure to regional-specific mesoderm. Indeed, co-culture of midgut epithelium with heterologous mesenchymal tissues does not influence its specification, as indicated by the retained expression of midgut epithelial digestive enzymes [43, 47–49]. This difference in the ability of midgut and foregut endoderm to undergo complete heterologous differentiation demonstrates that the mechanisms involved in the gut epithelium cyto-differentiation are different along the AP axis, with midgut endoderm displaying cell-autonomous features.

Signaling pathways and transcription factors involved in GI tract mesenchymal–epithelial interactions

Mesenchymal–epithelial interactions during GI tract development are regulated by several signaling pathways and transcription factors involved in its specification

(Fig. 1). As previously commented, Roberts and colleagues demonstrated that ectopic *Shh* expression in the mesoderm induces *Hoxd11* and *Hoxd13* in the visceral mesoderm [18]. *Hox* genes are homeobox-containing transcription factors that regulate pattern formation during development (for review see [50]). A number of *Hox* genes are expressed very early in the developing gut (mainly in the mesoderm) before the onset of regionalization. Their early mesodermal expression plays an important role in gut morphogenesis by regulating its regionalization along the AP axis and the mesenchymal–epithelial interactions that are required later for normal epithelium differentiation [5, 49]. During gut development, the expression of *AbdB* class *Hox* genes is spatially and temporally regulated in the posterior gut mesoderm, from the post-umbilical portion of the midgut through the hindgut [5, 18, 49, 51]. For example, *Hoxd13* is expressed in the distal-most region of the hindgut mesoderm (anorectum in the mouse and cloaca in the chick), where it controls the differentiation of the underlying endoderm [5, 49]. Experiments in mouse and chick embryos indicate that in the hindgut, *Hoxd13* plays a major role in the mesoderm to endoderm signaling that drives the final epithelial phenotype. Indeed, *Hoxd*13 ectopic expression in the midgut mesoderm is sufficient to induce the differentiation of the underlying endoderm towards a hindgut type [5, 49]. Moreover, *Hoxd13*$^{-/-}$ mouse mutants show malformations in the muscular and epithelial layers of the rectum [24]. *Hoxa5* is expressed in a dynamic fashion in the mesenchymal compartment of the developing gut [18, 52]. In *Hoxa5*$^{-/-}$mice, cell specification during stomach development is perturbed, resulting in gastric epithelial and enzymatic defects. This indicates that *Hoxa5*-driven mesenchymal–epithelial signaling is required for the stomach regional specification [52]. Indeed, in these mutants, the expression of genes encoding signaling molecules, such as SHH, Indian hedgehog (IHH), transforming growth factor β (TGF-β) family members and Fibroblast growth factor 10 (FGF10), is altered. Other *Hox* genes (such as *Hoxd8)* are expressed in the small and large intestine mesenchyme; however, no functional studies have been undertaken so far [53–55].

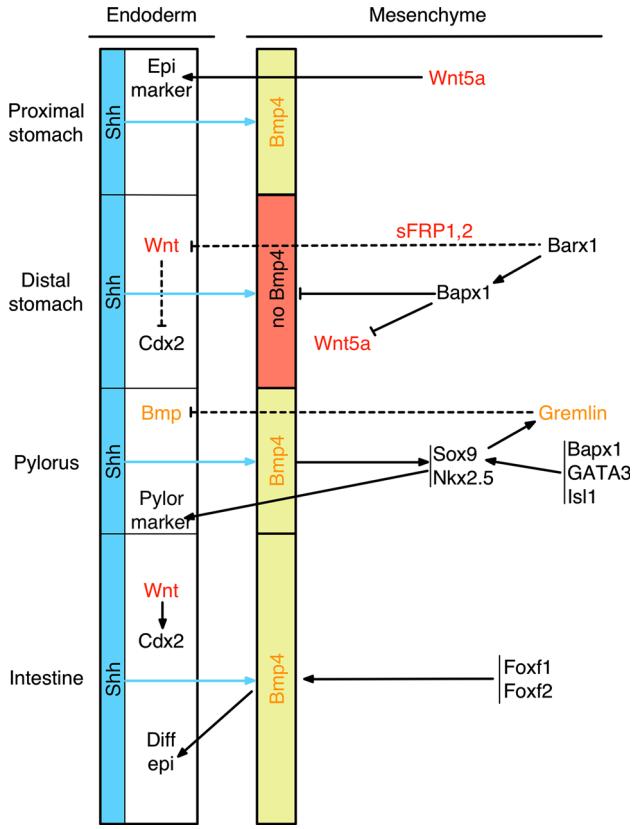


Fig. 1 Molecular control of the mesenchymal–epithelial interactions in the developing GI tract in vertebrates. Throughout the AP axis, epithelial *Shh* induces *Bmp4* expression in the adjacent mesenchyme, with the exception of the distal stomach where *Bapx1* represses *Bmp4* and *Wnt5a* expression. In the distal stomach, *Barx1*, which is upstream of *Bapx1*, regulates the mesenchymal expression of the WNT antagonists sFRP1 and sFRP2 that inhibit WNT activity and Cdx expression in the gastric epithelium. In the small intestine, *Foxf1* and *Foxf2* activate *Bmp4*, leading to BMP activity in both mesoderm and endoderm. In the pyloric sphincter, *Bmp4* activates the expression of *Nkx2.5* and *Sox9* that induce the pyloric epithelial phenotype through modulation of mesenchymal–epithelial signaling. In addition, *Bapx1*, *Gata3* and *Isl1* regulate *Sox9* expression in the pyloric structure. SOX9 controls Gremlin expression in the pyloric sphincter mesenchyme. Gremlin, a diffusible factor, modulates the activation of the endodermal BMP pathway to induce the specific pyloric epithelium differentiation

Another important factor involved in visceral mesenchyme development is Bone Morphogenetic Protein 4 (BMP4) the expression of which is induced by SHH (Fig. 1) [49]. *Bmp4* is detected in the whole early primitive GI mesenchyme with the exception of the stomach [49, 56]. BMP ligands are members of the TGF-β superfamily of signaling molecules and play major roles during embryogenesis and organogenesis. BMP signaling is quite complex. Indeed, it integrates positive and negative signals coming from many different ligands, inhibitors and receptors that are widely expressed in the gut, ultimately leading to activating phosphorylation of SMAD1, 5 and 8. Activated SMAD proteins are translocated into the nucleus where they induce their transcriptional targets [57]. Thus, detection of phosphorylated SMAD1/5/8 (P-SMAD1) is commonly used to monitor BMP activity in vertebrates [58, 59]. As expected from BMP4 expression, active BMP signaling is observed in all developing digestive mesenchymal tissues, but not in the stomach. Surprisingly, mesodermal BMP4 can induce BMP activity in the adjacent endoderm [56, 60] and high BMP activity is detected in the developing small intestine endoderm. Given the restricted expression of BMP ligands in the mesoderm layer at this stage, BMP activation in the endoderm

probably is regulated by signaling molecules present in the mesoderm. In the colon, P-SMAD1 detection is delayed compared to the small intestine, probably due to transient expression of the transcription factor *Bapx1*, a BMP inhibitor [61–63]. Inhibition of BMP activity through sustained *Bapx1* expression in the hindgut mesoderm results in persistence of undifferentiated endoderm associated with stenosis of the hindgut lumen [64]. These data demonstrate that BMP activity must be tightly regulated for the correct development and differentiation of colon epithelium. Recently, a similar stenosis phenotype was observed upon misexpression of a dominant negative form of LEF1 (a protein involved in WNT signaling) in the hindgut/cecal mesoderm [65], suggesting a connection between the BMP and WNT pathways in this process.

The forkhead transcription factors FOXF1 and FOXF2 are detected early in the splanchnic mesoderm and their expression, which is induced by SHH, persists in the intestinal mesenchyme (Fig. 1) [66–69]. *Foxf2*$^{-/-}$ and *Foxf2*$^{+/-}$ mouse embryos show multi-visceral abnormalities, including colorectal muscle hypoplasia. In these animals, the mesenchymal expression of *Bmp4* and of extracellular matrix components is reduced, whereas *Wnt5a*, which is normally inhibited by BMP4 in the mesenchyme, is up-regulated [70]. This is associated with increased proliferation and resistance to apoptosis of intestinal epithelial cells, leading to epithelial disorganization [70].

Many pathways are at play during the development/differentiation of the intestinal epithelia and these data highlight the importance of the cross-talk between endoderm and mesoderm for epithelial integrity. Other transcription factors have been described as specific intestinal mesenchymal markers, such as the homeotic factor NKX2.3, but their exact function and involvement in mesenchymal–epithelial interactions have not been elucidated yet [71–73].

In all vertebrates, the pyloric sphincter connects the stomach to the duodenum [3]. This sphincter is a mesodermal structure that holds food in the stomach when contracted and controls the gastric content flow into the duodenum. The molecular events required for the establishment and the differentiation of the stomach/duodenum boundary are regulated by the BMP signaling pathway (Fig. 1) [56, 64, 74, 75]. Mesodermal BMP4 activates the specific mesenchymal pyloric expression of the transcription factors NKX2.5 [56, 75] and SOX9 [74, 76]. Ectopic expression of BMP4, NKX2.5 and SOX9 in the stomach mesenchyme is sufficient to redirect differentiation of the underlying epithelial layer towards a pyloric sphincter type, suggesting a cross-talk between these genes (Fig. 1) [56, 74–76]. Interestingly, SOX9 misexpression in the stomach had no effect on BMP4 or NKX2.5 expression, suggesting that SOX9 and NKX2.5 present distinct, but complementary features [74]. This is in agreement with the finding that the *Drosophila* homologs of genes of the *Sox* and *Nkx2* families act in concert to regulate cell fate in the fly neuroectoderm [77]. Expression of WNT ligands, such as WNT11 and Gremlin (a modulator of the BMP pathway), is restricted to the pyloric sphincter mesoderm, and is essential for the establishment of the pyloric epithelium through mesenchymal–epithelial interactions (Fig. 1) [65, 75]. The function of SOX9 in this mechanism was investigated because it is a potent regulator of diffusible WNT and BMP proteins and their related inhibitors. *Wnt11* expression is not affected by gain or loss of SOX9 function; conversely, *Gremlin* expression is controlled by SOX9 [74]. Moreover, ectopic expression of Gremlin in the stomach mesenchyme partially phenocopies SOX9 misexpression, leading to the differentiation of the stomach epithelium into a pyloric sphincter-type epithelium [74]. In addition, BMP activation in the pyloric sphincter epithelium is tightly regulated and Gremlin certainly acts as a modulator of BMP activity. Indeed, pyloric epithelium patterning needs low levels of BMP activity, whereas formation of the gizzard (the chick muscular stomach) is associated with complete absence of BMP activity [64, 74] (Fig. 1). Other transcription factors involved specifically in the pyloric sphincter development, such as GATA3 and the LIM homeodomain transcription factor ISL1, have been recently described and connected with the BMP4/NKX2.5 and SOX9 pathways [78–80]. Their requirement or involvement in mesenchymal–epithelial interactions has not been addressed yet.

The development of the vertebrate stomach also is controlled by inductive and reciprocal interactions between the endoderm and the adjacent mesoderm. The homeotic transcription factor *Bapx1* (previously named *Nkx3.2*) is expressed in the mesenchyme of the developing gizzard in chicken embryos and in the distal stomach in mouse embryos [61, 63, 81] (Fig. 1). Ectopic expression of *Bapx1* in the mesenchyme of the developing proventriculus (glandular stomach in chicken embryos) results in important morphological defects (thicker mesenchymal layer and loss of proventricular glands associated with *Wnt5*a down-regulation) [63]. The stomach of *Bapx1*$^{-/-}$ mouse embryos is modestly reduced in size and loses the constriction of the pyloric sphincter. Moreover, the stomach epithelium does not have antral gland units, leading to a significant hind stomach truncation [81]. *Barx1*, another homeodomain-containing protein, is specifically expressed in the mesenchyme of the entire developing stomach in vertebrates [3, 82]. *Bapx1* expression is lost in the absence of *Barx1* [81], suggesting that *Barx1* is upstream of *Bapx1*. In *Barx1*$^{-/-}$ mice, the stomach is reduced in size and shows homeotic transformation of the epithelium associated with

aberrant expression of the intestinal marker CDX2 [83, 84]. Indeed, *Barx1* is required for mesenchymal expression of secreted frizzled-related protein 1 and 2 (sFRP1 and sFRP2), two WNT antagonists that attenuate the activity of the WNT pathway and contribute to the normal development and regionalization of the stomach epithelium [83, 84] (Fig. 1).

Influence of smooth muscle differentiation and vagal enteric neural crest cells on mesenchymal–epithelial interactions

In addition to its regionalization along the AP axis, the GI tract differentiates through the radial axis, giving rise (from the outer to the inner part of the gut wall) to the longitudinal and circular muscle layers, the submucosa and the muscularis mucosae, close to the epithelial lining [32, 64, 85–87]. The elongation and clustering of digestive mesenchymal cells are the first sign of their differentiation into smooth muscle cells (SMCs). Expression of SMC-specific lineage markers, such as αSMA, smooth muscle protein 22 (SM22) and Calponin, is followed by the acquisition of contractile capacities [88, 89]. In the colon and small intestine, SMCs organize sequentially into three different muscle layers (Fig. 2). The first to form is the circular smooth muscle layer, located in the middle of the intestinal mesenchyme. It is quickly followed by the formation of the longitudinal smooth muscle layer in the outer part of the mesenchyme. Finally, around week 14–20 in humans, the muscularis mucosae forms and constitutes the third smooth muscle layer, close to the epithelium (Fig. 2) [90–92]. Concomitantly with the smooth muscle layer differentiation, the uniform and multi-stratified intestinal endoderm forms villi that project into the lumen. Specifically, the endoderm is transformed into epithelial ridges that are then organized into parallel zigzags and finally into intestinal villi [93]. The link between epithelial morphogenesis and smooth muscle layer differentiation was nicely investigated step by step by Shyer and colleagues [93]. By chemical and

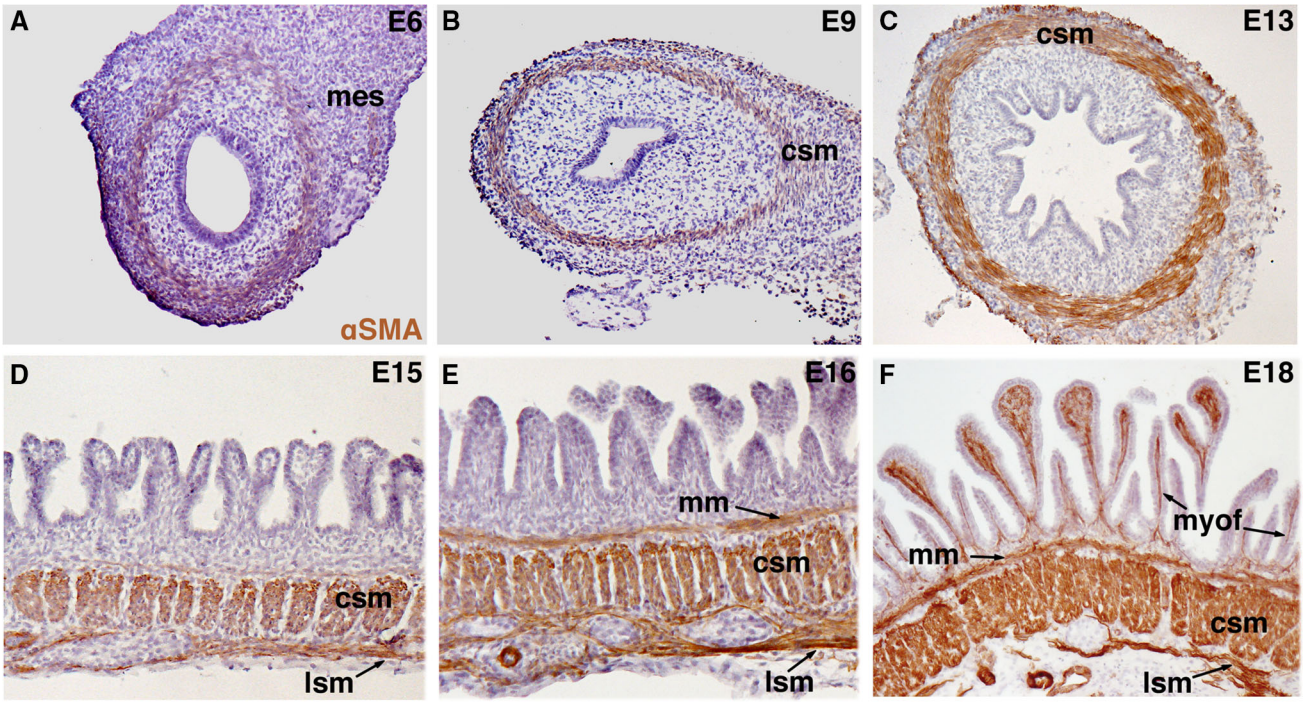


Fig. 2 Smooth muscle cell differentiation in the small intestine of chicken embryos. Smooth muscle cells (SMC) were detected by immunostaining with an antibody against the SMC-specific marker αSMA. **a** Non-organized and undifferentiated mesenchymal cells in the small intestine of a 6-day/old (E6) chick embryo. **b** αSMA-positive cells are present in the smooth muscle layer in the small intestine of an E9 chick embryo. **a**, **b** During early embryonic development, the visceral endoderm is uniform with stratified layers of cells. **c** αSMA-positive cells in the circular smooth muscle layer of the small intestine of an E13 chick embryo. The ongoing intestinal epithelial cytodifferentiation leads to the formation of an epithelial monolayer. **d** αSMA-positive cells in the longitudinal and circular smooth muscle layers of the small intestine of an E15 chick embryo. **e** αSMA-positive cells in the longitudinal, circular and submucosal smooth muscle layers in the small intestine of an E16 chick embryo. **f** αSMA-positive cells (myofibroblasts) are detected also in the lamina propria of the small intestine in a E18 chick embryo. **d**–**f** Intestinal epithelial cytodifferentiation is marked by mesodermal growth into the lumen and villi formation, characterized by specific long and thin villi in the small intestine. Abbreviations: *mes* mesenchyme, *csm* circular smooth muscle, *lsm* longitudinal smooth muscle, *myof* myofibroblast, *αSMA* alpha smooth muscle actin, *mm* muscularis mucosae

physical ablation of the different smooth muscle layers, they showed that the differentiation of the circular smooth muscle layer is necessary for the development and maintenance of the epithelial ridges, probably by enforcing the mechanical constraint to limit mesenchymal and endodermal growth. The longitudinal smooth muscle layer and muscularis mucosae are required, respectively, for the development of the zigzags and of the villi. Villi formation also requires localized changes in endodermal and mesenchymal cell proliferation. Altogether, these authors demonstrated that the intestine epithelial morphogenesis is dependent on the sequential differentiation of the three smooth muscle layers present in the gut [93].

Concomitantly with these early patterning events, the primitive GI tract is colonized by neural crest precursor cells that give rise to the ENS, the intrinsic innervation of the GI tract. In vertebrates, the ENS is predominantly derived from vagal enteric neural crest cells (vENCCs) that are initially located adjacent to somites 1 to 7 [94–98]. These cells migrate away from the neural tube and enter the esophageal mesenchyme. Their AP migration in the GI tract is promoted and regulated through interactions with extracellular matrix proteins produced by mesenchymal cells [99, 100]. Ultimately, vENCCs populate the entire GI tract, from the esophagus to the terminal colon, but also the lungs and pancreas, two associated GI organs. During their migration along the GI tract, neural crest cells proliferate and differentiate into neurons and glial cells, the main ENS components. These cells form two concentric ganglionic plexuses localized within the muscle layers of the gut wall [101], while in the pancreas, ENCCs are found close to the epithelium [102]. Surprisingly, ENCC genetic ablation leads to aberrant morphogenesis of the pancreas epithelium, suggesting the existence of instructive signals from ectoderm-derived cells to the endoderm-derived developing pancreas [102]. In the stomach, ENS influence on the developing mesenchyme and epithelium was recently demonstrated. Indeed, vENCC ablation in chick embryos impairs smooth muscle development and induces transdifferentiation of both stomach mesenchymal and epithelial layers into a mixed stomach-intestine structure [72]. Constitutive activation of Notch signaling is sufficient to induce the expression of intestinal mesenchymal and epithelial markers in the stomach, suggesting an additional role for Notch signaling in the regulation of intestinal development [72]. vENCCs contribute to the inhibition of the Notch pathway in the mesenchyme, which is a prerequisite for the establishment of gastric identity (Fig. 3). These findings support the involvement of specific tissue and molecular interactions in the establishment of gastric features [72]. Altogether, these observations are in line with previous studies demonstrating that intestinal specification is the default state of the gut endoderm [83] and that reciprocal mesenchymal–epithelial interactions are

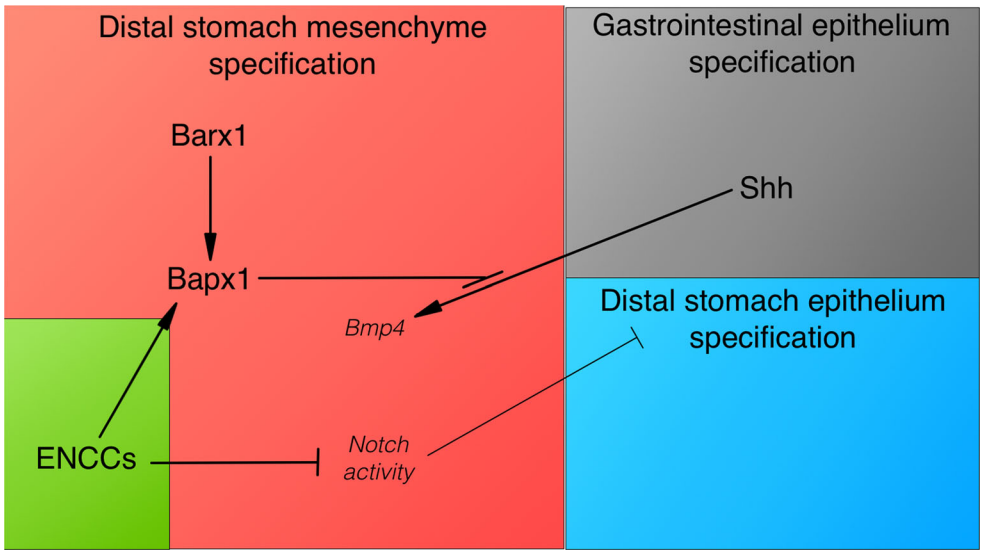


Fig. 3 Model of vENCC role in reciprocal epithelial–mesenchymal interactions during distal stomach development. The schematic shows the molecular pathways and their potential interactions during stomach patterning in vertebrates. *Shh* from the gastrointestinal epithelium induces *Bmp4* expression in the adjacent mesenchyme, with the exception of the distal stomach, where *Bapx1* prevents its expression. *Bapx1* expression in the distal stomach mesenchyme requires *Barx1*. vENCCs are required for *Bapx1* expression in the distal stomach mesenchyme, independently of *Barx1*. Importantly, inhibition of Notch activity in the distal stomach mesenchyme is essential for conferring stomach identity to the mesenchymal and epithelial layers. Indeed, ectopic activation of the Notch pathway in the stomach leads to a mixed stomach–intestinal phenotype. In conclusion, in the vertebrate stomach, mesenchymal–epithelial interactions involve also vENCCs required for stomach patterning during development

crucial for its morphological and functional regionalization. They also demonstrate ENS role in these interactions, a contribution that was previously unappreciated.

Mesenchymal–epithelial interactions in stem cell regeneration and GI tract pathologies

In adults, the main morphological patterning of the intestinal epithelium occurs along the crypt-villus axis. Proliferative progenitor cells are at the bottom and differentiated/functional/apoptotic cells in the lumen [2, 103]. In the gastric mucosa, epithelial cells are organized in vertical flask-shaped structures [104, 105] with progenitor/proliferative cells at the bottom and differentiated/functional/apoptotic cells in the lumen [2, 103]. The organization of these structures is established during embryogenesis and maintained throughout life, despite the continuous renewal of the digestive epithelium from stem cells [2, 93, 106, 107]. The genetic control of the adult digestive epithelium homeostasis (reviewed in [108]) involves reciprocal interactions between epithelial cells and also between epithelium and mesenchyme [109, 110]. However, the influence of mesenchymal–epithelial interaction mechanisms on stem cell renewal and epithelial architecture has only been partially elucidated. Disruption of this morphological organization results in bowel dysfunction, potentially leading to tumor development. Many factors that are involved in intestine morphogenesis during embryonic life also contribute to the maintenance of its pattern in adult life. In this section, we will describe and discuss the different pathways and factors that control cell fate decision and cell differentiation through mesenchymal–epithelial interactions, during digestive epithelium regeneration and in GI tract pathologies.

Myofibroblasts are a component of the neonate and adult mesenchymal layer in close contact with the digestive epithelium. Intestinal subepithelial myofibroblasts (ISEMFs) reside within the mesenchymal layer, just below the epithelium of both villi and crypts and next to the muscularis mucosae, along the whole length of the intestine (colon and small intestine). In humans, they start developing from week 21 of gestation [111] and form a sheath between the epithelium and the muscularis mucosae, where they contribute to the composition of the extracellular matrix (ECM) and basal membrane [112, 113]. ISEMFs express vimentin and αSMA, but not (or weakly) desmin [114, 115]. Although still debated, they may originate from neuroepithelial cells [116], bone-marrow-derived stem cells [117, 118], or trans-differentiation of resident fibroblasts [119] or of intestinal SMCs [120]. In mouse embryos, ISEMFs are detected from E18.5 [121] and in avian embryos from E16 (Fig. 2) [122]. Similar to epithelial cells, ISEMFs differentiate as they migrate from the crypt along the villus axis [123]. There are increasing evidences that ISEMFs are major players in GI epithelium regeneration in normal and pathological conditions. ISEMFs envelop the intestinal crypts [124] and support the intestinal epithelium growth ex vivo and in tissue xenografts [113, 125]. It was suggested that growth factors essential for the development of intestinal stem cells are in part secreted by ISEMFs [126]. For example, in the intestine, epimorphin, a membrane-associated protein that regulates late epithelial morphogenesis [127], is produced by ISEMFs [127, 128]. The intestine length is increased in adult *Epimorphin*$^{-/-}$ mice due to enhanced crypt cell proliferation and crypt fission during the neonatal (suckling) period. This is mediated at least in part through changes in the BMP and WNT/beta-catenin signaling pathways [129]. In *Epimorphin*$^{-/-}$ mice, ISEMFs are less responsive to Hedgehog signals, leading to decreased BMP activity associated with strong expression of Chordin (an inhibitor of the BMP pathway) and reduction of stromal IL-6 secretion [127, 130].

Recent studies also highlighted key roles of microRNAs (miRs) in the control of intestinal epithelial regeneration through mesenchymal–epithelial interactions. miR-143/145 are strongly expressed in the colon and are down-regulated in colorectal cancer [131]. In the absence of miR-143/145, colon epithelial development occurs normally. However, following injury, epithelial regeneration is strongly impaired [132]. Moreover, miR-145 is a positive regulator of intestinal SMC differentiation and maturation through its capacity to directly target *Gata6* mRNA [133]. In agreement, miR-143 and miR-145 are also detected in the intestine mesenchyme, mostly in smooth muscles and ISEMFs [132]. Interestingly, miR-143/145 ablation exclusively in the mesenchyme phenocopies the defective epithelial regeneration observed in miR-143/145 knockout animals, while epithelial loss of miR-143/145 does not. This demonstrates that the control of epithelial regeneration is governed in part through mesenchymal–epithelial interactions in an miR-143/145-dependent manner.

Less is known about the potential differences in the subepithelial myofibroblast populations along the AP axis. Comparison of the expression of genes associated with the HOX, Hedgehog, Notch, BMP, FDF and WNT pathways in ISEMFs and gastric subepithelial myofibroblasts (GSEMFs) did not highlight any significant difference in the expression of activators and inhibitors of the WNT pathway [134]. This is in agreement with the finding that WNT secretion by ISEMFs is not required for the intestinal stem cell niche homeostasis [135]. In contrast, growth arrest-specific gene 1 (*Gas1*), a SHH receptor [136], is up-regulated in GSEMFs, while *Hoxc8* and *Notch1* are strongly expressed in ISEMFs, suggesting that the Notch pathway is more active in ISEMFs. These features are reminiscent of the recent finding that Notch pathway activation is required during the

development of the intestinal, but not of the gastric mesenchyme [72]. They also demonstrate the existence of GSEMF- and ISEMF-specific intrinsic molecular pathways and raise the question of whether these specific differences modulate their paracrine influence on the renewal of digestive epithelial stem cells.

Epithelial metaplasia is frequently observed in the adult GI tract [104]. This corresponds to a change of the epithelium characteristics towards a different type of epithelium. For instance, the esophagus epithelium shows features of intestinal epithelium and pyloric-type gastric glands in patients with Barrett's esophagus syndrome or following *Helicobacter pylori* infection [104, 137, 138]. Epithelial metaplasia is the result of homeotic transformations (i.e., the aberrant differentiation of one region into another, implying misregulation of homeotic genes) [139]. The mechanisms ensuring the maintenance of the regional epithelial differentiation in the GI tract are partially known and involve the same factors found in early GI patterning. Many studies on the intestinal metaplasia of the stomach have focused on the misregulation and function of key actors of intestinal epithelial patterning and differentiation, such as the homeotic transcription factors CDX2 and SOX9 [140, 141]; for review [104, 142]. Interestingly, a switch from gastric to intestinal morphology is observed in both epithelium and mesenchyme in the stomach of patients with intestinal metaplasia and in $Cdx2^{-/-}$ mice [143]. In addition, *Helicobacter pylori* infection in the stomach induces gastric-intestinal metaplasia associated with CDX2 misexpression and an increase in αSMA-positive myofibroblasts [144, 145]. Although no molecular analysis of these myofibroblasts was done, we can hypothesize that stromal myofibroblats participate in or at least maintain this tissue-specific epithelial transformation through the same mechanisms based on mesenchymal–epithelial interactions used during GI tract development.

Conclusion and perspectives

The importance of mesenchymal–epithelial interactions has been first demonstrated and studied during the development of the chicken GI tract by dissociating and recombining the mesenchymal and endodermal layers. These pioneering approaches were followed by the identification of the molecular mechanisms involved in the communication between these interdependent tissues. Several major signaling pathways (BMP, WNT, Notch and FGF) and transcription factors (HOX, SOX, FOXF) are involved in the interactions between these tissues along the AP axis. Moreover, recent studies have shown the impact of smooth muscle differentiation on the regulation of intestinal epithelium morphogenesis and on the regionalization of the gastric epithelium.

In adults, the reciprocal influence of mesenchyme and epithelium has been hypothesized for a long time, but investigations on these interactions have really started only recently. It has been already demonstrated that mesenchyme-derived cells, such as subepithelial myofibroblats, actively participate in epithelial stem cell regeneration and could also be involved in the maintenance of the digestive epithelial regionalization. Different studies have found a correlation between epithelial and mesenchymal alterations in metaplasia. Moreover, genes expressed by stromal cells rather than by epithelial tumor cells define subtypes of colorectal cancer (CRC) with poor prognosis [146], supporting the mesenchyme influence in GI tract pathologies.

During development, the digestive mesenchyme is colonized by vENCCs, endothelial and lymphatic cells [147–150]. Unexpectedly, the number of vENCCs contributes to the maintenance of stomach identity and differentiation through inhibition of the Notch signaling pathway [72]. This demonstrates that by regulating mesenchyme identity, vENCCs act as a new mediator of mesenchymal–epithelial interactions in the control of gastric epithelial regionalization. The close association of endothelial and lymphatic cells with ENCCs during the establishment of their respective networks and also in adults [148–150] opens the way for investigating new potential actors in the regulation of mesenchymal–epithelial interactions during development, but also in adults. Besides smooth muscle, enteric and endothelial cells, the GI wall contains many other cell types (for instance, fibroblasts, lymphocytes and leukocytes) [124] that could be involved in mesenchymal–epithelial interaction mechanisms to maintain the regionalization and homeostasis of digestive epithelia in adults. Their real involvement needs to be investigated and the underlying mechanisms identified.

Altogether these challenging questions must be addressed in the context of GI tract development, stem cell and pathology research, and collaborative efforts will give us a better understanding of these complex and exciting three-dimensional structures.

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