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Foxf2: a mesenchymal regulator of intestinal adenoma development

Gijs R. van den Brink[1] and **Deborah C. Rubin**[2]

[1]Department of Gastroenterology & Hepatology and Tytgat Institute for Liver and Intestinal Research, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands. Tel +31-20-5669111

[2]Division of Gastroenterology, Washington University School of Medicine, St Louis, 660 South Euclid Ave Box 8124, MO 63110, USA. Tel +1-314-362-8935

Epithelial-mesenchymal interactions by morphogenetic signaling molecules dictate patterning of the gastrointestinal tract during development and maintain homeostasis of the adult intestinal epithelium.[1] The development of an intestinal adenoma can be viewed as a morphogenetic patterning event.[2] During this process of adenomagenesis, the epithelial stem cell compartment is clonally expanded by genetic mutations that activate the Wnt signaling pathway. Despite the fact that these activating mutations act cell autonomously, the relevance of epithelial-mesenchymal interactions for morphogenetic events suggests that mesenchymal genes may still be required for adenomagenesis to occur. In this issue of Gastroenterology, Nik et al., now demonstrate the critical role of mesenchymal Foxf2 expression in adenoma initiation and growth.[3]

Negative feedback loops control intestinal epithelial homeostasis

The epithelial cells that cover the intestinal mucosa are continuously replenished from a pool of rapidly cycling cells. The fate of these proliferating cells depends on Wnt signaling which drives nuclear accumulation of β-catenin. In the nucleus β-catenin complexes with DNA-bound Tcf transcription factors and activates a transcriptional program specific for both intestinal stem cells and transit amplifying cells.[4] Careful expression analyses of Wnt target genes has established that a subset of these genes such as *Lgr5* and *Ascl2*marks the intestinal stem cells[5] but the precise mechanisms that differentiate stem cells from transit amplifying cells have not been identified.

Homeostatic systems such as the rapidly renewing intestinal epithelium are self regulating systems that depend on negative feedback loops. The output (differentiated cells) produces a signal that restricts the input (rate of proliferation).This results in a tight restriction of epithelial proliferation which is dependent on the integrity of the surface epithelium. Wounding will result in loss of the negative feedback signal allowing the proliferating cells to rapidly increase the production of differentiated cells which restores the production of the feedback signal and restores homeostasis. The negative feedback loops that control intestinal

Email for correspondence: g.r.vandenbrink@amc.nl; drubin@dom.wustl.edu.

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epithelial homeostasis have only partially been resolved but appear to include both epithelial and mesenchymal factors.[6, 7] One of the factors that plays a key role is Indian Hedgehog. Indian Hedgehog is a morphogen produced by differentiated enterocytes of the adult small intestine[8] and colon.[9] Conditional activation of Hedgehog signaling in the adult gut leads to reduced Wnt signaling and depletion of proliferating cells in the crypt whereas conditional loss of Indian Hedgehog signaling results in activation of Wnt signaling and increased epithelial proliferation. This is sufficient to result in features of epithelial repair such as crypt lengthening and fissioning.[8, 10] Effects of Hedgehog signaling on the proliferating cells are indirect and exclusively mediated via the mesenchyme. During development Foxf1 and Foxf2 are mesodermally expressed transcription factors that are regulated by Hedgehog signaling. The relevance of Foxf expression to mediate effects of Hedgehog signaling during development of the gut is underscored by two previous findings of the Carlsson lab. The first is that *Foxf1* heterozygous mice have a foregut and airway phenotype that is identical to that of *Sonic Hedgehog* mutant mice.[11, 12] The second is the developing intestine of *Foxf1* and *Foxf2* mutant mice shows ectopic activation of Wnt signaling on the villi and expansion of epithelial precursor cells,[13] a phenotype similar to mice in which Hedgehog is specifically inhibited in the intestine during development.[14–16] In their publication in this issue of Gastroenterology the group shows for the first time that Foxf2 is a mesenchymally expressed negative regulator of the epithelial precursor cells in the adult intestine. Nik et al. examine a $Foxf2^{+/-}$ mutant mouse that was previously generated by their group. In addition they generated a novel BAC transgenic mouse in which extra copies of Foxf2 and surrounding genomic region were introduced and Foxf2 expression is therefore driven from its own regulatory sequences. This technique therefore allows overexpression of genes in a spatiotemporal manner that approaches the regulation of expression of the endogenous gene. Using these tools to modulate expression of Foxf2, the authors find that the gene dose of Foxf2 negatively correlates with the rate of epithelial proliferation and number of Lgr5 positive intestinal epithelial stem cells (Figure 1).

So what is the nature of the mesenchymal negative feedback signal that is regulated by Hedgehog signaling and Foxf2? This is likely not a linear pathway, multiple mechanisms may exist that are most likely not completely overlapping between Hedgehog signaling and Foxf2. One of the proteins that have been examined as a potential Hedgehog/Foxf2 regulated factor is Bone Morphogenetic Protein (Bmp)-4. During development Bmp4 is expressed exclusively in the mesenchyme,[15] regulated by Hedgehog signaling,[15] and completely absent in $Foxf2^{-/-}$ mice.[13] However in contrast to the remarkable intestinal developmental phenotype of Hedgehog mutant and Foxf2 mutant mice, two independent groups have shown that mice that overexpress the Bmp antagonist Noggin in the intestine do not have a phenotype until after the suckling to weaning transition around postnatal day 21.[15, 17] Noggin overexpressing and *Bmpr1a* mutant mice develop polyps in adult mice.[17, 18] However these polyps are hamartomas that have a remarkable mesenchymal expansion rather than the epithelial expansion that is typical for adenomas (for discussion see [19]) and that is not observed in Hedgehog or Foxf mutant mice. In their current publication, Nik et al. perform an extensive analysis of potential modulators of Wnt signaling that could be controlled by Foxf2. They find no change in Bmp4 expression but find that Foxf2 controls the expression of Sfrp1 a soluble antagonist of Wnt signaling in the intestinal mesenchyme. This suggests that Sfrp1 may mediate at least some of the negative regulation by Foxf2 on the epithelial stem cells and rate of proliferation. However, as the authors point out, Foxf2 is a transcriptional regulator that will more broadly affect the behavior of the mesenchymal cells that express it and other mechanisms are therefore likely to exist.

In conclusion, Indian Hedgehog and Foxf2 seem to be components of an epithelial-mesenchymal negative feedback loop that controls intestinal epithelial homeostasis.

Although Foxf2 may control the epithelial precursor cell compartments via multiple mechanisms, Sfrp1 may be an important epithelial to mesenchymal signal in this loop.

Mesenchymal modulation of adenoma initiation and growth

Most sporadically occurring intestinal adenomas form by mutations that activate the Wnt signaling pathway. Since Wnt signaling drives stem cell and transit amplifying cell fate, such mutations abnormally expand the size of the epithelial precursor cell compartment.[4] The most frequent mutations that activate the Wnt pathway are mutations in *APC*. Germ line mutations in *APC* are also the cause of the rare Familiar Adenomatous Polyposis syndrome in humans, and cause a similar syndrome in the $Apc^{min/+}$ mouse mutant that was used in the studies of Nik et al. Even though mutations in *APC* expand the precursor cell compartment this expansion is not a linear phenomenon. Adenomas are very slow growing and can in fact regress. A study in which adenomas of <10 mm in size where left in situ to be removed at a follow up endoscopy 3 years later showed that there was no significant increase in adenoma size over the three year observation period.[20] This indicates that despite the increased Wnt signaling that results from an *APC* mutation, negative regulatory mechanisms are still in place. Some may even be specifically activated by the increased size of proliferating cells. We know very little about such barriers to adenoma progression that have to be overcome during the adenoma to carcinoma sequence. The work by Nik et al. now suggests that at some of these factors are mesenchymally derived and transcriptionally regulated by Foxf2 (Figure 1).

In conclusion, negative feedback loops act to maintain homeostasis in the normal intestinal epithelium. Such negative regulatory mechanisms also affect epithelial cells that have already acquired the genetic mutations that initiate adenoma growth. They are a likely explanation of the slow progression of adenomas and the observation that adenomas can even regress. Clearly some of the restraints on normal epithelial proliferation and the adenoma to carcinoma progression are imposed by epithelial mesenchymal cross talk. Nik et al have now identified Foxf2 as a key mesenchymal transcriptional regulator in this interaction. The further characterization of such negative regulatory mechanisms will be a key area of research to understand the adenoma to carcinoma sequence and may point to novel avenues in chemoprevention and chemotherapy.

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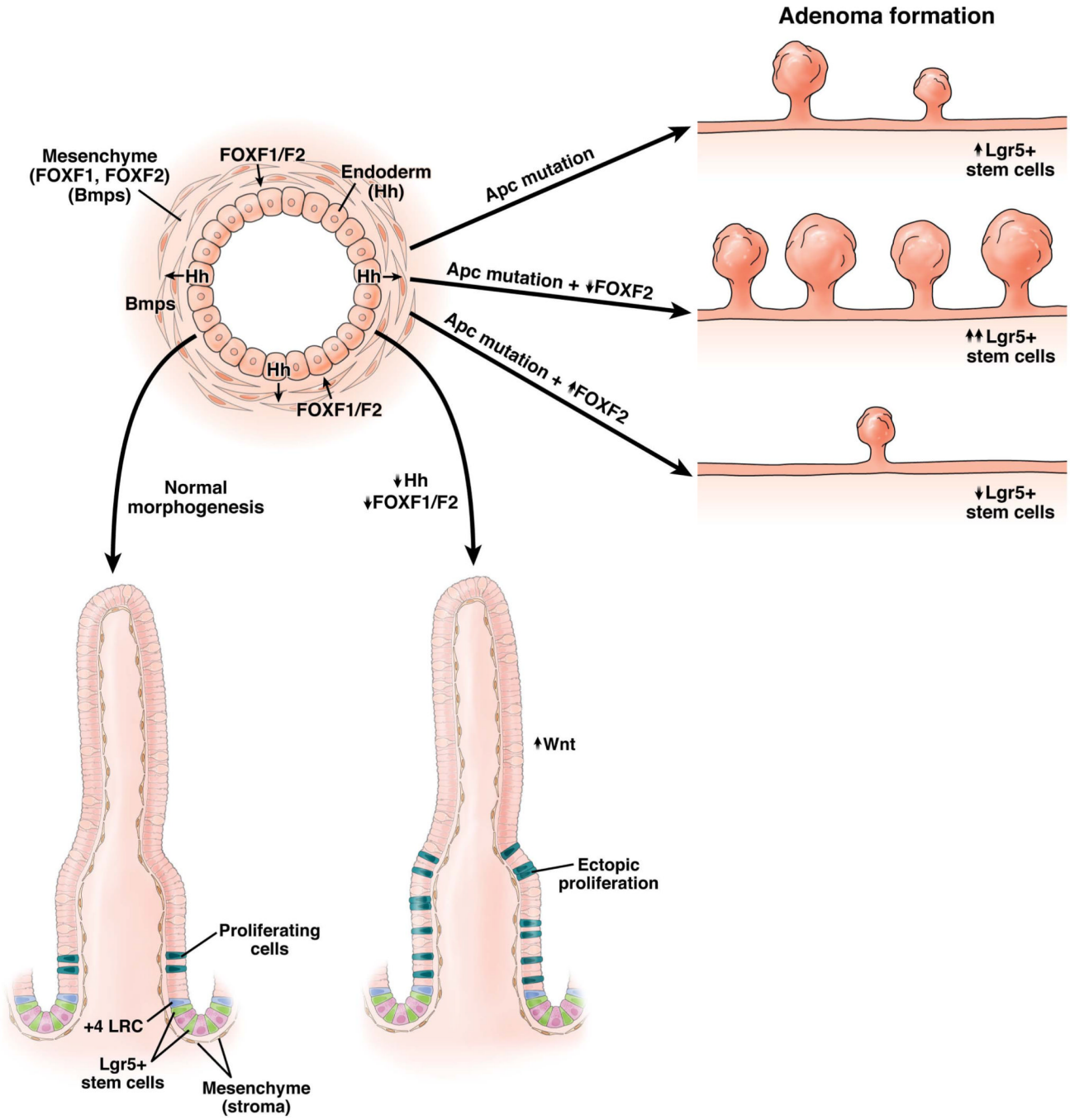

Fig. 1.

Mesenchymal FoxF2 regulates intestinal epithelial proliferation and modulates adenoma initiation and growth in Apcmin/+ mice. Epithelial-mesenchymal cross-talk plays a critical role in crypt-villus morphogenesis. Hedgehog signals to mesenchyme. Bmps and FoxF1/F2 are key mesenchymal factors that crosstalk to epithelium. Reduced FoxF1/F2 expression results in increased proliferation and increased Wnt signaling during villus morphogenesis (bottom left), and FoxF2 gene dosage of regulates adenoma initiation and growth (top right).