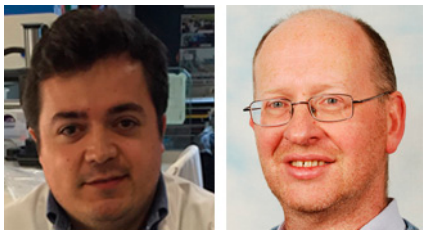
Metaplastic Cdx2-depleted cells can be very disruptive neighbors

In this issue of JEM, Balbinot et al. (https://doi.org/10.1084/jem.20170934) describe an original mechanism where *Cdx2* inactivation regulates intestinal metaplastic to neoplastic transition in a paracrine fashion. Surprisingly, the target cells are neighboring "normal" Cdx2-positive cells.



Insight from Filipe C. Lourenço and Douglas J. Winton

The homeodomain or homeobox protein family are transcription factors that contain a conserved DNA-binding domain that play a vital role in spatial patterning during embryogenesis. Their function has been first described in *Drosophila* (Scott and O'Farrell, 1986) and is conserved throughout evolution to vertebrates (De Robertis et al., 1989; Wright et al., 1989). Cdx2 is a homeodomain protein whose expression in the murine late embryo and adult is confined to the epithelium of the intestine. It has a pivotal role in the establishment of endodermal anterior-posterior (AP) asymmetry and more specifically intestinal identity. Cdx2-null mice are not viable and die before gastrulation. Heterozygote Cdx2-deficient mice are viable and present developmental abnormalities such as stunted growth or rib malformation (Chawengsaksophak et al., 1997). Mosaic conditional *Cdx2* knockout in the adult intestine results in impairment of stem cell differentiation with gradual loss of villous morphology and formation of cystic vesicles. When aged, these mice present cecal polyps with Cdx2-negative regions displaying gastric features (Stringer et al., 2012).

Motivated by the recent classification of human colorectal cancers into subgroups based on transcriptional signatures Balbinot and colleagues revisit the mosaic *Cdx2* conditional knockout mouse model described above (Stringer et al., 2012). Strong CDX2 down-regulation was shown to be associated with one particular subtype characterized by serrated precursor neoplasia, stromal infiltration, stem-cell–like features, and poor prognosis (Marisa et al., 2013). In the current mouse study, Balbinot et al. (2018) found the expected, cecal polypoid lesions with some degree of gastric metaplasia. This was confirmed by transcriptomic analysis where intestinal markers such as Cdx1 or Alpi were down-regulated and several gastric markers (e.g., Tff1/2, Cldn18, Muc1/6) were up-regulated. The absence of other typical gastric markers (e.g., Sox2 or Muc5a) suggests incomplete metaplasia. Often, metaplasia persistence can lead to the development of dysplasia and sporadically to neoplasia (Giroux and Rustgi, 2017). However, this does not seem to be the case for Cdx2-deficient gastric metaplasia, despite the tumor predisposition associated with its heterozygous loss (Chawengsaksophak et al., 1997). To further test the protumorigenic role of Cdx2 deficiency, the authors next crossed the mosaic *Cdx2* inactivation model to mice deficient in the intestinal tumor suppressor gene *Apc*. These $Apc^{+/\Delta 14}$ mice develop adenomas and a few adenocarcinomas along the intestinal tract. These compound mice had a reduced lifespan with more lesions in the small intestine, colon, and cecum when compared with mice with only the $Apc^{+/\Delta 14}$ mutation. The lesions displayed a mixed phenotype combining the gastric metaplasia and some pathological features reminiscent of carcinomas. Transcriptomic analysis showed an up-regulation of the Wnt pathway as well as gastric-type genes. However, the most striking results were obtained when the authors performed lineage tracing of the cells that had undergone *Cdx2* inactivating recombination by crossing a conditionally activated fluorescent cassette (tdTomato) into the model. Surprisingly, they observed a total exclusion of red cells (Cdx2 negative) from tumor regions displaying high nuclear β-catenin. This observation led the authors to suggest that Cdx2-negative cells generate a tumor-prone microenvironment that induces neoplastic conversion of Cdx2 intact cells. By this definition, Cdx2 is acting as a "non–cell-autonomous tumor suppressor."

In the search for a mechanism, Balbinot et al. (2018) looked again at the transcriptome of gastric metaplasia present in the cecum. They found up-regulation of several cytokines and chemokines previously associated with malignancy. Moreover, they show that NF-κB, a key node for cytokine and chemokine signaling, was translocated and activated only in the nucleus of Cdx2-positive cells present at the surface epithelium adjacent to the metaplastic lesions. These Cdx2-positive cells displayed high levels of nitric oxide synthase (iNOS), a protein previously shown to induce genomic instability and accelerate allelic loss of *Apc*, leading to enhanced tumor development (Shaked et al., 2012). To test the therapeutic relevance of these findings the authors treated the compound mice with aminoguanidine (AG), an iNOS inhibitor, which reduced the tumor load. Interestingly, there was no effect in $Apc^{+/\Delta 14}$ mice treated with AG, strengthening the putative role of *Cdx2* inactivation

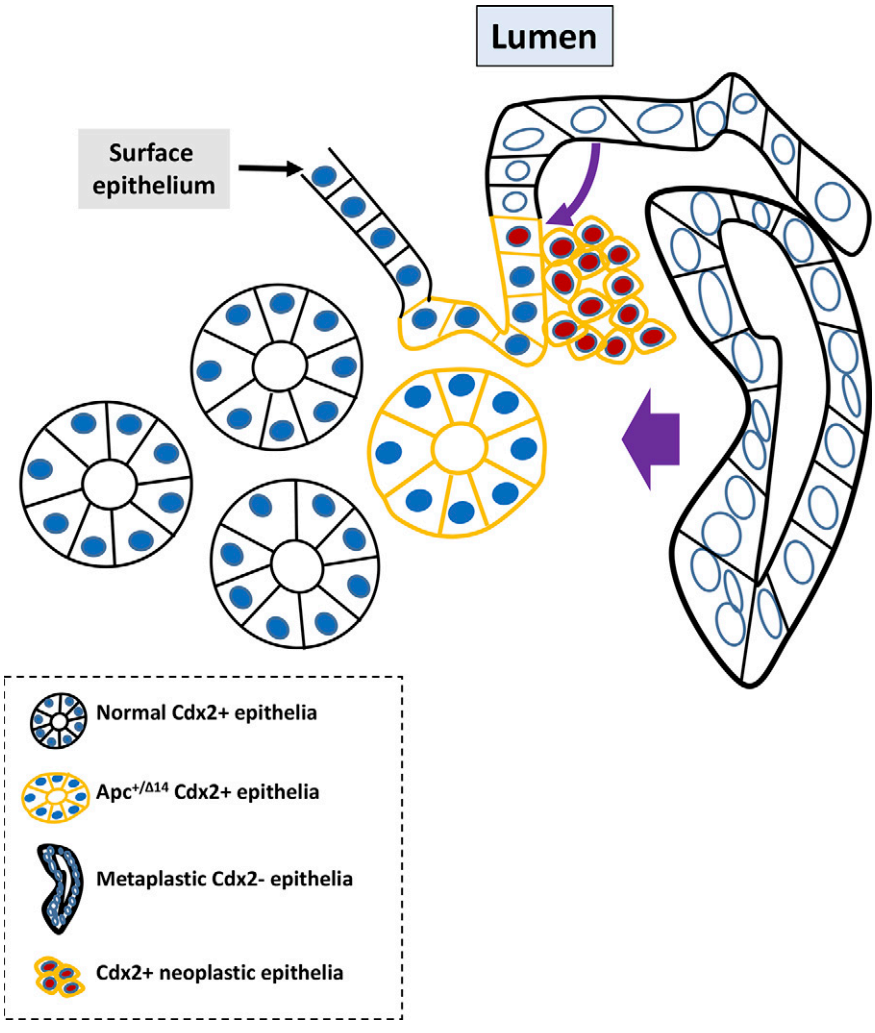
Filipe C. Lourenço and Douglas J. Winton, Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Cambridge, England, UK: Filipe.Lourenco@cruk.cam.ac.uk; Doug.Winton@cruk.cam.ac.uk

in the induction of nitrosative stress in Cdx2-positive neighboring cells.

Over many years, there has been a huge effort on understanding how the microenvironment created by the stroma influences tumor initiation, progression, and therapeutic responses. However, less is known about the interactions within the epithelium. This work adds a new perspective on how neighboring metaplastic epithelial cells can shape the transition between different tissue states and in fine cancer initiation by otherwise "normal" cells. The "field cancerization" concept (Curtius et al., 2018) may need to be refined to include a mechanism whereby cancers initiate from cells residing at clonal boundaries adjacent to epithelial cells expressing alternative tumor promoting programs. In this case a Cdx2-negative metaplastic clone bordering a Cdx2-positive $Apc^{+/\Delta 14}$ clone (see figure).

One of the pending questions Balbinot et al. (2018) did not address relates to why Cdx2-negative cells do not themselves initiate neoplasia. Speculatively, the answer may lie in the way the intestinal epithelium is renewed. Upon Cdx2 patterned differentiation, cells retain Cdx2 expression and move along the crypt toward the lumen where they are shed. During this terminal differentiation and removal from the epithelium, they shut down essential functions such as the DNA damage response. This contrasts to stem cells that need to ensure genomic stability. The soft spot for neoplastic transition is likely to be the transition state where cells are still cycling and differentiating, especially in premalignant cells displaying oncogenic mutations (Schwitalla et al., 2013). Conceivably, aberrant differentiation associated with partial metaplasia allows programs for genomic stability to be maintained.

The association between CDX2 inactivation and the serrated-type carcinoma has focused attention on the different molecular pathways driving this pathology. A significant majority of these lesions do not display activation of the WNT pathway, reflecting the lower prevalence of somatic mutations in *APC* or *β-Catenin* (Sakamoto et al., 2017). Instead, these lesions often have mutations in *BRAF*, a key MAPK pathway mediator. Two recent independent studies using mouse models show that combined conditional *Cdx2* inactivation and expression of mutant *Braf* ($Braf^{V600E}$) promoted serrated benign and invasive tumors (Sakamoto et al., 2017; Tong et al., 2017). One of these studies provides evidence that the differentiation status of the normal colonic mucosa is a good predictor of susceptibility to serrated tumors (Tong et al., 2017). A possible way of unifying all these observations would be that CDX2-deficient cells in the "normal" or metaplastic mucosa preferentially favor the emergence of BRAF mutant serrated lesions cell autonomously and WNT-driven lesions in a non–cell-autonomous fashion.



Neoplastic transformation at clonal borders. Cdx2-negative metaplastic cells induce paracrine signals (arrows) that increase genomic instability in preneoplastic Cdx2-positive cells at the luminal surface, leading to malignant transformation.

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