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Converting intestine into esophagus

Anterior-posterior patterning of the vertebrate gut requires Cdx2

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After the primitive gut tube is formed during gastrulation of the vertebrate embryo, its derivative organs, including liver, stomach, pancreas or colon, have to be elaborated at precise anterior-posterior positions. The epithelial cells of the gut are derived from the endoderm, one of the three germ layers. The primitive gut endoderm is a pseudostratified epithelium with little evidence of cytodifferentiation. Genetic experiments, expression studies and lineage tracing over the past fifteen years have yielded insights into how transcriptional regulators control the differentiation processes that lead to the elaboration of the various organ systems and cell types in the gastrointestinal tract. A major remaining knowledge gap involved the question of how the specification of the primitive gut endoderm to intestinal fates is controlled.

Anterior-posterior patterning of the gut tube can first be detected by expression of the homeobox transcription factor *Cdx2* in the hindgut from the moment the organ is formed.[1] *Cdx2*, just like its vertebrate paralogues *Cdx1* and *Cdx4*, is orthologous to the Drosophila gene *caudal*. As the name implies, *caudal* is required for the establishment of anterior-posterior regional identity in the fly.[2-4] The mammalian *Cdx1* and *Cdx2* genes are expressed in the intestinal and colonic epithelia from the onset of gut development throughout adulthood.[5] Anteriorly, expression of the *Cdx* genes forms a sharp boundary at the pyloric/duodenal junction, suggesting that these factors play a role in the anterior/posterior patterning of the primitive gut tube. Additional evidence for this notion comes from the fact that the *Cdx2* gene forms part of the evolutionarily conserved "parahox cluster", in which the three homeobox genes *Gsh*, *Pdx1* and *Cdx2* are spaced closely together on mouse chromosome 5 (human chromosome 13). Like the standard four homeobox gene clusters, the three genes of the parahox cluster are activated in an anterior to posterior fashion, with *Gsh* activated in the CNS and pituitary, the *Pdx1* expression domain encompassing the distal stomach and proximal duodenum, and *Cdx2* localized from duodenum to colon.[6,7]

Gene ablation studies have proven that *Pdx1* plays an essential role in establishing the patterning of the foregut field, as mice deficient for *Pdx1* display only a small pancreatic rudiment, a lack of Brunner's glands in the duodenum, misspecification of enteroendocrine cells in the rostral duodenum and caudal stomach, and an abnormal and frequently blind-ending pylorus.[8-10] Likewise, *Gsh* is essential for pituitary development.[11] In contrast, the contribution of *Cdx2* to the patterning of the gut tube has not yet been fully explored. Mice null for *Cdx2* die before implantation, due to essential functions of *Cdx2* in the preimplantation embryo.[12,13] *Cdx2*$^{+/-}$ mice have an interesting phenotype, with the formation of squamous metaplasia most often in the proximal colon where *Cdx2* expression is highest;[12] however, these mice were not informative regarding the function of *Cdx2* in earliest gut patterning.

To address the function of *Cdx2* in gastrointestinal development specifically, we developed a conditional allele for the gene, and derived mice in which *Cdx2* was deleted from the endoderm beginning on embryonic day 8.5 using the *Foxa3*-Cre YAC transgenic line we had developed previously (*Cdx2*$^{loxP/loxP}$; *Foxa3*Cre mutant mice).[14,15] Embryos thus lacking *Cdx2* in the gut tube from its inception developed to term, but died shortly thereafter due to their inability to feed. Histological and molecular analyses revealed major deficiencies in gastrointestinal architecture and cell differentiation. Normally, the intestinal epithelium elaborates four differentiated cell types—absorptive enterocytes, hormone-secreting enteroendocrine cells, mucin-secreting goblet cells and Paneth cells located in the base of the crypts involved in the innate immune response. None of the differentiated cells were present in the *Cdx2*-deficient epithelium (See **Fig. 1** for an example). In addition, expression of important transcriptional regulators of the intestinal epithelium, such as *Cdx1*, *HNF4*α and *HNF1*α, were absent from the mutant intestine, indicating that *Cdx2* acts near the top of the transcription factor hierarchy in the intestine.

So what had become of the intestinal epithelium? While anterior structures, such as the duodenum, retained some semblance of normality, with the elaboration of—albeit abnormal—intestinal villi; posteriorly, the phenotype was even more dramatic. *Cdx2* mutants displayed complete colonic atresia, such that the gut tube ended in a malformed cecum. Just anteriorly, in the ileum, villi were missing, and a smooth, squamous epithelium was elaborated. This *Cdx2*-deficient "ileum" expressed cytokeratin 13 and p63, markers normally restricted to the keratinocytes of the esophagus. This apparent anterior homeotic transformation was confirmed

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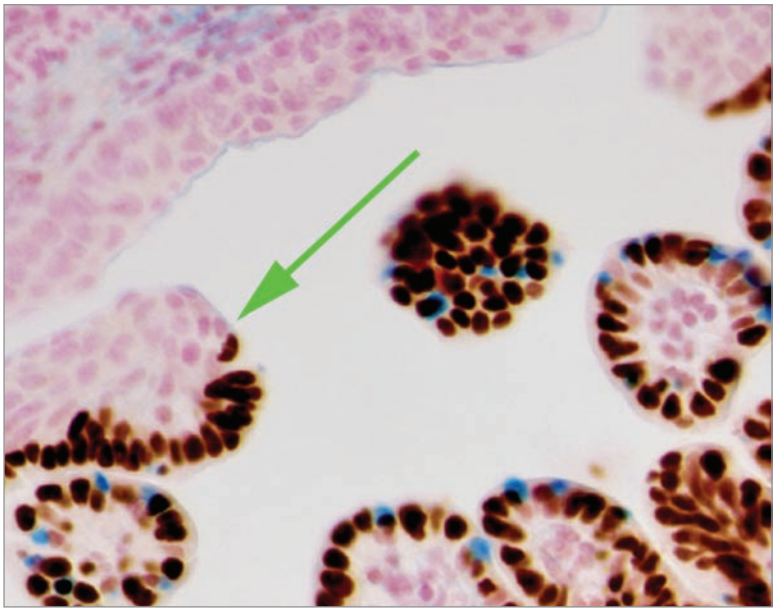

Figure 1. Lack of intestinal epithelial differentiation in absence of *Cdx2*. A section of small intestine from *Cdx2*$^{loxP/loxP}$; *Foxa3*-Cre mice with rare mosaic deletion was stained for *Cdx2* by immunohistochemistry (brown nuclei). Goblet cells were visualized by Alcian blue staining (blue). The green arrow points to a villus with contribution from a *Cdx2*-positive and a *Cdx2*-negative crypt. Epithelial cells that retain *Cdx2* form the normal columnar epithelium with the presence of ample goblet cells (blue). Sections of the gut tube where *Cdx2* has been efficiently deleted elaborate a disorganized, squamous epithelium with no goblet cells, indicative of homeotic transformation.

by global expression profiling, which showed complete conversion of the gene expression profile of the mutant ileum. In fact, the change was so dramatic that unsupervised clustering sorted the mutant ileum next to wild type esophagus, instead of wild type ileum. Among the genes that were activated in the *Cdx2*-deficient ileum were dozens encoding cytokeratins and other keratinocyte markers. Conversely, transcripts for the nutrient and ion transporters, digestive enzymes and secreted proteins normally found in the ileum were absent from the *Cdx2* mutants. These findings extend previous observations in transgenic mice ectopically expressing Cdx2 protein in the gastric epithelium.[16] In this gain-of-function experiment, the transgenic stomach displayed intestinal metaplasia, a sign of posterior homeotic transformation.

In conclusion, the *Cdx2*$^{loxP/loxP}$; *Foxa3*Cre mouse is the first mouse model with a complete conversion of an intestinal phenotype into esophagus. These findings add to our knowledge of the developmental roles of the members of the parahox gene cluster, and establish *Cdx2* at or near the top of the transcription factor hierarchy that controls intestinal differentiation.

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