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EDITORIAL

CFTR and the Regulation of Crypt Cell Proliferation

Gastrointestinal manifestations of cystic fibrosis (CF) may be quite severe and a cause of significant morbidity. Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) result in meconium ileus, and, in later life, small-bowel bacterial overgrowth, bowel obstructions, and an increased incidence of intestinal cancers. A 2013 study of more than 40,000 patients from 250 US CF centers showed an increased risk of colon cancer (standardized incidence ratio, 6.2) and small-bowel cancer (standardized incidence ratio, 11.5).[1] Other investigators have observed an increased incidence of polyp detection and progression after age 40,[2,3] suggesting that, as long-term patient survival rates improve, enhanced colon cancer screening procedures will be required. Loss of CFTR expression in early stage human colorectal cancer patients was associated with reduced disease-free survival.[4]

Relevant to these observations in human beings has been the discovery that, unlike pulmonary disease, many of the gastrointestinal manifestations are recapitulated in mouse models of cystic fibrosis. Increased crypt cell proliferation in *Cftr* gene knockout (KO) mice has been well documented.[5] *Cftr* knockout mice have high mortality rates and must be maintained on oral osmotic laxatives for survival. Mice with intestine-specific *Cftr* deletion spontaneously develop adenomas in the small bowel and colon and there is enhanced tumorigenesis in Adenomatous polyposis coli multiple intestinal neoplasia ($Apc^{min/+}$) mice lacking intestinal *Cftr* expression.[4] Although the intestinal phenotype is robust, the precise mechanisms by which *Cftr* deletion results in increased crypt epithelial cell proliferation and adenoma/cancer remain unclear.

In this issue of *Cellular and Molecular Gastroenterology*, Strubberg et al[6] propose a novel mechanism of Wnt signaling dysregulation in *Cftr* KO mice as the basis for the observed increased crypt cell proliferation. By using *Cftr* KO mice, intestinal crypt and enteroid culture, and advanced imaging techniques, Strubberg et al[6] showed increased transit-amplifying and Leucine rich repeat containing g protein coupled receptor 5 stem cell populations in *Cftr* KO crypts, and showed that *Cftr* KO enteroids have increased 5-ethynyl deoxyuridine (Edu) incorporation. *Cftr* KO crypts and passaged enteroids also had increased expression of dephosphorylated β-catenin and Lef1, suggesting an increase in Wnt β-catenin signaling. Fluorescence-activated cell sorting for SRY-Box9 (Sox9)^{EGFPlo} stem cells, which contain an Lgr5-enriched population, had increased CFTR expression compared with the Sox9^{EGFPsublo} (transit-amplifying) population. The authors then used elegant intracellular pH monitoring to show that normal enteroid crypt base columnar cells are responsive to CFTR inhibition, and that *Cftr* KO enteroid crypt base cells show an increase in pH compared with wild-type crypt base columnar cells. Furthermore, they explored mechanisms by which Wnt signaling might be modified in these cells. Wnt ligands interact with cell surface receptors Frizzled and Lrp5/6, followed by recruitment of Dishevelled (Dvl) to the Frizzled receptor. Recruitment of Dvl to the plasma membrane is facilitated by the interaction of Dvl's polybasic DVL, Egl-10, Pleckstrin (DEP) domain to the negatively charged phospholipids of the inner leaflet of the plasma membrane. Previous studies have shown that an acidic pH inhibits these interactions, which in turn reduce Wnt signaling activity. The authors thus hypothesized that alkaline pH also might regulate the electrostatic attraction of the DEP domain to the plasma membrane. Among other interesting observations, they showed that Dvl association is pH-responsive, with enhanced plasma membrane association in response to alkalinization and reduced association in an acid pH milieu. In addition, an interesting asymmetry in Dvl plasma membrane association in WT cells was observed.

A limitation of these studies was that they do not directly connect the observed changes in Dvl localization resulting from alkalinization and pH change on gut Wnt signaling activity. However, these data can be placed in the context of the few studies that have attempted to determine the underlying mechanism for increased crypt cell proliferation in *Cftr* KO intestine. Than et al[4] showed enhanced colonoid growth and enrichment in changes in intestinal stem cell and Wnt signaling pathway genes in $Apc^{min/+}$ mice lacking intestinal CFTR. microRNA profiling of CF intestine showed differential expression of microRNA clusters (miR-17-92 and miR-106b-25), which have been shown to regulate cell proliferation.[7] Other investigators have suggested that ongoing inflammation and small-bowel bacterial overgrowth all contribute to the increased risk of intestinal cancer, but specific mechanisms remain unknown.

In addition to presenting a novel mechanism for CFTR's effects on crypt cell proliferation, this elegant study adds to the list of innovative uses of enteroids for studying fundamental questions in intestinal biology. These studies set the stage for future investigations using human small-bowel and colon crypt enteroids from CF patients to further elucidate the pathogenesis of this important gastrointestinal manifestation of CFTR deficiency.

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