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ORIGINAL RESEARCH

Cftr Modulates Wnt/β-Catenin Signaling and Stem Cell Proliferation in Murine Intestine

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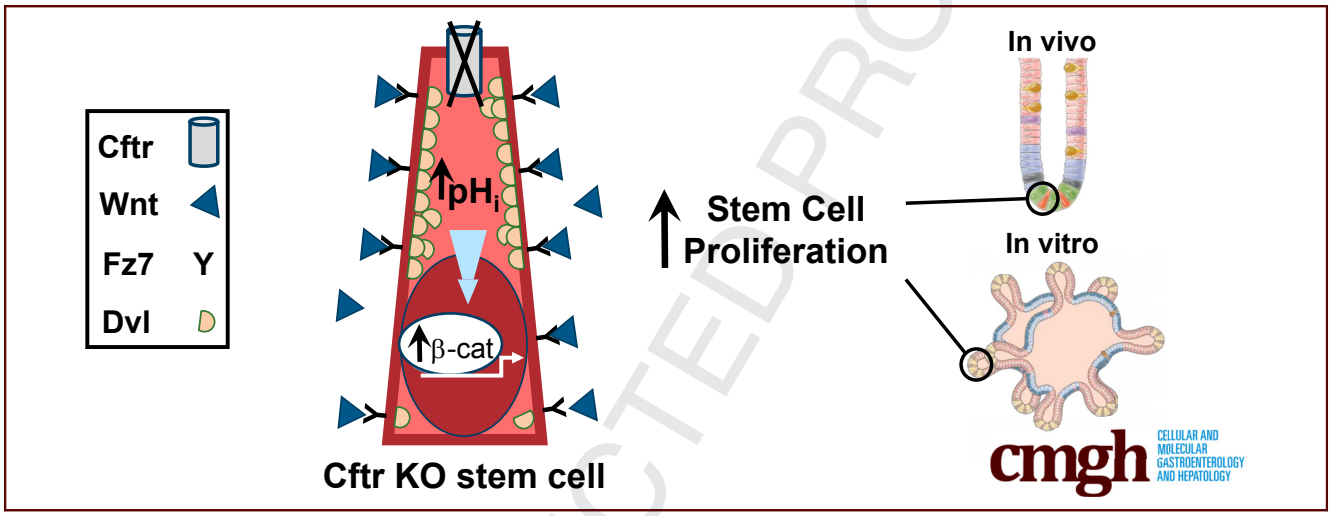

SUMMARY

This study documents the functional activity of cystic fibrosis transmembrane conductance regulator (Cftr) in the active intestinal stem cell population of murine intestine. In the absence of Cftr, the intracellular pH, surface localization of the Wnt-transducer Disheveled, Wnt/β-catenin signaling, and stem cell proliferation are all increased, which may contribute to increased gastrointestinal cancer risk in cystic fibrosis.

BACKGROUND & AIMS: Cystic fibrosis (CF) patients and CF mouse models have increased risk for gastrointestinal tumors. CF mice show augmented intestinal proliferation of unknown etiology and an altered intestinal environment. We examined the role of the cystic fibrosis transmembrane conductance regulator (Cftr) in Wnt/β-catenin signaling, stem cell proliferation, and its functional expression in the active intestinal stem cell (ISC) population. Dysregulation of intracellular pH (pH$_i$) in CF ISCs was investigated for facilitation of Wnt/β-catenin signaling.

METHODS: Crypt epithelia from wild-type (WT) and CF mice were compared ex vivo and in intestinal organoids (enteroids) for proliferation and Wnt/β-catenin signaling by standard assays. Cftr in ISCs was assessed by immunoblot of sorted Sox9$^{enhanced\ green\ fluorescent\ protein(EGFP)}$ intestinal epithelia and pH$_i$ regulation by confocal microfluorimetry of leucine-rich G-protein–coupled receptor 5 (Lgr5)+-EGFP ISCs. Plasma membrane association of the Wnt transducer Disheveled 2 (Dvl2) was assessed by fluorescence imaging of live enteroids from WT and CF mice crossed with Dvl2-EGFP/Rosa$^{mT/mG}$ mice.

RESULTS: Relative to WT, CF intestinal crypts showed an ~30% increase in epithelial and Lgr5+ ISC proliferation and increased Wnt/β-catenin signaling. Cftr was expressed in Sox9^{EGFPLo} ISCs and loss of Cftr induced an alkaline pH$_i$ in Lgr5+-EGFP ISCs. CF crypt-base columnar cells showed a generalized increase in plasma membrane Dvl2-EGFP association as compared with WT. Dvl2-EGFP membrane association was charge- and pH-dependent and increased in WT crypt-base columnar cells by Cftr inhibition.

CONCLUSIONS: CF intestine shows increased ISC proliferation and Wnt/β-catenin signaling. Loss of Cftr increases pH$_i$ in ISCs, which stabilizes the plasma membrane association of the Wnt transducer Dvl, likely facilitating Wnt/β-catenin signaling. Absence of Cftr-dependent suppression of ISC proliferation in the CF intestine may contribute to increased risk for intestinal tumors. *(Cell Mol Gastroenterol Hepatol 2017;■:■–■; https://doi.org/10.1016/j.jcmgh.2017.11.013)*

Keywords: Cystic Fibrosis; Disheveled; Organoids; Intracellular pH; Neoplasia.

Cystic fibrosis (CF) is a heritable genetic disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The gene product (CFTR) is a major anion channel of fluid transporting epithelia where it functions in transepithelial Cl^- and HCO_3^- secretion.[1,2] CF affects multiple organs, in particular the airway epithelia where failure of mucociliary clearance results in bacterial colonization of the lung. However, intestinal disease is one of the earliest manifestations of CF and presents with life-long conditions, including small intestinal bacterial overgrowth,[3] low-grade small-bowel inflammation,[4,5] obstructive bowel disease,[6,7] and an increased incidence of gastrointestinal (GI) cancer.[8,9] Cftr knockout (KO) mice recapitulate CF intestinal disease without significant manifestations of pancreatic, liver, or lung disease. The disease phenotype includes a high incidence of bowel obstruction,[10] low-grade bowel inflammation,[11] small intestinal bacterial overgrowth,[3] dysbiosis,[12] and the spontaneous development of intestinal tumors with age, inclusive of invasive forms.[13]

Previous in vivo studies have shown that Cftr KO mice show increased intestinal epithelial proliferation without a corresponding increase in apoptosis,[14] a condition that may predispose to intestinal neoplasia.[15] Recent epidemiologic studies have shown strong correlations between the rate of stem cell division and the incidence of cancer.[16] Because the intestine has one of the highest rates of epithelial turnover in the body, pathologic manifestations of CF that enhance the rate of epithelial turnover and contribute to intestinal inflammation are predicted to increase the risk of GI cancer. However, a mechanistic understanding linking the absence of Cftr with enhanced proliferation of the intestinal epithelium, particularly the stem cell population, has not been advanced.

Cftr is highly expressed in intestinal crypts,[17,18] the proliferative compartment of the intestine, and by providing apical membrane Cl^- and HCO_3^- ion permeability has an impact on the regulation of epithelial intracellular pH (pH_i). Loss of Cftr function by acute channel blockade or in Cftr KO enteroids results in an incompletely compensated alkaline pH_i in the crypt epithelium.[19] Compensation of the alkaline pH_i is impaired by a corresponding increase in intracellular Cl^- concentration, which reduces cellular anion exchange activity.[20] Several aspects of cell proliferation are known to be facilitated by an alkaline pH_i, including cell-cycle phase progression at G2/M,[21] optimization of DNA replication,[22] cytoskeleton remodeling and cell migration,[23,24] and membrane biogenesis.[25] Cell alkalinity also has been shown to facilitate Wnt signaling,[26,27] which may directly affect stem cell proliferation.

Wnt/β-catenin signaling is essential for homeostasis and proliferation of intestinal stem cells[28] and often aberrantly is activated in intestinal cancer. In *Drosophila* species, pH_i changes can alter Wnt signaling by modulating the interaction of the initial signal mediator Disheveled (Dvl) with the Wnt receptor Frizzled (Fz) at the plasma membrane.[26] The critical binding of Dvl's PDZ domain with the PDZ binding domain of Fz is facilitated by a stable interaction of Dvl's polybasic DEP domain to negatively charged phospholipids (phosphatidic acid, phosphatidylglycerol) at the inner leaflet of the plasma membrane. Phospholipid interaction is pH_i- and charge-dependent such that proton electrostatic interference at an acidic pH_i reduces DEP domain membrane binding and subsequent Wnt signaling.[26] We hypothesized that an alkaline pH_i in Cftr KO intestinal stem cells (ISCs) stabilizes Dvl interaction at the plasma membrane, thereby facilitating Wnt/β-catenin signaling.

The present study investigated augmented proliferation of the intestinal epithelium in a Cftr KO mouse model. Studies first examined whether hyperproliferation persists in Cftr KO enteroid culture, which isolates the epithelium from the immediate consequences of an abnormal Cftr KO intestinal environment (inflammation, dysbiosis) and provides the technological advantage of live crypt cell imaging.[3,11,29] Second, studies evaluated the activation status of Wnt/β-catenin signaling in the Cftr KO intestine and the functional activity of Cftr in ISCs, specifically, leucine-rich G-protein–coupled receptor 5 (Lgr5)+ stem cells.[30] Third, live cell imaging was used to examine the hypothesis that alkalinity of Cftr KO intestinal crypt base columnar stem cells was conducive to increased interaction of Dvl (ie, the major isoform Dvl2[31]) with the plasma membrane for Wnt signaling.

Materials and Methods

Mice

Mice with gene targeted disruptions of the murine homolog of *Cftr* (*abcc7*, Cftr KO) and sex-matched wild-type (WT, +/+ or +/-) littermates were used.[10] Mice were outbred to Black Swiss (Charles River) mice at generational intervals and resultant F1 heterozygotes were crossed to generate F2 offspring for experimentation. The Cftr KO mouse line was crossed with Lgr5–*enhanced green fluorescent protein* (EGFP)-IRES-creERT2 (Lgr5-EGFP; Jackson Laboratories) mice to generate WT/Lgr5-EGFP and Cftr KO/Lgr5-EGFP mice. The Cftr KO mouse line also was crossed with both Dvl2 KO/Dvl2-EGFP BAC transgenic[32] and Gt(ROSA)$^{26Sortm4(ACTB-tdTomato,-EGFP)Luo}$/J ($Rosa^{mT/mG}$; Jackson Laboratories) mouse lines to generate WT and Cftr KO/Dvl2 KO/Dvl2-EGFP/$Rosa^{mT/mG}$ mice. Genotypes were identified by polymerase chain reaction analysis of tail-snip DNA as previously described for mutant Cftr,[33] Dvl2 KO and Dvl2-EGFP expression,[34] and $Rosa^{mT/mG}$ (Jackson Laboratories). Copy number for the Dvl2-EGFP transgene was verified by TaqMan GFP copy number assay (ThermoFisher Scientific). Only mice expressing 2 copies of the Dvl2-EGFP

Abbreviations used in this paper: **CBC, crypt-base columnar cell; CCH, carbachol; CF, cystic fibrosis; Cftr, cystic fibrosis transmembrane conductance regulator; DEP, _____; Dvl, Disheveled; EdU, 5-ethynyl-2'-deoxyuridine; EGFP, *enhanced green fluorescent protein*; Fz, Frizzled; GI, gastrointestinal; ISC, intestinal stem cell; KO, knockout; Lgr5, leucine-rich G-protein–coupled receptor 5; PBS, phosphate-buffered saline; PDZ, _____; pHi, intracellular pH; PH3, phospho-histone H3; ROI, region of interest; WT, wild type.**

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