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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

# Requirement of $G\alpha_q/G\alpha_{11}$ Signaling in the Preservation of Mouse Intestinal Epithelial Homeostasis

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## SUMMARY

Gut hormones are important in coordinated actions of intestine and they exert actions through G-protein–coupled receptors. We show that $G\alpha_{q/11}$-mediated signaling plays a pivotal role in the maturation and positioning of Paneth cells and in the maintenance of intestinal homeostasis.

**BACKGROUND & AIMS:** Proliferation, differentiation, and morphogenesis of the intestinal epithelium are tightly regulated by a number of molecular pathways. Coordinated action of intestine is achieved by gastrointestinal hormones, most of which exert these actions through G-protein–coupled receptors. We herein investigated the role of $G\alpha_{q/11}$-mediated signaling in intestinal homeostasis.

**METHODS:** Intestinal tissues from control (*Gnaq*$^{flox/flox}$ *Gna11*$^{+/+}$), Int-$G_q$ knock-out (KO) (*VilCre*$^{+/-}$*Gnaq*$^{flox/flox}$ *Gna11*$^{+/+}$), $G_{11}$ KO (*Gnaq*$^{flox/flox}$*Gna11*$^{-/-}$), and Int-$G_q/G_{11}$ double knock-out (DKO) (*VilCre*$^{+/-}$*Gnaq*$^{flox/flox}$*Gna11*$^{-/-}$) mice were examined by microscopy, transmission electron microscopy, and immunohistochemistry. The effect of $G\alpha_{q/11}$-mediated signaling was studied in the cell lineage, proliferation, and apoptosis. Dextran sodium sulfate (DSS) colitis was induced to study the role of $G\alpha_{q/11}$ in colon.

**RESULTS:** Paneth cells were enlarged, increased in number, and mislocalized in Int-Gq/G11 DKO small intestine. Paneth cells also reacted with PAS and Muc2 antibody, indicating an intermediate character of Paneth and goblet cells. The nuclear $\beta$-catenin, T-cell factor 1, and Sox9 expression were reduced severely in the crypt base of Int-Gq/G11 DKO intestine. Proliferation was activated in the crypt base and apoptosis was enhanced along the crypt. Int-Gq/G11 DKO mice were susceptible to DSS colitis. Proliferation was inhibited in the crypt of unaffected and regenerative areas. Cystic crypts, periodic acid–Schiff–positive cells, and Muc2-positive cells were unusually observed in the ulcerative region.

**CONCLUSIONS:** The $G\alpha_{q/11}$-mediated pathway plays a pivotal role in the preservation of intestinal homeostasis, especially in Paneth cell maturation and positioning. Wnt/$\beta$-catenin signaling was reduced significantly in the crypt base in $G\alpha_q/G_{11}$-deficient mice, resulting in the defective maturation of Paneth cells, induction of differentiation toward goblet cells, and susceptibility to DSS colitis. *(Cell Mol Gastroenterol Hepatol 2016;2:767–782; http://dx.doi.org/10.1016/j.jcmgh.2016.08.001)*

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The epithelium of small intestine is composed of 4 distinct cell lineages: absorptive enterocytes and 3 secreting cell types including mucus-secreting goblet cells, antimicrobial peptide-secreting Paneth cells, and hormone-secreting enteroendocrine cells. All of these cell types originate from multipotent stem cells residing in niches in the lower parts of the crypt. Cell renewal, lineage commitment, and cell differentiation in the intestinal epithelium are coupled to cell migration in a precise, spatially organized manner.[1] Stem cells give rise to progenitor cells, which are amplified by constant division along the bottom two thirds of the crypts.[2] These daughter cells migrate up as they proliferate. In the transit-amplifying zone near the top of the crypt, these cells terminally differentiate into the 4 main cell types. Then, absorptive enterocytes, goblet cells, and enteroendocrine cells migrate up the villi and Paneth cells migrate down to reside at the crypt base. The epithelium of large intestine lacks Paneth cells.

The digestive tract consists of a variety of tissues, each with a specific function necessary for the effective handling of a meal. The coordination of the complex functions of digestion, absorption, and excretion of a meal is achieved largely by molecules of neuroendocrine origin.[3] Gastrointestinal hormones, including cholecystokinin, gastrin, secretin, histamine, glucose-dependent insulinotropic polypeptide, glucagon-like-peptide-1, and vasoactive intestinal peptide, regulate their target cells through guanine

***Abbreviations used in this paper:*** **Atoh1, atonal homolog 1; BrdU, bromodeoxyuridine; Defa1, defensin $\alpha$1; Dll1, delta-like 1; DSS, dextran sodium sulfate; FGF, fibroblast growth factor; Fzd, frizzled; Hes, hairy/enhancer of split; IEC, intestinal epithelial cell; Ihh, Indian hedgehog; mRNA, messenger RNA; NICD, Notch intracellular cytoplasmic domain; PAS, periodic acid–Schiff; PCR, polymerase chain reaction; PKC, protein kinase C; qPCR, quantitative real-time polymerase chain reaction; Tcf, T-cell factor; TEM, transmission electron micrograph; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.**

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nucleotide-binding protein-coupled receptors (G-protein–coupled receptors). Another important group of modulators are neurotransmitters, such as acetylcholine, which are released from vagal nerve terminals and exert their roles through the muscarinic receptor subtype M3. Secretin, glucose-dependent insulinotropic polypeptide, and glucagon-like-peptide-1 exert their signals through the $G\alpha_s$ family of heterotrimeric G proteins.[4] In contrast, cholecystokinin, gastrin, and acetylcholine exert their signals through the $G\alpha_q$ family of G proteins. Mammals express 4 $G\alpha_q$ class $\alpha$-subunits, of which 2, $G\alpha_q$ and $G\alpha_{11}$, are widely expressed[5]; their activation results in the stimulation of phospholipase C-$\beta$ isoform and consequent inositol 1,4,5-triphosphate-mediated intracellular calcium mobilization and protein kinase C (PKC) activation.[6] The expression of $G\alpha_{14}$ and $G\alpha_{15/16}$ is restricted to certain tissues, such as kidney and hematopoietic organs, respectively.[7,8]

The gastrointestinal system is a rich source of neuroendocrine hormones that interact with at least 10 families of G-protein coupled receptors containing more than 30 known receptor subtypes.[3] Although the physiological relevance in the regulation of intestinal homeostasis remains unclear, the sheer number of potential $G\alpha_{q/11}$-coupled receptors suggests an importance of this G-protein family in the intestine.

Proliferation, differentiation, and morphogenesis in the intestinal epithelium are tightly regulated by a number of molecular pathways. Stem cells generate daughter cells that undergo lineage commitment and maturation through the combined action of the Wnt/$\beta$-catenin and Notch signaling pathways. Cells adopt either an absorptive or a secretory cell fate according to the balance between Wnt and Notch signaling.[9] Both pathways also regulate transcription networks that further define the differentiation of intestinal epithelial cells (IECs).[10]

The most established effects of Wnt/$\beta$-catenin in IECs are those involved in cell proliferation, in particular by maintaining the proliferative state of progenitors.[11] However, Wnt/$\beta$-catenin signaling is not confined to proliferating immature cells, but also is active in fully differentiated Paneth cells.[11] Wnt/$\beta$-catenin signaling maintains the correct positioning of the Paneth cells by controlling the expression of genes encoding ephrin B2/B3 receptors and the ephrin B1 ligand[1] and terminal maturation of Paneth cells.[11–15]

The Notch cascade mediates cell-to-cell signaling and has been shown to be essential for the maintenance of the proliferative crypt compartment, as well as for the formation of absorptive enterocytes.[10] Notch signaling occurs when the transmembrane Notch receptor is bound by ligands expressed on adjacent cells. The intracellular cytoplasmic domain of the receptor is cleaved from the transmembrane domain by $\gamma$-secretase and translocates to the nucleus, where it associates with the transcriptional activator protein RBP-Jk (CSL/CBF/Su[H]/Lag-1) to stimulate the expression of target genes, such as Hairy/enhancer of split 1 (Hes1) and Hes5.[16] Progenitor cells that express Hes1 will differentiate into absorptive enterocytes, whereas progenitors that express atonal homolog 1 (Atoh1, also known as Math1) are committed to the secretory lineage, differentiating into goblet, Paneth, or enteroendocrine cells. Goblet and Paneth cells continue to share similar characteristics, whereas the differentiation of secretory precursors into endocrine fate is regulated by neurogenin3.[17]

To study the role of the $G\alpha_q$ class of G proteins in intestinal homeostasis, we generated and examined mice with intestine-specific knockout of the genes encoding $G\alpha_q$ and $G\alpha_{11}$. We herein show that the $G\alpha_{q/11}$-mediated signaling pathway plays a pivotal role in Paneth cell maturation and positioning. It also plays a critical role in the maintenance of intestinal homeostasis.

## Materials and Methods

### Animals

C57BL/6 (B6) $Gnaq^{flox/flox}Gna11^{-/-}$ mice were kindly provided by Dr Stefan Offermanns of the Institute of Pharmacology, University of Heidelberg (Germany). To delete *Gnaq* in intestinal epithelial cells in adult mice, we crossed $Gnaq^{flox/flox}Gna11^{-/-}$ mice to Villin-Cre transgenic mice, which express Cre recombinase under the control of the villin promoter.[18] The villin promoter drives stable and homogeneous expression of Cre recombinase in nearly all epithelial cells in the small intestine and, to a lesser extent, the large intestine.[18]

All mice were maintained in a specific pathogen-free animal facility with free access to food and water, except for during experiments on dextran sodium sulfate (DSS)-induced colitis. All experiments using mice were approved by the Institutional Animal Care and Use Committee of Akita University.

### Polymerase Chain Reaction for Genotyping, Conventional Reverse-Transcription Polymerase Chain Reaction, and Quantitative Real-Time Polymerase Chain Reaction

The primers used in this study are listed in Supplementary Table 1. Genomic DNA was isolated from mouse tails and amplified by standard polymerase chain reaction (PCR). Total RNA was obtained from IECs using an RNeasy Mini kit (Qiagen, Valencia, CA), which were scraped off the intestinal tissue with a spatula. First-stranded complementary DNA was synthesized from total RNA using Superscript First-stranded Synthesis System for reverse-transcription PCR (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Quantitative real-time PCR (qPCR) was performed using ABI PRISM 7900HT (Applied Biosystems, Foster City, CA): denaturation at 94°C for 30 seconds, annealing at 60°C for 30 seconds, and extension at 72°C for 30 seconds.

### Materials

The primary antibodies used in the studies are listed in Supplementary Table 2. Horseradish-peroxidase–conjugated donkey anti-mouse IgG, horseradish-peroxidase–conjugated donkey anti-rabbit IgG, horseradish-peroxidase–conjugated donkey anti-goat IgG, and Cy3-conjugated donkey anti-rabbit IgG were from Jackson Immuno Research (West Grove, PA). DSS (molecular weight, 36–50 kilodaltons) was purchased from MP Biomedicals (Solon, OH).