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Inhibiting RHOA Signaling in Mice Increases Glucose Tolerance and Numbers of Enteroendocrine and Other Secretory Cells in the Intestine

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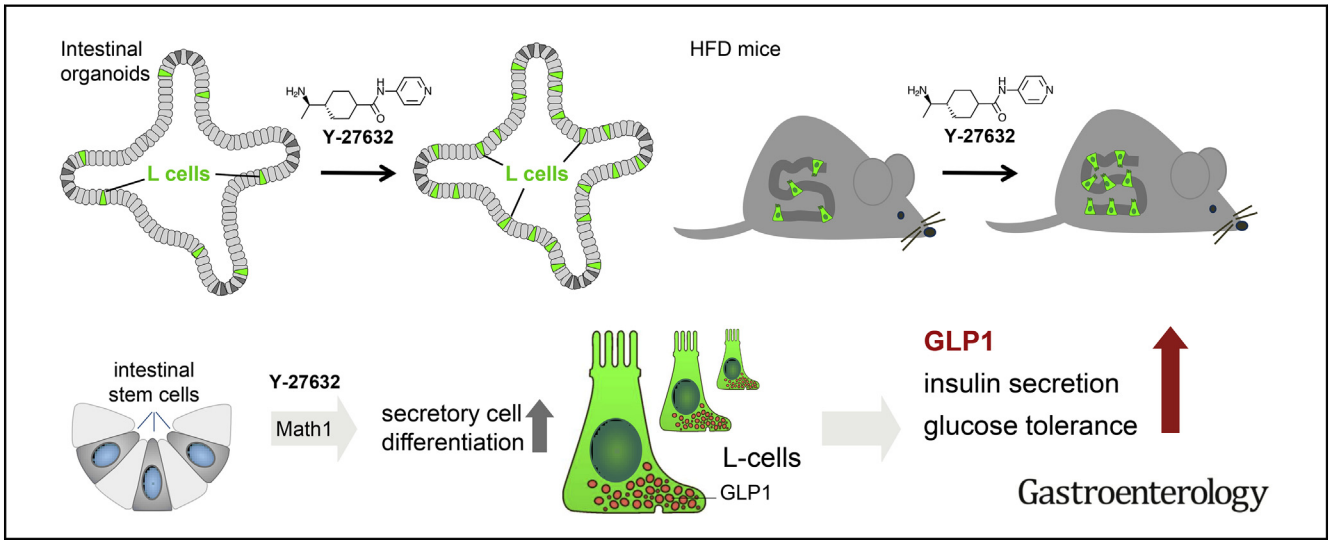

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BACKGROUND & AIMS: Glucagon-like peptide 1 (GLP1) is produced by L cells in the intestine, and agonists of the GLP1 receptor are effective in the treatment of diabetes. Levels of GLP1 increase with numbers of L cells. Therefore, agents that increase numbers of L cell might be developed for treatment of diabetes. Ras homologue family member A (RhoA) signaling through Rho-associated coiled–coil-containing protein kinases 1 and 2 (ROCK1 and ROCK2) controls cell differentiation, but it is not clear whether this pathway regulates enteroendocrine differentiation in the intestinal epithelium. We investigated the effects of Y-27632, an inhibitor of ROCK1 and ROCK2, on L-cell differentiation. **METHODS:** We collected intestinal tissues from GLU-Venus, GPR41-RFP, and Neurog3-RFP mice, in which the endocrine lineage is fluorescently labeled, for in vitro culture and histologic analysis. Small intestine organoids derived from these mice were cultured with Y-27632 and we measured percentages of L cells, expression of intestinal cell-specific markers, and secretion of GLP1 in medium. Mice were fed a normal chow or a high-fat diet and given Y-27632 or saline (control) and blood samples were collected for measurement of GLP1, insulin, and glucose. **RESULTS:** Incubation of intestinal organoids with Y-27632 increased numbers of L cells and secretion of GLP1. These increases were associated with upregulated expression of genes encoding intestinal hormones, neurogenin 3, neurogenic differentiation factor 1, forkhead box A1 and A2, and additional markers of secretory cells. Mice fed the normal chow diet and given Y-27632 had increased numbers of L cells in intestinal tissues, increased plasma levels of GLP1 and insulin, and lower blood levels of glucose compared with mice fed the normal chow diet and given saline. In mice with insulin resistance induced by the high-fat diet, administration of Y-27632 increased secretion of GLP1 and glucose tolerance compared with administration of saline. **CONCLUSIONS:** In mouse intestinal organoids, an inhibitor of RhoA signaling increased the differentiation of the secretory lineage and the development of enteroendocrine cells. Inhibitors

of RhoA signaling or other strategies to increase numbers of L cells might be developed for treatment of patients with type 2 diabetes or for increasing glucose tolerance.

Keywords: Mouse Model; Type 2 Diabetes; Enteroendocrine Cells; Signal Transduction.

WHAT YOU NEED TO KNOW

BACKGROUND AND CONTEXT

Synthetic analogues of GLP1, a hormone produced by intestinal L-cells, are effective against type 2 diabetes. A modulation of L-cell number can increase the body's own GLP-1 production and become a therapeutic alternative, however, the pharmacological targets are unknown.

NEW FINDINGS

The ROCK inhibitor Y-27632 increases the numbers of L-cells and other secretory cells in intestinal organoids. Y-27632 increased L cell numbers, GLP1 secretion and glucose tolerance in normal chow and high-fat diet-fed mice.

LIMITATIONS

The ROCK inhibitor increased the number of other secretory cells, which might result in excessive mucous production and reduced absorptive capacity of the intestine.

IMPACT

The RhoA/ROCK pathway is important for differentiation of L cells and other secretory cells and may form the basis for modulation of L-cell numbers for type 2 diabetes treatment.

Introduction

Synthetic analogues of the intestinal hormone glucagon-like peptide 1 (GLP1) are widely used for the treatment of type 2 diabetes because of the insulinotropic and beneficial metabolic properties of the hormone. The continued discovery of new beneficial effects of GLP1, in various tissues, indicates its potential in the treatment of systemic metabolic disorders.[1,2] The endogenous GLP1, mainly produced by intestinal epithelial L cells, is very short lived in the blood stream because of efficient inactivation by dipeptidyl peptidase-4. Studies of patients with type 2 diabetes undergoing gastric bypass operations suggest that it is possible to restore glucose tolerance in part through increased secretion of GLP1 and other gut hormones.[3] Moreover, our previous work showed that enhancing L-cell differentiation could provide a possible treatment strategy for patients with type 2 diabetes.[4] The apparent benefit of this approach would be enhanced release of GLP1 in addition to contributions of other L-cell hormones (oxyntomodulin and peptide YY [PYY]) and their beneficial metabolic action at their effective sites (ie, the sensory vagal afferents).[5,6] In addition, because the intestinal epithelium renews itself every 4–5 days, with secretory lineages showing a significantly longer retention, the modulation of cell numbers probably would not require frequent drug administration, such as injected GLP1 analogues, some of which are taken before meals.

L cells originate from a common secretory progenitor, marked by the expression of the transcription factor mouse atonal homologue 1 (Math1).[7,8] At commitment to an enteroendocrine fate, cells start expressing neurogenin 3 (Ngn3),[9,10] and as cells irreversibly commit to the specialized enteroendocrine fate, cells become postmitotic and decrease Ngn3 expression. These cells subsequently express neurogenic differentiation factor 1 (NeuroD1), which is specific for the enteroendocrine cell (EEC) lineage,[8] and common markers of secretory linages including forkhead box A1 and A2 (Foxa1 and Foxa2), which control the differentiation of L cells, D cells, and goblet cells.[11] The terminally differentiated EEC will secrete a complement of hormones, of which GLP1-producing cells typically coexpress secretin, cholecystokinin (CCK), PYY, and neurotensin.[12,13] These hormones have multiple beneficial effects on metabolism, because they regulate insulin secretion, appetite and food intake, and modulate gastrointestinal motility and gastropancreatic secretions. Thus, this branch of EECs could be exploited for the correction of pathophysiologic conditions such as type 2 diabetes and obesity.

Therapeutically applicable pharmacologic targets for modulation of L-cell differentiation have not been identified. However, recent advances in understanding the mechanisms driving overall differentiation of intestinal epithelial cell types[14] have allowed modulation of tissue composition. As an example, inhibition of Notch causes a profound increase in the number of secretory cells. This results in larger numbers of L cells and enhanced secretion of GLP1, leading to curative effects in a type 2 diabetes mouse model.[6] A recent study by Basak et al[15] showed that preventing growth and differentiation of absorptive enterocytes, Paneth cells, and goblet cells by combined inhibition of wingless-type MMTV integration site family (Wnt), Notch, and epidermal growth factor receptor and mitogen-activated protein kinase pathways in vitro results in enhanced differentiation toward the EEC lineage. This provides conceptual evidence for the directed differentiation of epithelial cells in vitro. However, this approach is difficult to translate to an in vivo setting, because signaling by Wnt, Notch, and epidermal growth factor receptor are essential

Abbreviations used in this paper: **CCK, cholecystokinin; Ctgf, connective tissue growth factor; Cyr61, cysteine-rich angiogenic inducer 61; Dll1, delta-like protein 1; DBZ, dibenzazepine; EdU, 5-ethynyl-2′-deoxyuridine; EEC, enteroendocrine cell; Foxa1 and 2, forkhead box A1 and A2; GCG, preproglucagon; GIP, glucose-dependent insulinotropic peptide; GLP1, glucagon-like peptide 1; Hes1, hairy and enhancer of split-1; I-Fabp, intestinal fatty acid binding protein; ITF, intestinal trefoil factor; Lgr5, leucine-rich repeat-containing G protein-coupled receptor; Lyz1, lysozyme 1; Math1, mouse atonal homologue 1; Ngn3, neurogenin 3; PYY, peptide YY; qPCR, quantitative polymerase chain reaction; RFP, red fluorescent protein; RhoA, Ras homologue family member A; ROCK, Rho-associated protein kinase; Wnt, wingless-type MMTV integration site family; YAP, yes-associated protein.**

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