Using Enteroendocrine Cell–Enriched Human Enteroids to Evaluate Responses to Gut Stimuli

Enteroendocrine cells produce and secrete peptide hormones in response to luminal content that act locally or systemically to regulate various functions in the gastrointestinal (GI) tract including food intake, digestion, and motility. Although enteroendocrine cells comprise less than 1% of epithelial cells in the GI tract, together they make up the largest endocrine organ in the body. Enteroendocrine cells are characterized (at least 15 types) on the basis of which peptide hormones they secrete, with enterochromaffin (EC) cells that secrete serotonin making up the largest percentage (~ 40%).\(^1\) Until recently, much of our understanding of how enteroendocrine cells respond to various gut stimuli has come from animal models and cancer-derived cell lines.

Actively dividing, Lgr5\(^+\) intestinal stem cells give rise to all the major epithelial cell types. Neurogenin 3 (NGN3) is the major transcription factor that directs secretory progenitors to differentiate into enteroendocrine cells. Although the pathways responsible for specialization of each EC subtype remain to be determined, segment-specific expression of enteroendocrine cell subtypes in murine enteroids has been demonstrated. NGN3 transgenic mice exhibited enhanced expression of enteroendocrine cells that correlated with a decrease in goblet cell numbers.\(^2\) Similarly, expression of NGN3 in human induced pluripotent stem cell derived intestinal organoids also enriched the expression of enteroendocrine cells that could sense and respond to increased luminal glucose levels.\(^2\) Alternatively, enteroendocrine cells can be preferentially differentiated in murine enteroids after pharmacologic inhibition of Wnt and Notch signaling as well as blocking MEK activity.\(^4\) Whether the population of enteroendocrine cells could also be similarly increased in human enteroids had not yet been established.

In this issue of *Cellular and Molecular Gastroenterology and Hepatology*, Chang-Grahame et al\(^3\) transduced a human jejunal enteroid line with a tet inducible-NGN3 construct to enhance the expression of enteroendocrine cells, which was similar in three-dimensional (ie, as spheroids) and planar (ie, monolayer) conformations. On induction, the authors observed increased differentiation of chromogranin A–positive and serotonin-positive cells, indicating increased expression of enteroendocrine cells, which was correlated with the detection of enteroendocrine cell hormones and enzymes. In contrast to overexpression of NGN3 in mice, the increase in enteroendocrine cells in human enteroids was not at the expense of absorptive or other secretory cell lineages, suggesting that subtle differences may exist between mice and humans related to secretory cell differentiation. Although only jejunal enteroids were used in this study, future characterization of NGN3-induced human enteroids from other regions of the small intestine and stomach to confirm segment-specific expression and function of enteroendocrine cell subtypes could expand the utility of this model.

To probe their ability to sense and respond to various stimuli, NGN3-induced enteroid monolayers were exposed to norepinephrine or human rotavirus. When compared with norepinephrine treatment, enteroendocrine cell–enriched enteroids differentially responded to rotavirus infection as demonstrated by increased polarized secretion of additional hormones, including PYY and ghrelin, confirming that multiple enteroendocrine cell subtypes (eg, EC cell, K cell, and L cell) are present in NGN3-induced enteroids. Importantly, this differential response indicates that this model may be a powerful tool to screen how enteroendocrine cells respond to other luminal gut contents including nutrients, drugs, commensal and pathogenic microbes, as well as mucosal factors from underlying mesenchymal, immune, and neuronal cells in the intestinal mucosa.

The recent development of various bioengineered platforms, matrices, and co-culture systems to generate more complex enteroid models could provide an opportunity to further understand how enteroendocrine cell–secreted hormones affect non-epithelial components of the intestinal mucosa. Moreover, because human enteroids also preserve the epithelial phenotype of the donor from which they were derived, enteroendocrine cell–enriched enteroids could also be used to understand how enteroendocrine cell function is altered in conditions such as metabolic disease. The ability to “program” human intestinal stem cell–derived enteroids to specifically increase the prevalence of a particular epithelial cell type is a unique feature of these cultures that further expands their potential for scientific discovery.

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Conflicts of interest
The author discloses no conflicts.

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