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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

EDITORIAL

A New Role for Endocrine Cells in the Intestinal Crypt

The stem cells of the crypt are a major determinant of gut growth, with increased proliferation resulting in expansion of the functional intestinal epithelium. In the small intestine, the crypt of Lieberkühn is relatively isolated from the luminal contents, because of a narrow neck, a mucus barrier, and defensive products secreted by the Paneth cells. Several signaling molecules, including Wnts and BMPs, play critical roles in regulating intestinal stem cell proliferation and differentiation.[1] However, nutritional status is also well-established to affect stem cell number and function in the gut. For example, feeding of a high-fat diet increases proliferation of the stem cells and switches their key metabolic pathway toward fatty acid oxidation.[2] Paradoxically, the same changes are observed in the fasting state, presumably as an anticipatory mechanism to prepare for subsequent food intake.[3] However, despite this knowledge, the mechanisms underlying the regulation of the stem cells by ingested nutrients have remained unclear. The recent study by McCauley et al[4] in *Cellular and Molecular Gastroenterology and Hepatology* now demonstrates an essential role for enteroendocrine cells in linking luminal nutrients to stem cell proliferation and activity in the small intestine.

McCauley et al[4] developed a gut endocrine cell-depletion model by crossing inducible *Villin-creERT2* mice with *Ngn3*$^{fl/fl}$ animals, neurogenin-3 being a master regulator of intestinal endocrine cell differentiation.[5] Unlike humans with inactivating mutations in neurogenin-3,[6] the mice did not demonstrate marked changes in whole-body metabolism. They also did not exhibit diarrhea, likely because of greater expression of the *Villin* promoter in the small intestine as compared with the colon. Consistent with this finding, no changes in fecal short-chain fatty acids were detected, suggestive of normal microbial function, although that of the small intestine was not assessed. The results of metabolomics on jejunal crypts lacking endocrine cells demonstrated reduced production of ATP and ADP, and an increase in oxygen consumption rate caused by enhanced fatty acid oxidation. In parallel, increased non-Paneth cell mitochondrial activity was observed in crypts from the mutant mice, similar to that observed in crypts from fasted, normal mice. Decreased activation of mTORC1, an inhibitor of lipolysis, was also detected in Paneth cells from endocrine cell-deficient crypts and fasted animals, further suggesting increased reliance on fatty acid metabolism. Importantly, these findings were consistent with the demonstration of increased expression of lipid metabolic genes in the stem, progenitor, and Paneth cells from a neurogenin-3 null human organoid. Finally, increased proliferation and expression of the stem/progenitor cell marker, olfactomedin-4, was observed in the absence of enteroendocrine cells, in addition to enhanced enteroid-forming capacity, a change that was reversed by incubation in a nutrient-rich media.

The results of this study are consistent with previous studies demonstrating that intestinal stem cell fatty acid oxidation and proliferation and activity are increased by fasting in mice.[3] However, unexpectedly, the dependence on reduced mTORC1 for the changes observed in the present study differs from the finding that activation of mTORC1 increases stem cell proliferation and adaptation in a murine model of short bowel.[7] Other studies have also demonstrated that increased mTORC1 activity in either the Paneth cells or, alternatively, the neighboring stem cells is responsible for their enhanced proliferation in the setting of chronic caloric restriction.[8,9] When taken together, these findings suggest that multiple mechanisms underlie the changes in stem cell behavior in response to differential nutrient supply. Nonetheless, the current study extends these previous findings by the demonstration that enteroendocrine cells provide essential signals regulating the relationship between nutrient ingestion and stem cell behavior, at least in the fasted state.

Although an exciting new finding, many questions remain, the most notable being the identity of the specific enteroendocrine cells and of the secreted product that mediates this "nutrient-crypt" axis. Intestinal endocrine cells are well-known to release a diversity of peptide hormones, depending on their localization along the aboral axis. For example, the upper gut produces mainly secretin and cholecystokinin, and glucose-dependent insulinotrophic polypeptide, whereas the distal small intestine releases neurotensin and glucagon-like peptide-1 and -2. Collectively, these hormones play essential roles in intestinal nutrient digestion, absorption, transit, and growth, and in whole-body metabolism and feeding behavior.[10] However, recent studies have indicated that many enteroendocrine cells are polyhormonal and, indeed, can undergo hormone-switching during migration up the crypt-villus axis, whereas others have demonstrated that some of these cells release additional bioactive compounds, such as ATP, L-glutamate, and serotonin.[11-14] Nonetheless, 2 recent studies have linked neurotensin and glucagon-like peptide-2, hormones released in response to nutrient ingestion, to the enhancement of stem cell proliferation in the gut.[15,16] Whether these or other gut hormones or bioactive molecules can account for the findings of the present study, and if they are mediated through paracrine or endocrine actions from villus and/or crypt endocrine cells, remains to be determined. In addition, it is unclear as to whether altered nutrient handling by the small intestine may have accounted for some of the observed changes in crypt cell activity. It is well-established that crypt cell proliferation follows a circadian rhythm, with increased proliferation occurring during the normal feeding period in mice.[17] Similar rhythms in secretion of several intestinal hormones have also been

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demonstrated.[18] Hence, loss of the gut hormones may have altered stem cell proliferative activity indirectly, through alterations in the timing of nutrient availability to the crypt cells, including not only the stem cells, but also the supportive Paneth cells. Finally, whether the findings of the present study have relevance to disease states, such as inflammatory bowel disease and/or cancer, remains an interesting question, particularly given the known changes in mitochondrial function and cellular metabolism that are associated with both of these morbidities.[19] These questions, and likely others, will be answered by future studies using hormone- and/or hormone receptor–specific null models, under physiological conditions and in disease states.

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Conflicts of interest
Over the past 2 years, Patricia L. Brubaker has served as consultant to GLyPharma Therapeutic, Novome Biotechnologies, Prolynx Inc, Takeda Pharmaceuticals, VectivBio AG, and Zealand Pharma A/S.

Funding
Studies in the Brubaker laboratory are supported by operating grants from the Canadian Institutes of Health Research (PJT-14853 and PJT-11454).

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2352-345X
https://doi.org/10.1016/j.jcmgh.2023.01.012

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