In vitro effects of folate deficiency on intestinal crypt morphology and stem cell proliferation

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Introduction: Environmental enteropathy (EE), a subclinical manifestation of the undernutrition-infection cycle, is histologically characterized by chronic intestinal inflammation, villous atrophy, and intestinal crypt hypertrophy. In patients with persistent diarrhea and EE, folate supplementation and antibiotics ameliorate diarrhea. However, EE tends to persist or recur following nutritional and antimicrobial therapy. Rat models of folate deficiency show an irreversible small intestinal crypt hypertrophy, suggesting that folate is required to achieve intestinal epithelial homeostasis. We sought to evaluate mechanistic links between dietary folate deficiency, intestinal stem cell proliferation, small intestinal crypt hypertrophy, and EE pathogenesis in a mouse enteroid model.

Methods: Enteroids were prepared by isolating fresh mid-jejunal crypts from well-nourished C57BL/6 mice. Enteroid cultures were randomized to either standard minigut media or media devoid of folate and choline and maintained for 6-9 days before being propagated into additional wells or utilized for experimental procedures. Brightfield images were taken after two passages to assess enteroid morphology. Following a third passage, cell proliferation was assessed using a Click-iT™ EdU imaging kit with Hoechst nuclear stain. Confocal images were analyzed using Imaris™ software.

Results: Enteroids were viable under both standard conditions and conditions of folate and choline deficiency. Cell proliferation in enteroid cultures was 2.2-fold higher in standard media than in folate- and choline-deficient media ($P < 0.0001$). The number of crypt buds per enteroid was 1.6-fold higher in standard media versus deficient media ($P = 0.0007$). The width of the neck of each crypt bud was 1.3-fold greater in standard media than in deficient media ($P = 0.0002$).

Conclusion: Complementary to published findings from rodent models of methyl donor deficiency, the absence of folate and choline from mouse enteroid culture media causes significant alterations in intestinal crypt morphology and stem cell proliferation. Therefore, enteroids have the potential to serve as an *ex vivo* model with which to study the molecular and cellular mechanisms underlying EE and how these mechanisms can be reversed therapeutically.

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