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Critical intestinal cells originate from the host in enteroid-derived tissue-engineered intestine

Barrett P. Cromeens, DO, PhD,[a] *Yijie Wang, MS,*[a] *Yanchun Liu, MD,*[a] *Jed Johnson, PhD,*[b] *and Gail E. Besner, MD*[a,*]

[a] *Department of General Pediatric Surgery, Nationwide Children's Hospital, Columbus, Ohio*
[b] *Nanofiber Solutions, Inc, Hilliard, Ohio*

ARTICLE INFO

Article history:
Received 6 August 2017
Received in revised form
25 September 2017
Accepted 3 November 2017
Available online xxx

Keywords:
Tissue engineering
Short-bowel syndrome
Intestine
Enteroids
Epithelial culture
Intestinal subepithelial myofibroblast

ABSTRACT

Background: Enteroid-derived tissue-engineered intestine (TEI) contains intestinal subepithelial myofibroblasts (ISEMFs) and smooth muscle cells (SMCs). However, these cell types are not present in the donor enteroids. We sought to determine the origin of these cell types and to quantify their importance in TEI development.

Materials and methods: Crypts from pan-EGFP or LGR5-EGFP mice were used for enteroid culture and subsequent implantation for the production of TEI. TEI from pan-EGFP enteroids was labeled for smooth muscle alpha actin (SMA) to identify ISEMFs and SMCs and green fluorescent protein (GFP) to identify cells from pan-EGFP enteroids. Fluorescence *in situ* hybridization (FISH) for the Y chromosome was applied to TEI from male LGR5-EGFP enteroids implanted into female nonobese diabetic/severe combined immunodeficiency mice. To identify chemotactic effects of intestinal epithelium on ISEMFs, a Boyden chamber assay was performed.

Results: Immunofluorescence of TEI from pan-EGFP enteroids revealed GFP-positive epithelium with surrounding SMA positivity and no colocalization of the two. FISH of TEI from male LGR5-EGFP enteroids implanted into female nonobese diabetic/severe combined immunodeficiency mice revealed that only the epithelium was Y chromosome positive. Chemotactic assays demonstrated increased ISEMF migration in the presence of enteroids (983 ± 133) compared to that in the presence of either Matrigel alone (357 ± 36) or media alone (339 ± 24; $P \leq 0.05$).

Conclusions: Lack of GFP/SMA colocalization suggests that ISEMFs and SMCs are derived from host animals. This was confirmed by FISH which identified only epithelial cells as being male. All other cell types originated from host animals. The mechanism by which these cells are recruited is unknown; however, Boyden chamber assays indicate a direct chemotactic effect of intestinal epithelium on ISEMFs.

Introduction

Stem cells are the progenitors of healthy tissue and reside in specific anatomic and functional locations known as the stem cell niche. Although stem cells differentiate into the healthy cells that comprise complex tissues, it is not the stem cells themselves but the complex interplay of these cells within their niche that determines restitution and maintenance.

* *Corresponding author*. Department of Pediatric Surgery, Nationwide Children's Hospital, FOB6135, 700 Children's Drive, Columbus, OH 43205. Tel.: +1 614 722 3930; fax: +1 614 722 3903.
E-mail address: gail.besner@nationwidechildrens.org (G.E. Besner).
0022-4804/$ – see front matter
https://doi.org/10.1016/j.jss.2017.11.015

With increased interest in stem cells as therapeutic targets, understanding the determinant relationships within the niche is of paramount importance for therapeutic success.[1]

The intestine is lined by epithelium comprised of differentiated cells, including enterocytes, enteroendocrine cells, goblet cells, and Paneth cells, all derived from intestinal stem cells which reside in the crypt domain. Like many other stem cell compartments, the intestinal stem cell has been the subject of increasing interest in clinical applications for intestinal pathologies.[2-4] This increased interest has resulted in focused research in understanding the intestinal stem cell niche composed of Paneth cells, extracellular matrix, and varying pericryptal cell types.[5-8] Of particular importance within this niche are the intestinal subepithelial myofibroblasts (ISEMFs) which are aligned below the basement membrane and concentrated around the crypts.[9] The ISEMFs express numerous noncanonical Wnt signals which are implicated in epithelial proliferation and differentiation.[10,11] The importance of ISEMFs is underscored in studies performed using three-dimensional epithelial culture systems known as enteroid cultures. Enteroid cultures are purely epithelial cultures that are maintained without the mesenchymal niche through exogenous replacement of critical growth factors such as R-spondin which contribute to Wnt/β-catenin signaling. Coculturing of enteroids with a feeder layer of ISEMFs results in larger, more robust enteroids that proliferate at a faster rate. In addition, in the presence of ISEMFs, enteroids continue to grow and proliferate in the absence of some of these exogenous factors.[12]

In the production of tissue-engineered intestine (TEI), the importance of each component of healthy functioning intestine is critical. The main goal of producing TEI is to correct short-bowel syndrome, where patients have lost the majority of their bowel, resulting in loss of absorption of vital nutrients.[13-17] Ideally, this would require the development of an entirely functional length of intestine from the patient's native tissue to replace that which has been lost. To do so, all components critical to normal intestinal function must be present. One method of TEI production uses cultured enteroids to seed onto scaffolds and implant into host animals. Using these general methods, the TEI produced has been shown to contain all the major epithelial lineages.[12,18-20] Additional components have also been identified such as ISEMFs and smooth muscle cells (SMCs).[20] The presence of ISEMFs and SMCs within the TEI was unexpected because the enteroids from which the TEI was grown are a purely epithelial culture, and these supportive cells are not present at the time of implantation. This suggests that these critical cell types are either transdifferentiated from donor epithelial lineages through an epithelial-mesenchymal transition or are recruited from the host animal.

In this study, we investigated the origins of ISEMFs and SMCs in enteroid-derived TEI. We set out to determine if these cell types were being recruited from the host animal or transdifferentiated from donor epithelium. In addition, we investigated whether the supplementation of ISEMFs to TEI produces more robust epithelial growth, analogous to that seen with *in vitro* studies. We hypothesized that supplementing TEI with additional ISEMFs will result in a more robust product.

Materials and methods

Animal use

All animal experiments were carried out in accordance with the National Institutes of Health guide for the care and use of laboratory animals, with the approval of our Institutional Animal Care and Use Committee under the protocol # AR12-00001. These experiments were performed with LGR5-EGFP transgenic mice ($n = 20$; male = 11 and female = 9), pan-EGFP transgenic mice ($n = 3$; all male), or nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice ($n = 43$; male = 16 and female = 27), depending on the experimental design. All mice were bred in-house. The LGR5-EGFP and pan-EGFP colonies were obtained from The Jackson Laboratory (Bar Harbor, ME). The NOD/SCID colony was obtained from Charles River Laboratories (Wilmington, MA). All enteroid cultures were obtained from either LGR5-EGFP mice or pan-EGFP mice depending on experimental design. All seeded scaffolds were implanted into NOD/SCID mice.

Isolation of crypts and culture of enteroids

The methods used for the isolation of crypts and subsequent enteroid culture have been described previously.[20] In brief, small intestine was obtained from donor mice, opened longitudinally, minced, and washed with phosphate buffered saline (PBS; Corning, Manassas, VA) until clean. The minced tissue was then incubated in 2-mM ethylenediaminetetraacetic acid (Thermo Fisher Scientific, Waltham, MA) in PBS at 4°C for 30 min. After the incubation, the tissue underwent cycles of agitation by trituration to release mucosal fragments and settling to capture the mucosal fragment–containing supernatant until no further fragments were identified in the supernatant. The supernatant was then filtered through a 70-μm sieve (BioDesign Inc, Carmel, NY) to separate the crypts from the larger fragments. The crypt fraction underwent a series of washes and was resuspended in growth factor–reduced Matrigel (Corning, Manassas, VA) at 100-200 crypts/50 μL of Matrigel. They were then plated into 24-well culture plates at 50 μL/well. Each well was incubated with 500 μL of complete crypt culture medium at 37°C and changed every 3-4 d. The medium contained Advanced Dulbecco's Modified Eagle's Medium (DMEM)/F12 (Thermo Fisher Scientific, Waltham, MA), 2-mM GlutaMAX, 10-mM HEPES, 100-U/mL penicillin/100-μg/mL streptomycin, 1× N2 supplement, 1× B27 supplement (all from Thermo Fisher Scientific, Waltham, MA), 1-mM N-acetylcysteine (Sigma, St. Louis, MO), 50-ng/mL heparin-binding epidermal growth factor-like growth factor (R&D Systems, Minneapolis, MN), 100-ng/mL noggin (R&D Systems, Minneapolis, MN), and 500-ng/mL R-spondin (Sino Biological Inc, Beijing, China).

ISEMF isolation and culture

Donor mice underwent laparotomy, and small intestine from the ligament of Treitz to the terminal ileum was excised. The intestine was opened longitudinally, and the villi were scraped from the surface using a glass cover slip. The intestine was then minced and washed sequentially with PBS until no gross debris