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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Self-renewing Monolayer of Primary Colonic or Rectal Epithelial Cells

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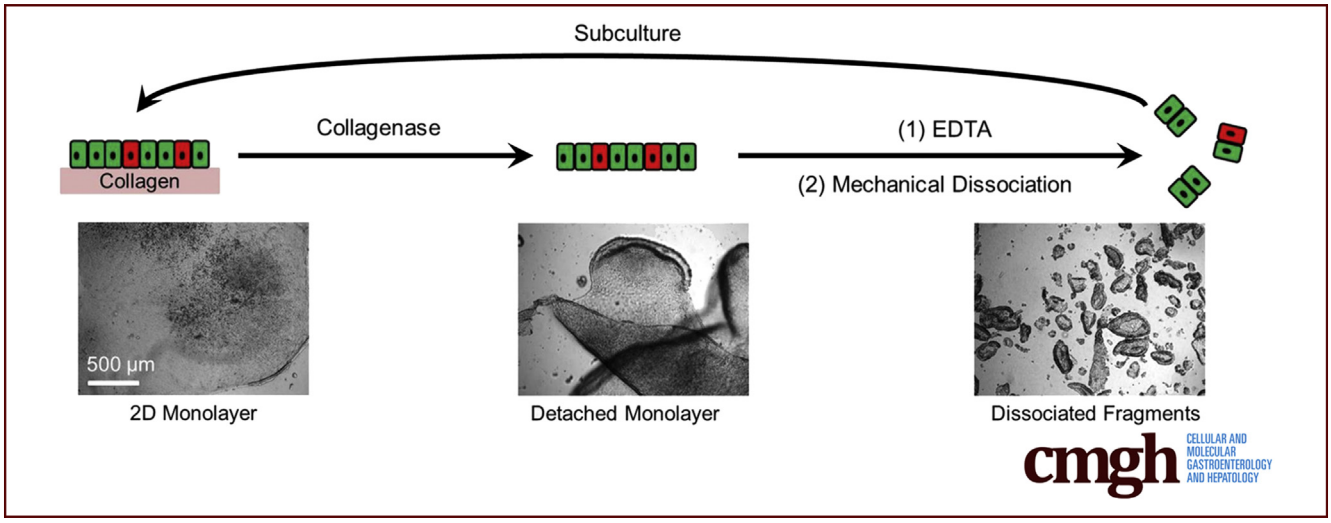

SUMMARY

Self-renewing, 2-dimensional primary colonic epithelial cells are sustained by a combination of surface matrix and chemical factors. The 2-dimensional culture platform is a physiologically relevant system to assay stem cell renewal and differentiation and to screen compounds.

BACKGROUND & AIMS: Three-dimensional organoid culture has fundamentally changed the in vitro study of intestinal biology enabling novel assays; however, its use is limited because of an inaccessible luminal compartment and challenges to data gathering in a three-dimensional hydrogel matrix. Long-lived, self-renewing 2-dimensional (2-D) tissue cultured from primary colon cells has not been accomplished.

METHODS: The surface matrix and chemical factors that sustain 2-D mouse colonic and human rectal epithelial cell monolayers with cell repertoires comparable to that in vivo were identified.

RESULTS: The monolayers formed organoids or colonoids when placed in standard Matrigel culture. As with the colonoids, the monolayers exhibited compartmentalization of proliferative and differentiated cells, with proliferative cells located near the peripheral edges of growing monolayers and differentiated cells predominated in the central regions. Screening of 77 dietary compounds and metabolites revealed altered proliferation or differentiation of the murine colonic epithelium. When exposed to a subset of the compound library, murine organoids exhibited similar responses to that of the monolayer but with differences that were likely attributable to the inaccessible organoid lumen. The response of the human primary epithelium to a compound subset was distinct from that of both the murine primary epithelium and human tumor cells.

CONCLUSIONS: This study demonstrates that a self-renewing 2-D murine and human monolayer derived from primary cells can serve as a physiologically relevant assay system for study of stem cell renewal and differentiation and for compound screening. The platform holds transformative potential for personalized and precision medicine and can be applied to emerging areas of disease modeling and microbiome studies. *(Cell Mol Gastroenterol Hepatol 2017;4:165–182; http://dx.doi.org/10.1016/j.jcmgh.2017.02.011)*

Keywords: Colonic Epithelial Cells; Monolayer; Organoids; Compound Screening.

See editorial on page 203.

Long-term culture of primary intestinal epithelial tissue as a planar monolayer has not been possible because of the rapid loss of stem and proliferative cells and rapid onset of apoptosis when primary epithelium is placed into culture.[1] Investigators have traditionally relied on colon cancer cell lines such as Caco-2 and its derivatives to study gut epithelial physiology because of their ability to grow indefinitely on conventional tissue culture plates.[2] Although cancer cell lines grow as confluent monolayers and can be efficiently passaged, they possess many non-physiologic characteristics including somatic mutations, chromosomal instabilities, altered metabolism, and aberrant proliferative and differentiation characteristics.[3] Together, these non-physiologic properties of Caco-2 cells call into question their predictive ability in assays designed to understand normal epithelial physiology.

Recent advances in epithelial culture conditions now promote intestinal stem cell (ISC) maintenance and indefinite culture of primary intestinal tissue as three-dimensional (3-D) organoids.[4–8] The organoid culture system uses soluble growth factors including Wnt-3A, R-spondin, noggin, and epidermal growth factor (EGF) to mimic the ISC niche environment that supports ISC survival, growth, and differentiation in a thick layer of Matrigel.[5,8–10] Like in vivo, organoid ISCs exhibit their defining properties by self-renewing and giving rise to progenitors that differentiate into absorptive colonocytes (water and electrolyte uptake), goblet cells (mucus production), enteroendocrine cells (hormones), and Paneth cells (antimicrobial and stem cell niche functions).[5] By virtue of their non-transformed condition, 3-D organoids represent a physiologically relevant model enabling novel assays and pharmaceutical and dietary compound screens that are not currently possible with colon cancer cell lines such as Caco-2.[3,11,12]

Although organoid culture technology has had a major positive impact on the in vitro study of primary gut epithelium, the 3-D geometry of organoids prevents access to the apical aspect of the epithelium, producing a number of challenges to physiologically relevant studies. The apical surface of the organoid is analogous to the lumen of the gut where digested contents and microbial communities interact with the epithelium. The spheroidal architecture of the organoids prevents access of exogenous compounds to the luminal epithelial surface, limiting studies focused on apical transporters, receptors, metabolic enzymes, and microbiota.[13] Matrigel embedded organoids exist in multiple planes, making collection of experimental readout by using conventional microscopy exceptionally challenging.[14,15] Unfolding the spherical organoid into a two-dimensional (2-D) planar tissue construct is a solution that addresses these major challenges and has the potential to further transform in vitro study of the gut epithelium.

We have previously demonstrated that primary intestinal epithelial cells can be cultured on polydimethylsiloxane (PDMS) and other artificial surfaces in the absence of a hydrogel.[4] Although they are supplied with the requisite soluble growth factors for growth within Matrigel, culture of primary epithelium on non-hydrogel surfaces produced a short-lived, non-proliferative monolayer of cells. Dissociated 3-D small intestinal and colonic organoids have been cultured on a porous membrane (coated with 0.1% gelatin or 10 $\mu g/cm^2$ collagen) to form a monolayer, but these monolayers were not self-renewing, suggesting that stem cells were lost from the monolayers over time and a self-renewing ISC compartment was not supported.[16,17] The failure of existing 2-D culture methods to produce long-term monolayers suggests that a biochemical environment composed of media and soluble growth factors alone is not adequate to sustain a self-renewing monolayer containing both stem and differentiated cells. To overcome the limitations in monolayer culture duration, we sought to identify parameters that would support self-sustaining monolayers.

Materials and Methods

Isolation of Crypts From Mouse Colon and Human Rectal Biopsies

Male mice were used at age 6–10 weeks. All experiments were performed in compliance with the relevant laws and institutional guidelines at the University of North Carolina (UNC). All experiments and animal usage were approved by the Institutional Animal Care and Use Committee (IACUC) at UNC. Mice were humanely killed by lethal dose of isoflurane, followed by cervical dislocation under the approved UNC IACUC-approved protocol #13-200. A cytomegalovirus enhancer plus chicken actin promoter (CAG)-DsRed mouse model in which all cells expressed the DsRed fluorescent protein was used to monitor the proliferation of colonic epithelial cells by fluorescence microscopy. CAG-DsRed heterozygous mice were bred on a CD-1 background, and wild-type mice were bred on a C57BL/6 background. Wild-type mice were used for fluorescence-based assays and compound screens. An Lgr5EGFPCreERT2xR26 confetti mouse was used for lineage tracing experiments on the 2-D monolayer. The confetti mouse was injected with 5 mg tamoxifen at 48 hours before death and isolation of crypts from the large intestine.[18] Human rectal biopsies were obtained from UNC Hospitals Meadowmont Endoscopy Center

Abbreviations used in this paper: **ALP, alkaline phosphatase; α-ChgA, anti-chromogranin A; α-Muc2, anti-mucin2; CAG, cytomegalovirus enhancer plus chicken actin promoter; CI, confidence interval; 2-D, two-dimensional; 3-D, three-dimensional; ECM, extracellular matrix; EDU, 5-ethynyl-2′-deoxyuridine; EGF, epidermal growth factor; ENR-W, cell medium with [Wnt-3A] of 30 ng/mL; ENR-w, cell medium with [Wnt-3A] of 10 ng/mL; HISC, human intestinal stem cell medium; IACUC, Institutional Animal Care and Use Committee; ISC, intestinal stem cell; PBS, phosphate-buffered saline; PDMS, polydimethylsiloxane; RFP, red fluorescent protein; SEM, scanning electron microscope; SSMD, strictly standardized mean difference; UNC, University of North Carolina.**

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