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EDITORIAL

A Scalable Human Intestinal Planar Culture Offers Opportunities for High-throughput Drug Testing

The small intestinal epithelium layer is the first line of entry for essential nutrients, such as dietary fatty acids. The uptake, metabolism, processing, and export of fatty acids by absorptive enterocytes are crucial for human health. Problems associated with fatty acid-handling are linked to a variety of metabolic diseases such as dyslipidemia and diabetes. To understand the fundamental regulatory mechanisms of fatty acid-handling and to facilitate drug screening, an in vitro culture system physiologically relevant to the human intestine is required. However, existing enterocyte culture techniques have drawbacks with mimicking the intestinal epithelial architecture due to the complex and dynamic nature of the human intestine.[1] Three-dimensional organoids, by virtue of their structure, restrict access to the apical layer, limiting their ability to model transport from apical to basal compartments. Additionally, intestinal epithelial cancer cell-lines such as Caco2 exhibit characteristics of undifferentiated enterocytes and fail to adequately model normal physiology. To overcome the drawbacks of current systems, Gomez-Martinez and coworkers,[2] in this issue of *Cellular and Molecular Gastroenterology and Hepatology*, developed a robust Transwell-based planar culture model from primary human small intestinal epithelium and demonstrated its feasibility for studying fatty acid oxidation and transport.

The authors first conducted a single-cell RNA sequencing analysis on human donor jejunum and ileum to establish transcriptomic profiles of absorptive enterocytes. By focusing on lipid-handling gene networks coupled with lineage trajectory analyses, the authors noted that human absorptive enterocytes progress through maturation stages with increasing abundances of fatty acid-handling transcripts. Informed by these findings, the team successfully optimized the culture and differentiation conditions that allowed for the generation of a highly pure monolayer of absorptive enterocytes on a Transwell. To achieve high-throughput applications, this planar monolayer culture was further scaled up to a 96-well Transwell format, which, over a time course, exhibited robust barrier and fatty acid-handling functions. This was validated by a number of assays, including transepithelial electrical resistance assay, bulk RNA analysis, and detection of a strong distribution of fluorescently conjugated fatty acid species across the apical, cellular, and basal layers. Using thin layer chromatography, the authors demonstrated the capability of quantifying BODIPY-fatty acids from the apical input media, as well as the metabolized and exported BODIPY-fatty acid products in the collected basal media. The effectiveness of this system for mechanistic study was shown by treatment of the culture with etomoxir, a known carnitine palmitoyl transferase 1 inhibitor that blocks fatty acid oxidation. Their results revealed that etomoxir did not decrease apical fatty acid uptake but reduced fatty acid export via impaired fatty acid oxidation. The authors further tested Metformin, an anti-diabetic drug, and C75, a weight-loss-inducing drug, both of which were shown to increase fatty acid oxidation in other cell types.[3,4] These experiments convincingly demonstrated a dependency of basal export of long-chain-derived fatty acids on fatty acid oxidation, providing a potential explanation as to why patients with abetalipoproteinemia exhibit distribution of major dietary fatty acids yet cannot secrete chylomicrons.[5]

Previously, Kondo et al had established a culture method using iPS-derived intestinal stem cells, which were then differentiated into enterocytes.[6] More recently, Kwon et al developed a protocol to induce human pluripotent stem cells into human intestinal epithelial progenitors and generated a subsequent working monolayer, which was demonstrated to be a physiologically relevant model in the context of drug absorption.[7] The difference between these studies and the current one is the establishment of an absorptive enterocyte monolayer from primary intestinal cells of human donors, which serves as a better model with respect to investigating patient-specific disease conditions of the intestine. The Transwell culture setup and differentiation conditions detailed by Gomez-Martinez et al appear to be crucial for the successful use of this platform. Other major strengths include the easy access to the apical and basal compartments for loading materials, obtaining of samples of interest, and reported high transepithelial electrical resistance displayed by the monolayer. These points illustrate the value of this model system for mechanistic and functional studies of the intestinal barrier and permeability. The use of this enterocyte-enriched model is certainly not limited to fatty acid research, but could be applied to study of carbohydrate, protein, and vitamin metabolism in primary human absorptive enterocytes. Future development of this system into a co-culture platform, as suggested by the authors, will be useful for studying enterocyte-enteroendocrine cell communications that are vital for nutritional, dietary, and lipid metabolic research. Thus, the prospective applications of this planar culture are promising for the future of intestinal research, including but not limited to screening and testing therapeutic drugs for dyslipidemia, diabetes, inflammatory bowel diseases, and other metabolic disorders.

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Conflicts of interest
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