LITHUANIAN UNIVERSITY OF HEALTH SCIENCES

International **Summer School** Practice

Human Intestinal Epithelial Responses to Tryptophan Metabolites: **Differences between Apical and Basolateral Exposure**

Inflammatory bowel disease (IBD) is characterized by chronic intestinal inflammation and impaired epithelial barrier function. Patient-derived intestinal epithelial organoids provide a human-relevant model for studying epithelial responses while preserving donor-specific biological features.

Tryptophan and its host- and microbiota-derived metabolites can modulate epithelial homeostasis and inflammatory signaling, including pathways associated with the aryl hydrocarbon receptor (AHR). As intestinal epithelial cells are intrinsically polarized, their responses may depend on whether exposure occurs from the apical or basolateral side. Polarized epithelial monolayers grown on transwell inserts enable separate apical and basolateral stimulation, longitudinal assessment of barrier integrity by transepithelial electrical resistance (TEER), and compartment-specific sample collection.

This summer practice will investigate whether selected tryptophan metabolites induce distinct functional and molecular responses depending on the side of exposure. Students will work with polarized transwell cultures derived from donor-specific intestinal epithelial organoids from IBD and non-IBD donors. The broader experimental panel includes tryptophan, tryptamine, nicotinamide, indole, indole-3-acrylic acid (IAcrA), and indole-3-propionic acid (IPA). During the practice, students will work with a prioritized subset, such as tryptamine, IPA, and IAcrA, selected according to preliminary data and experimental feasibility.

Student Involvement and Research Activities

1. Establishment of polarized intestinal epithelial monolayers

- Expansion and preparation of donor-derived intestinal epithelial organoids
- Seeding of epithelial cells onto transwell inserts and formation of polarized monolayers
- Culture maintenance, morphological assessment, and differentiation

2. Barrier assessment and pilot metabolite exposure

- Longitudinal TEER measurements to monitor monolayer formation and barrier integrity
- Pilot apical and/or basolateral exposure to selected tryptophan metabolites under homeostatic and/or inflammatory conditions
- Comparison of TEER responses across experimental conditions

3. Sample collection and molecular analysis

- Separate collection of apical and basolateral culture media for subsequent analyses
- Epithelial cell harvesting, RNA isolation, quality assessment, and cDNA synthesis
- Targeted gene expression analysis by RT-qPCR
- If experimental progress and sample availability permit, introduction to RNA-sequencing library preparation and quality control

Expected Ocomes and Training

The practice is expected to generate pilot TEER and targeted gene-expression data and paired apical/basolateral samples. These results will help determine whether the direction of metabolite exposure influences epithelial barrier and molecular responses and inform subsequent experiments within the main research project. Students will gain hands-on experience with polarized intestinal epithelial models, TEER measurements, experimental stimulation, sample collection, and molecular analyses.