Title: Investigating the role of DNA methylation in regulating cell signalling of the human intestinal epithelium during development and Inflammatory Bowel Diseases (IBD)

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**Background:** The human intestinal epithelium serves a wide range of diverse functions including digestion and absorption of nutrients as well as forming a critical barrier between environment and the host and regulating the host’s adaptation to the environment. Epigenetic mechanisms are known to play a role in this adaptation, by mediating environmental stimuli into potentially heritable changes in cellular function. Amongst these, DNA methylation, has been shown to play a major role in regulating gene transcription and cellular function of the intestinal epithelium during development and healthy homeostasis\(^1\). Moreover, the Zilbauer lab recently demonstrated distinct disease associated DNA methylation changes to be present in children diagnosed with Inflammatory Bowel Diseases (IBD) such as Crohn’s Disease (CD) and Ulcerative Colitis\(^2\). Given the critical importance of the intestinal epithelium in human health and IBD, gaining a detailed understanding of its function is an essential prerequisite in order to prevent associated diseases from developing and/or develop improved treatment strategies.

**Aims of the project:**
In this project we aim to investigate cell signalling pathways in the intestinal epithelium in response to distinct, physiologically relevant environmental factors such as dietary factors, viral or bacterial products as well as inflammatory cytokines. Specifically, we will:
1. Use existing large-scale datasets from IBD patients and patient/control-derived intestinal epithelial organoid lines\(^3\) to identify the drivers of variation between control and IBD samples
2. Identify the signalling pathways that are affected by these drivers of variation
3. Use organoids\(^3\) to validate and further explore the interplay between the various cellular regulatory networks (i.e. genotype, epi-genotype, transcription and signalling).

Patient cohort, tissue samples and datasets  
Through the Department of Paediatric Gastroenterology at Addenbrookes Hospital, Cambridge University we have recruited **>500 children** (IBD patients and matched healthy controls) into our research study obtaining the following tissue samples/purified cell subsets: 1)Purified intestinal epithelium from 3 gut segments (small bowel and 2 x large bowel), 2)Adjacent gut microbiota from each gut segment, 3)Purified CD8\(^+\) T-cells 4) Whole blood and serum. Additionally, we have generated over 300 human intestinal epithelial organoid lines from mucosal samples obtained from children (various age groups, different gut segments, disease and controls), neonatal gut and human fetal gut. A large selection of **datasets** has been generated from a subset of existing samples, including Genome wide DNA methylation profiles, 16s microbial profiling of gut microbiome, Genome wide expression profiles, miRNA profiles, Genotyping.
Identification of affected signalling pathways in IBD: As a first step of the project, the successful EBPOD candidate will apply or develop, if necessary, data integration approaches\(^4\)\(^5\), reduce the dimensionality of the datasets and reveal the drivers of variations in the datasets. The Petsalaki group has developed a diffusion-based approach to extract signatures of signalling pathways from phosphoproteomics datasets (unpublished). Using the available RNAseq profiles of all samples, the EBPOD will predict the transcription factor activities and identify differentially expressed cell surface receptors and signalling pathway components. These will be overlaid with pathway information\(^8\) and combined with the miRNA datasets. He/she will then expand and apply our diffusion-based approach to extract signatures of active signalling modules across all samples. A correlation/covariation approach will then be used to test the relationship between the identified drivers of dataset variation and these modules. Thus he/she will uncover potential effects of specific epigenetic, gene regulatory, or microbiome patterns on the signalling networks of the intestinal epithelial cells.

Influence of drivers of variation on context-specific signalling responses: To validate and further test the influence of the drivers of variation on the signalling pathways we will recall the relevant intestinal epithelial organoid lines from our collection plus control organoids. These will then be exposed to environmental factors, such as inflammatory cytokines or bacterial products, and their signalling response will be measured by phosphopeptide arrays, along with paired methylome and transcriptome. Our diffusion-based method mentioned above, will be used to identify signalling module activation in the selected lines vs the control lines. This will provide a picture of context-specific signalling regulation by epigenetic marks. It can also identify potential kinase targets for IBD, as well as (phospho)protein biomarkers, that can also be validated in the organoids and patient samples.

Through identifying the effect of specific epigenetic, genetic and gene expression variations on signalling pathways, we will contribute to an improved understanding of the mechanisms underlying IBD and suggest potential targets and biomarkers for the disease. The successful EBPOD fellow will benefit from the extensive experience of the Zilbauer group on human epithelial cell biology, epigenetics, IBD, organoid cultures and that of the Petsalaki group on cell signalling, network analyses and data integration.

References