Project 4: Gendoo

Name of lead academic supervisor: Dr Deena Gendoo

Name of at least one co-supervisor (e.g. other academics or research fellows): Prof Shishir Shetty, Dr Saad Rehman

Project title: Epigenetic Regulation of liver endothelial cells as a novel target to boost immunotherapy efficacy in hepatocellular cancer (HCC)

Project Description: Outline the broad research question and specific project goals, including the type(s) of data to be analysed, and skills to be developed.

Broad Research Question

The overarching research question is: "How can epigenetic regulation of the tumour endothelium be targeted to reprogramme the immune microenvironment of liver cancer?" This question addresses the challenges in treating hepatocellular carcinoma (HCC), a leading cause of cancer-related mortality globally, with rising incidence in the UK. The tumour microenvironment (TME) promotes immune evasion, limiting the efficacy of immunotherapy in HCC. Liver sinusoidal endothelial cells (LSECs) undergo endothelial-to-mesenchymal transition (EndoMT), fostering a pro-angiogenic state that contributes to immunosuppression. Preliminary bulk RNA-seq data indicates that the epigenetic regulator EHMT2 is significantly upregulated in LSECs in HCC, correlating with increased infiltration of pro-cancer immune cells and poorer patient outcomes in publicly available data. We hypothesize that EHMT2 drives EndoMT and immune cell recruitment through histone and non-histone methylation, suggesting that targeting EHMT2 could reverse these epigenetic changes to develop novel therapeutic strategies.

Specific Project Aims

This project aims to elucidate the mechanistic role of EHMT2 in modulating chromatin accessibility and gene expression in LSECs, with the following specific objectives:

1) Analyse ATAC-seq data from LSECs with and without EHMT2 knockdown to identify alterations in chromatin accessibility regions influenced by EHMT2-mediated methylation.
2) Correlate ATAC-seq findings with bulk RNA-seq data from the same samples to determine how EHMT2's downstream effects influence gene transcription, particularly for EndoMT markers (e.g., αSMA, vimentin) and immune cell recruitment pathways.
3) Identify key differentially accessible regions and associated genes that may explain EHMT2's role in promoting immune evasion in the HCC TME, supporting further experiments with human HCC samples and in vitro models (e.g., flow-based adhesion assays investigating EHMT2 impact on immune cell recruitment).

These aims will enhance understanding of EHMT2's impact on LSEC-mediated immune recruitment, contributing to the development of combination therapies, such as antiangiogenic agents and checkpoint inhibitors, to address HCC treatment resistance.

Types of Data to Be Analysed

ATAC-seq data: Chromatin accessibility assays from LSEC samples with and without EHMT2 knockdown, providing insights into open chromatin regions affected by EHMT2 methylation, including peak calling, differential accessibility analysis, and motif enrichment.
Bulk RNA-seq data: Transcriptomic profiles from LSECs with and without EHMT2 knockdown, used to evaluate differential gene expression and transcriptional outcomes. Much of this data is already processed, but will need to be analyzed against the ATAC-seq data.

These datasets will be integrated to correlate epigenetic modifications (from ATAC-seq) with transcriptional changes (from RNA-seq), potentially incorporating metadata from a human HCC biobank (e.g., expression correlations with immune markers such as Tregs, CD8+ T cells, macrophages, and neutrophils).

Skills to Be Developed

Students will gain skills working with varied next-generation sequencing and ‘omic datasets, including:
* Proficiency in processing high-throughput sequencing data, including quality control, alignment (e.g., using tools like Bowtie2 or STAR), differential analysis (e.g., DESeq2 for RNA-seq, DiffBind for ATAC-seq), and peak annotation.
* Multi-omics integration: Competence in correlating ATAC-seq and RNA-seq data using tools such as GREAT for functional annotation, HOMER for motif analysis, or R/Bioconductor packages for integrative visualization (e.g., heatmaps, volcano plots).
They will also gain experience in how to translate computational findings into biological insights, linking chromatin changes to immune-related pathways, and designing follow-up wet-lab validations.

Students will use R and Python for developing analysis pipelines, managing large datasets, and performing statistical modelling (e.g., hypothesis testing, pathway enrichment via GO or KEGG). Where needed, analyses will be performed using UoB’s high-performance cluster (BEAR) to run code and scripts.

Through this exciting work, students will also gain skills in scientific communication: how to presen complex data through reports, figures, and potential publications, and fostering effective collaboration between computational and experimental biology.

2 key References/background reading/relevant web pages

Llovet JM, Pinyol R, Kelley RK, et al. Molecular pathogenesis and systemic therapies for hepatocellular carcinoma. Nat Cancer. 2022;3(4). doi:10.1038/s43018-022-00357-2

Feng H, Zhuo Y, Zhang X, et al. Tumor Microenvironment in Hepatocellular Carcinoma: Key Players for Immunotherapy. J Hepatocell Carcinoma. 2022;
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Shetty S, Lalor PF, Adams DH. Liver sinusoidal endothelial cells —gatekeepers of hepatic immunity. Nat Rev Gastroenterol Hepatol. 2018;15(9). doi:10.1038/s41575-018-0020-y

Fernández-Barrena MG, Arechederra M, Colyn L, Berasain C, Avila MA. Epigenetics in hepatocellular carcinoma development and therapy: The tip of the iceberg. JHEP Reports. 2020;2(6). doi:10.1016/j.jhepr.2020.100167