PhD project proposal for the Graduate+ initiative InnovInOnco

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# Netrin-1 and TFF3 impact on colorectal cancer stem cells

## Background

Colorectal cancer (CRC) is the third leading cause of cancer-related mortality worldwide [1], with cancer stem cells (CSCs) driving chemoresistance, recurrence, and metastasis [2]. The secreted protein Netrin-1 and its receptor Uncoordinated-5 Homolog B (UNC5B) can regulate stemness and tumor survival [3-5]. Our recent work found that Trefoil factor (TFF) 3 is a downregulated effector in UNC5B-expressing cells upon anti-Netrin-1 treatment, suggesting that a Netrin-1/UNC5B/TFF3 may regulate CSC self-renewal and survival.

## Research Objectives

1. Investigate TFF3 Regulation by Netrin-1 using *in vitro* CRC Models

UNC5B-high CRC cells and patient derived tumor organoids (PDTOs) will be treated with netrin-1 to assess TFF3 expression changes. We will use CRISPR/Cas9 and RNAi to reduce TFF3 expression and investigate its role in netrin-1-mediated CSC self-renewal. We will also test the therapeutic potential of Netrin-1 antibodies.

2. Characterization of UNC5B and TFF3 co-expressing cell populations

scRNA-seq will enable the analysis of UNC5B+/TFF3+ cell heterogeneity in CRC tissues and PDTOs. Purified UNC5B+/TFF3+ tumor cells will be exposed to Netrin-1, followed by transcriptomic and spatial analyses to map Netrin-1-induced transcriptional signatures and associated histopathological defined regions such as invasive fronts.

3. *In Vivo* Validation of UNC5B/TFF3 Interplay

A luciferase-labeled CRC liver metastasis mouse model will be used to assess the effects of NP137 (Netrin-1 antibody) and TFF3 inhibitor, both alone and in combination with chemotherapy. Bioluminescence imaging will allow real-time tumor monitoring and evaluation of recurrence-free survival over six months. Molecular mechanisms driving tumor progression will be analyzed using IHC, IF, WB, RT-qPCR, and flow cytometry.

4. Correlate UNC5B and TFF3 expression with immune and clinical outcomes

CRC samples from Léon Bérard Center and Victoria Comprehensive Cancer Centre will be analyzed using spatial transcriptomics and multiplex IHC to examine the UNC5B/TFF3 axis, immune suppression, and spatial co-localization. Blinded pathological scoring and survival data will be leveraged to construct an integrated prognostic model, combining molecular, immune, and clinical data.

## Significance

This study will elucidate the role of Netrin-1 and TFF3 in CRC progression, with a particular focus on their impact on CSCs. It will identify new therapeutic targets and evaluate the efficacy of Netrin-1 and TFF3 inhibitors in combination with chemotherapy, providing novel strategies for treating metastatic CRC.

## References

[1] Kuipers EJ *et al*. Nat Rev Dis Primers. 2015 Nov 5 ;1 :15065. [2] Li F *et al*. Front Pharmacol 2023, 14 :1165666. [3] Cassier *et al*. Nature 2023, 620 :409. [4] Sun Y *et al*. Proc Natl Acad Sc 2021, 118 :e2103319118. [5] Ducarouge B T *et al*. Cell Death Diff 2023, 30:2201

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