



Stromal alteration and colorectal cancer initiation Dimitri HAMEL^{1,2}, Muriel QUARANTA¹, Julie FONCY², Laurent MALAQUIN², Audrey FERRAND¹

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I - INTRODUCTION & OBJECTIVES x7 fold risk



CRC initiation

CRC is initiated in ISC / progenitors (Bakers 2009, Vermeulen 2014) CSC phenotype is regulated by CAF secretions (CSC niche)



II – EXPERIMENTAL STRATEGIES

A - Fibroblasts & organoids co-culture Human colonic tissus :



B - 3D gut microdevise to reproduce colon topography



<u>3 – In vitro co-culture between primary fibrobla</u>sts & organoids



put on glass coverslip (10mm)

NAF or IAF or CAF

J6 after co-culture NORG/NAF

III – OUTPUT ANALYSIS



A - Cellular investigations : cellular growth & survival

> **Microscopy** (brightfield & immunostaining) **Organoid growth** (size and polarization)

300µm



Gibroblasts staining



Stress fibers (αSMA) FAPα

IV - CONCLUSION

To study a putative reprogramming of normal stem cell (ISC) to tumor-initiating stem cells (TIC) by fibroblasts depending on their (physio)pathological origin, we performed in *vitro* co-culture between organoids & fibroblasts provided from human colonic biopsis.

During the first year of this project, we established organoids & fibroblasts biological bank, adjusted culture protocoles for co-culture experimentation, immunostaining, transcriptomic analysis and first FACS analysis on organoids & fibroblasts markers.



LGR5

CD26







Intermediate Columnar structure Cystic structure

B – Molecular & phenotype investigations

Transcriptomic analysis (Fluidigm / RNAseq)

Epithelial phenotype ISC & CSC signature :

Lgr5, CD44, CD24, CD133, CD166, ALDH, KI67 ...

<u>Differenciation markers</u> : CK20, MUC2 ... Extracellular matrix : collagen, laminin, integrins ... Cytokines, GF : EpHB2, IL6, IL8, IL10, TGFB, PDGF...

Gibroblast phenotype

<u>Activation markers</u> : α FAP, α SMA, PDGFR β ... Extracellular matrix : collagens, laminin, GAG ... Cytokines, GF : IL6, IL8, IL10, TGFB, PDGF...

Protein expression analysis based on phenotype signature (FACS)

Epithelial marker EpCAM NCAD

ISC expression EphB2^{high} ALDH activity (AC)CD133 **Up-regulated in** Connexin 43 CSC / TIC

Mesenchymal marker

CD10

Fibroblasts (normal niche) CD90 αSMA **Activated fibroblasts** FAPα FSP1 PDGFRB **Up-regulated in CAF** GPR77

