Understanding the role of *Fusobacterium nucleatum* metabolism in colon cancer initiation and progression

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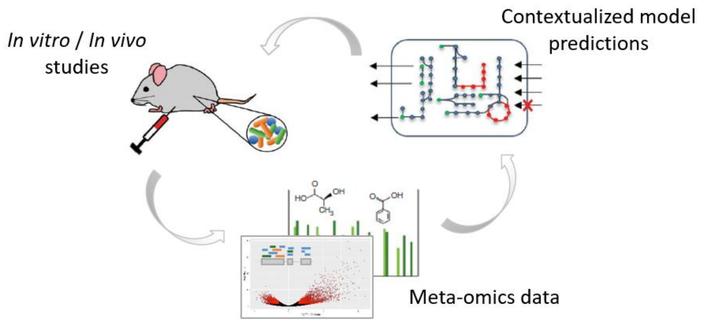
Microbiome in colorectal cancer: how to get from meta-omics to mechanism?

Accumulating evidence suggests that dysbiosis, a state of pathological imbalance in the human gut microbiome, is present in patients suffering from colorectal cancer (CRC). 16S rRNA gene sequencing, as well as metagenomic and metatranscriptomic analyses, identified specific bacteria being associated with CRC. Among others, *Fusobacterium* ssp. have been found to directly interact with cancer or immune cells of their host. However, only a limited number of CRC-associated microbes have been examined for host-microbial interactions and, as such, the role of bacteria in the etiology of the disease remains largely elusive.

Our aim is the development of predictive and experimental models that allow to not only study the host-microbiota interactions but are also amenable to high-throughput experimentation and large-scale omics-data integration. Ultimately, such models should help to get from meta-omics to cellular mechanism and, moreover, serve as tools for reproducible analyses of host-microbial interaction mechanisms of on a transcriptomic, proteomic, and metabolomic level.

Our research proposes an integrative study approach allowing us to bridge meta-omics with functional mechanisms by focusing on the interaction taking place between *F. nucleatum* and patient-derived CRC cells.



Project objectives

- *In vitro* co-cultures of primary CRC cells (T18) with *F. nucleatum*
- *In silico* predictions of host-microbe interactions
- *In vivo* phenotypic validations

Goal: Identification and validation of molecular targets involved in host-microbe interaction by combining multi-omics computational analyses, tailored to co-culture methods

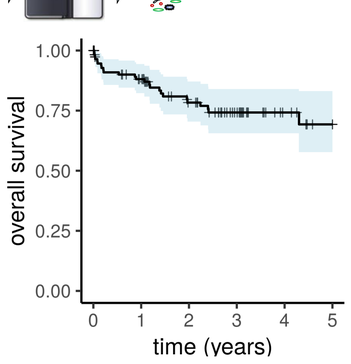
Fusobacterium nucleatum

- Gram-negative, anaerobe bacilli
- Indigenous to the oral cavity, but also present in tissue and stool of CRC patients

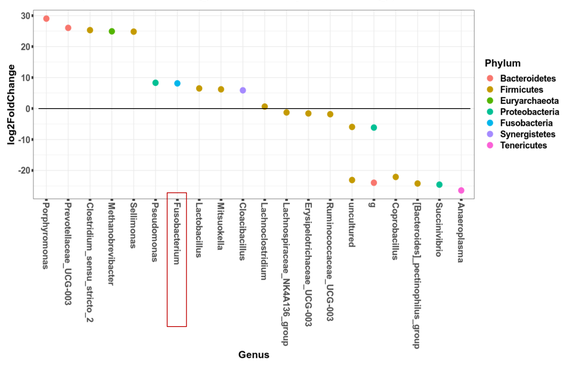
Fusobacterium is enriched in stool of Luxembourgish patients

Table 1: SOCS summary statistics

variable	n
Age	
≤ 65	33
> 65	92
Gender	
female	38
male	87
Living status	
alive	100
dead	25
TNM stage	
0	1
1	18
2	20
3	18
4	15
unknown	53
Tumor location	
proximal colon	36
distal colon	50
rectum	17
unknown	22

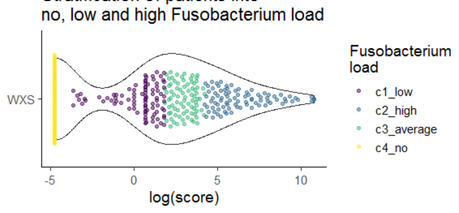


Stool microbiome (16S rRNA gene sequencing)

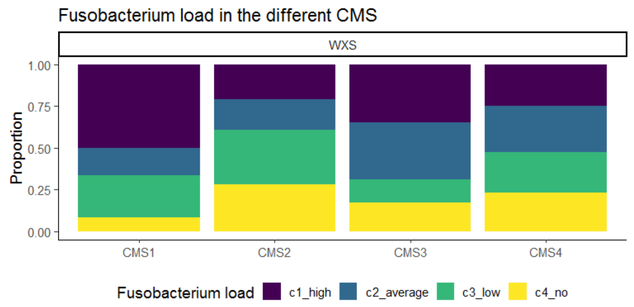


Fusobacterium^{high}-patients are associated with CMS1 and the "metabolic" CMS3 subtypes

Stratification of patients into no, low and high Fusobacterium load

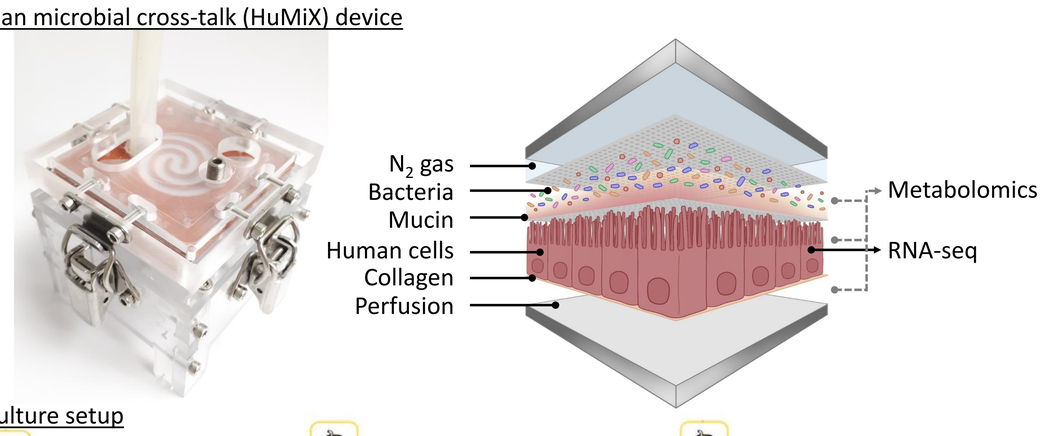


Fusobacterium load in the different CMS

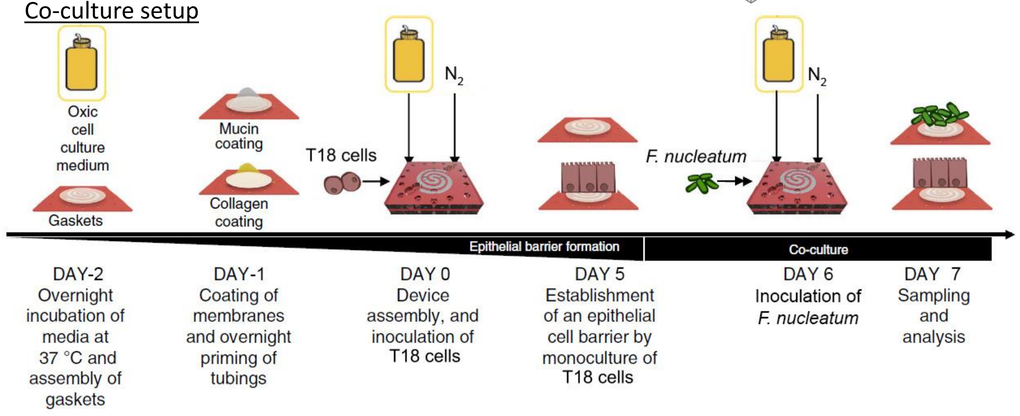


Multi-omics analysis of *F. nucleatum* co-cultures with patient-derived CRC cells

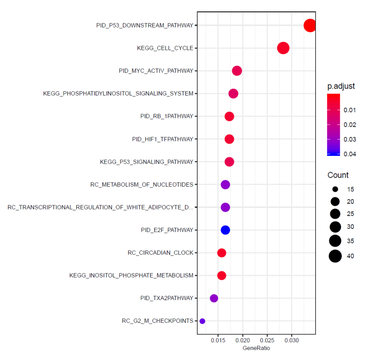
Human microbial cross-talk (HuMiX) device



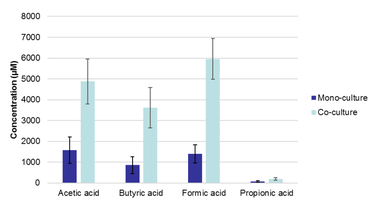
Co-culture setup



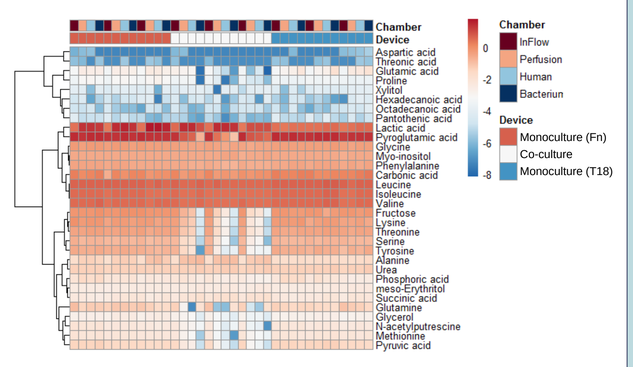
F. nucleatum induces pro-tumorigenic gene expression in CRC cells



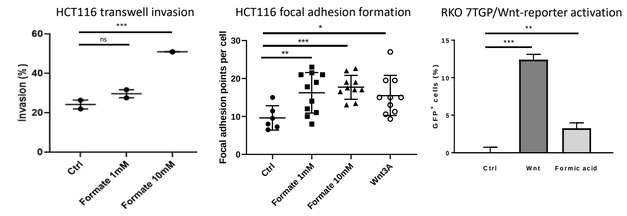
Increased SCFA production of *F. nucleatum* in co-culture with CRC cells



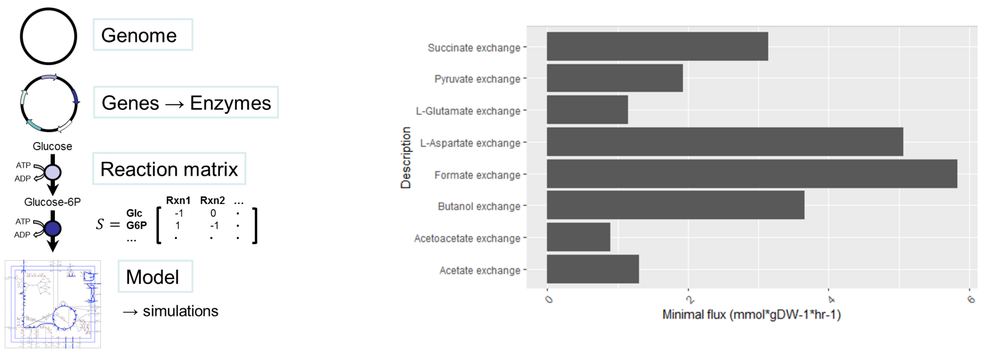
Untargeted metabolomics results suggest alteration of bacterial metabolism in co-cultures with CRC cells



Formic acid increases cellular invasion and focal adhesion potentially via Wnt signaling

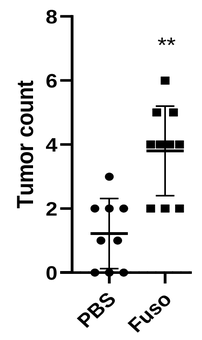


Constraint-based modeling approach suggests increased formic acid metabolism of *F. nucleatum*



Conclusion and outlook

- *In vitro* co-cultures of *F. nucleatum* with CRC cells suggest a metabolically-driven pro-tumourigenic phenotype
- *In silico* models can help to trace altered 'metabolic phenotypes'
- *In vivo* results indicate higher tumor incidence in GF (AOM) mice gavaged with *F. nucleatum*
  -> What is the role of formic acid?



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