FUNGAL DYSBIOSIS ASSOCIATED WITH COLORECTAL CANCER

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INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers worldwide. Although the incidence of colorectal cancer is currently decreasing in developed countries, mainly thanks to the preventive and screening programs (1), it is still a disease with **complicated treatment** and **high mortality**.

Is also known to harbour **considerable heterogeneity** (2). This comprises temporal and spatial differences in genetic mutations, epigenetic regulation, tumour microenvironment and much more. The analyzing amount, the distribution and frequency of epigenetic, microenvironment or genetics differences within a given tumour, turned out to be critical for the better stratification of patients and the development of new therapeutic methods (3). Although most of the studies have proved the benefit of epigenetic and **molecular analysis** as a predictive and prognostic factor, still is it **not beneficial for all** CRC patients (4,5).

Nowadays seems that **tumour microenvironment** plays a critical role in CRC initiation and promotion, with the dietary intake and the intestinal microbiota being the most dominant factors of the luminal microenvironment in the gut. As interest in the gut microbiome has expanded, there have been new links established between microbiome and the development of CRC(6) (Fig. 1.) .

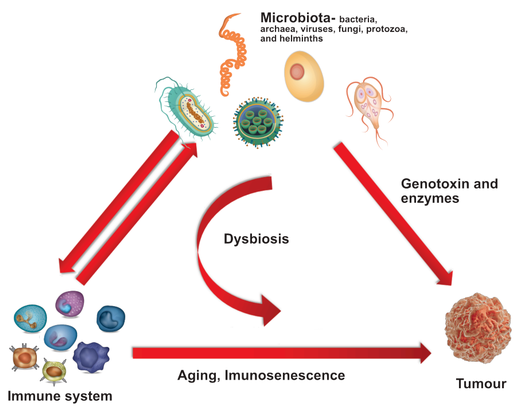

Fig. 1. The relationship between immune system, microbiota and tumorigenesis

The **mycobiome** probably contributes to the progression of fungus-associated diseases and plays an important role in health and disease, by interfacing with other biomes, as well as with the host (7). Nevertheless, the gut fungal microbiota not only in CRC remains **largely unexplored**. Most of the studies neither elucidate the cause or the consequences of the dysbiosis in CRC nor do they provide mechanistic insights by which the intestinal microbiota influences the development of CRC. Increased understanding of microbial community linked to epigenetic, genetics, immune differences in context CRC, will allow for future therapeutic and preventive strategies.

OBJECTIVES

Here I present study design of my dissertation thesis, which will be focused on the search for associations between microbial composition, namely gut fungal microbiome, and the data obtained from the analysis of molecular pathology and immune profiles of the tumour.

STUDY DESIGN

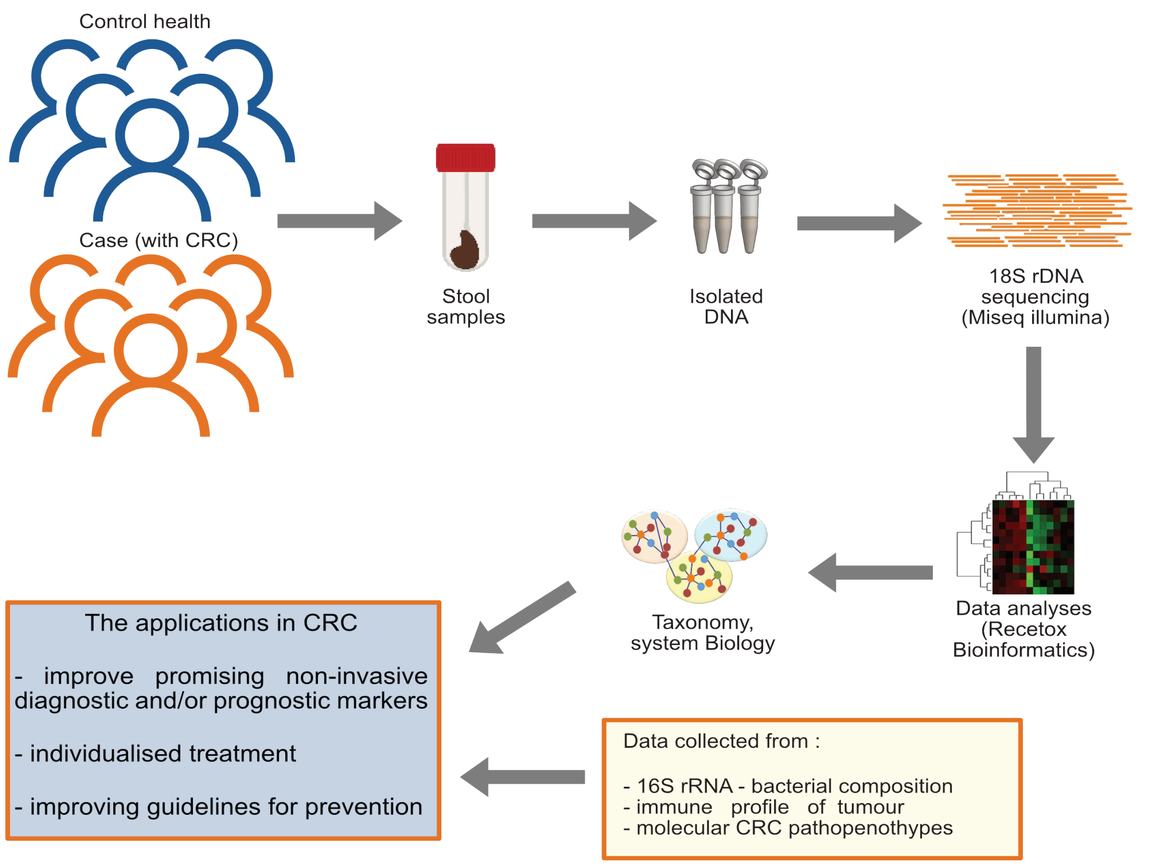

Fig. 2. Design of the study aiming fungal (mycobiome) composition

METHODS AND TECHNIQUES

The pilot phase of the study will be processing stool samples. I will analyse the fungal composition on samples based on **sequencing 18S rRNA** gene. I will associate my results with the data produced and processed from the analysis of molecular pathology, immune profile and bacterial composition.

Samples:

- **Patients:** 150 with colorectal cancer, stage I –IV,
- **Mycobiome:** 150 stool swab samples

Methods:

DNA has been isolated with DNeasy PowerLyzer PowerSoil Kitt. Isolated DNA will be used for the preparation of the 18S rDNA library (Fig.2). The massive parallel sequencing will be carried out on **MiSeq Illumina** tool (placed at the CEITEC laboratory).
Mycobiome composition will be estimated by sequencing of **18S rRNA** gene in prospectively collected stool.

Statistics:

Software Qiime2 for data analysis will be used for bioinformatics analysis of the reads in order to extract taxonomical assignments of the Fungi in the samples. These results will be compared to data collected from 16S rDNA, whole shotgun sequencing, molecular pathology and immune profile of the tumour (Fig.3.)

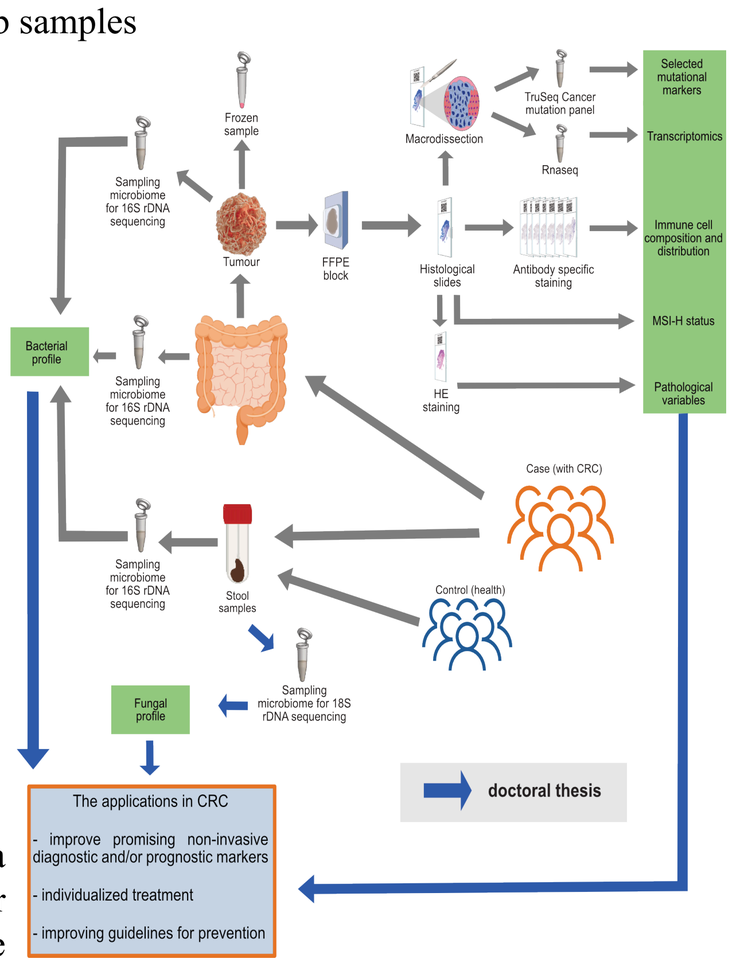

Fig. 3. Design of the complete study

EXPECTED RESULTS

My overall aim is to **improve prognostic** and **predictive stratification** of CRC, by finding new links between the microbiome, immune and molecular phenotypes of CRC.

Primary aims:

- Contribute to improving guidelines for prevention and develop usable, potentially more cost-effective, individualized treatment.
- Contribute to improve promising non-invasive diagnostic and/or prognostic markers for CRC and advanced adenoma.

Secondary aims:

- Innovation of the established methods for 18S rRNA gene sequencing.

REFERENCES

1. Arnold, Melina, Mónica S Sierra, Mathieu Laversanne, Isabelle Soerjomataram, Ahmedin Jemal, a Freddie Bray. 2017. "Global Patterns and Trends in Colorectal Cancer Incidence and Mortality". *Gut* 66 (4): 683–91. https://doi.org/10.1136/gutjnl-2015-310912.
2. McGranahan, Nicholas, a Charles Swanton. 2017. "Clonal Heterogeneity and Tumor Evolution: Past, Present, and the Future". *Cell* 168 (4): 613–28. https://doi.org/10.1016/j.cell.2017.01.018.
3. Ruiz-López, Lidia, Isabel Blancas, José M Garrido, Nuria Mut-Salud, Marta Moya-Jódar, Antonio Osuna, a Fernando Rodríguez-Serrano. 2018. "The Role of Exosomes on Colorectal Cancer: A Review: Exosomes and Colorectal Cancer". *Journal of Gastroenterology and Hepatology* 33 (4): 792–99. https://doi.org/10.1111/jgh.14049.
4. Malapelle, Umberto, Pasquale Pisapia, Roberta Sgariglia, Elena Vigliar, Maria Biglietto, Chiara Carlomagno, Giuseppe Giuffrè, Claudio Bellevicine, a Giancarlo Troncone. 2016. "Less Frequently Mutated Genes in Colorectal Cancer: Evidences from next-Generation Sequencing of 653 Routine Cases". *Journal of Clinical Pathology* 69 (9): 767–71. https://doi.org/10.1136/jclinpath-2015- 203403.
5. Sepulveda, Antonia R., Stanley R. Hamilton, Carmen J. Allegra, Wayne Grody, Allison M. Cushman- Vokoun, William K. Funkhouser, Scott E. Kopetz, et al. 2017. "Molecular Biomarkers for the Evaluation of Colorectal Cancer". *The Journal of Molecular Diagnostics* 19 (2): 187–225. https://doi.org/10.1016/j.jmoldx.2016.11.001.
6. Dahmus, Jessica D., Drew L. Kotler, David M. Kastenberg, a C. Andrew Kistler. 2018. "The gut microbiome and colorectal cancer: a review of bacterial pathogenesis". *Journal of Gastrointestinal Oncology* 9 (4): 769–77. https://doi.org/10.21037/jgo.2018.04.07.
7. Coker, Olabisi Oluwabukola, Geicho Nakatsu, Rudin Zhenwei Dai, William Ka Kei Wu, Sunny Hei Wong, Siew Chien Ng, Francis Ka Leung Chan, Joseph Jao Yiu Sung, a Jun Yu. 2019. "Enteric Fungal Microbiota Dysbiosis and Ecological Alterations in Colorectal Cancer". *Gut* 68 (4): 654–62. https://doi.org/10.1136/gutjnl-2018-317178.