**Multi-cohort faecal metagenomic analysis reveals the altered fungal signatures in colorectal cancer and the pathogenic *Aspergillus rambellii***

Yufeng Lin1, Yali Liu1, Harry Cheuk-Hay Lau1, Xing Kang1, Nick Lung-Ngai Ting1, Changan Liu1, Sunny Hei Wong1,2, Joseph JY Sung1,2, Jun Yu1

1 Institute of Digestive Disease and Department of Medicine and Therapeutics, State Key Laboratory of Digestive Disease, Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

2 Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

**Background & Aims:**

Enteric fungi is a major component of human gut microbiota, but its role in colorectal cancer (CRC) remains largely elusive. We aimed to conduct a meta-analysis to uncover the contribution of fungal mycobiota to CRC progression and their clinical implication.

**Methods:**

We retrieved faecal metagenomic datasets from seven previous publications and established an additional in-house metagenomic cohort, totalling 1,329 metagenomes (454 CRC, 350 adenoma and 525 healthy subject). Assignment of taxonomies was performed by exact k-mer alignment against an integrated microbial reference genome database. DGCA was used to calculate intra- and trans-kingdom interactions. The classification model was performed by random forest across leave-one-set-out. CRC cell lines and immunodeficient mice were used for functional investigation.

**Results:**

Our multi-cohort analysis revealed that alteration in enteric mycobiota was occurred in CRC. We identified a core set of 33 fungal species (10 CRC-enriched and 23 CRC-depleted), of which their abundances were consistently changed in CRC patients compared to healthy subjects (false discovery rate < 0.01) across multiple cohorts. *Aspergillus rambellii* was the top enriched fungi in CRC patients compared to healthy subjects (*p* < 0.05 in 7 out of 8 cohorts). Abundances of 24 fungal species (16 CRC-enriched and 8 CRC-depleted) were also significantly altered in CRC patients compared to adenoma patients, and *A. rambellii* remained the top enriched fungi. Co-occurrence interactions amongCRC-enriched fungi became stronger in CRC compared to adenoma and healthy subjects. Our correlation analysis also demonstrated trans-kingdom interactions between enteric fungi and bacteria in CRC progression, of which *A. rambelli* was closely associated with well-established CRC-enriched bacteria including *Fusobacterium nucleatum* and *Parvimonas micra*. CRC cell(HT29 and SW480) . Moreover, we found that a diagnostic panel with trans-kingdom fungal and bacterial biomarkers (5 fungi including *A. rambellii,* and 9 bacteria including *F. nucleatum* and *P. micra*; area under the curve (AUC) = 82.52%) was more accurate than panel with pure bacterial species (12 bacteria; AUC = 80.82%) to discriminate CRC patients from healthy subjects (AUC increased by 1.44%-10.60%).

**Conclusions:**

This meta-analysis reveals enteric fungi signatures in stages of colorectal tumourigenesis. *A. rambellii* is a potential novelpathogenic enteric fungi critical to CRC progression. Faecal fungi can be utilized, in addition to bacteria, for non-invasive diagnosis of colorectal neoplasia.