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

ABSTRACT

**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.

**Methods:** We retrieved fecal metagenomic datasets from 7 previous publications and established an additional in-house metagenomic cohort, totaling 1,329 metagenomes (454 CRC, 350 adenoma and 525 healthy controls). Analyses on mycobiota composition, fungal interactions, and trans-kingdom interactions between fungi and bacteria were conducted. Performance of fungal and bacterial biomarkers in CRC diagnosis was assessed.

**Results:** Our multi-cohort analysis revealed that alteration in enteric mycobiota was occurred in CRC. Abundances of 33 fungal species (10 enriched, 23 depleted) were significantly altered in CRC patients compared to healthy controls (false discovery rate (FDR) < 0.01). *Aspergillus rambellii* was the top enriched fungi in CRC patients and maintained its performance in all studies. To validate the in-silico findings, we proved *Aspergillus rambellii* promoted colon cancer cell promotion *in vivo* and *in vitro* experiment. Whereas co-occurrence interactions among *A. rambellii* and other CRC-enriched fungi were stronger in CRC. Our correlation analysis also demonstrated trans-kingdom interactions between enteric fungi and bacteriain CRC progression, of which *A. rambelli* was closely associated with well-established CRC-enriched bacteria. Moreover, we found that a diagnostic panel with combined fungal and bacterial biomarkers was more accurate than panels with pure bacteria to discriminate CRC patients from healthy individuals (relative change area under the curve increased by 1.44%-10.60%).

**Conclusions:** This study revealed the involvement of enteric fungi and their trans-kingdom interactions with bacteria in CRC, implying the importance of fungal mycobiota in colorectal tumorigenesis. Our finding also established a reproducible bacterial and fungal biomarkers panel for predicting CRC, which can be used to develop clinical diagnostic tests.