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

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**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 fecal metagenomic datasets from seven 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 also assessed. 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 significantly altered in CRC patients compared to healthy controls (false discovery rate < 0.01) across multiple cohorts. And seven of them were also distinguished in comparison between CRC and adenoma. *Aspergillus rambellii* was the top enriched fungi in CRC patients compared to health control (*p* < 0.05 in 7 out of 8 cohorts). Whereas co-occurrence interactions among *A. rambellii* and other CRC-enriched fungi became 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 including *Fusobacterium nucleatum* and *Parvimonas micra*. Moreover, we found that a diagnostic panel with combined 14 fungal and bacterial biomarkers (5 fungi and 9 bacteria) was more accurate than panels with pure bacteria (12 candidates) to discriminate CRC patients from healthy individuals (relative change of area under the curve increased by 1.44%-10.60%). In addition, *A. rambellii* was the most important fungal feature, ranked top 4th. Mechanically, *A. rambellii* promoted *in vitro* growth of CRC cells and CRC progression in tumor-bearing mice.

**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 panel with bacterial and fungal biomarkers panel for CRC diagnosis which could be used in clinical application.

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