**Background**

Gut microbiota alterations are associated with colorectal cancer (CRC) pathogenesis. However, the role of enteric fungi, an essential component of gut microbiota, in CRC remains largely elusive. We aim to characterize the contribution of enteric fungi to the development of CRC.

**Methods**

We performed shotgun metagenomic analyses of 1325 fecal samples from seven public datasets and one new cohort (454 CRC patients, 350 Adenoma patients and 216 healthy controls).

**Results**

We identified 33 differentially abundant fungal species in CRC versus healthy individuals (false discovery rate (FDR) < 0.01), of which Aspergillus rambellii showed the most significant difference (FDR = 5.13E-18). In seven of the eight cohorts, the combined bacterial and fungal biomarkers classified CRC from healthy individuals with an AUC 1.44% - 10.60% higher than the bacterial classifier. Among 14 biomarkers in the combined classifier, *A. rambellii* was the most important fungal species. Further abundance correlation analyses of the 64 differentially abundant species (33 fungi and 31 bacteria) showed that cross-kingdom interactions are associated with CRC. Particularly, strong differential correlations were shown between *A. rambellii* and two CRC-associated pathogens, *Fusobacterium* *nucleatum* and *Parvimonas* *micra*. The role of *A. rambellii* in colorectal carcinogenesis was further confirmed by our colorectal cancer stem cell proliferation experiment.

**Conclusions**

This study revealed the mycobiome alterations in CRC particularly the enrichment of *A. rambellii* implying that the role of mycobiome in CRC is not negligeable.