Tissue-associated bacterial alterations in early-stage rectal carcinoma patients revealed by 16S rRNA community profiling

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BACKGROUND: Colorectal cancer (CRC) can be classified as inherited (due to genetic instability), inflammatory (due to presence of chronic inflammation of the gastrointestinal tract, e.g. Crohn's disease) or sporadic, which accounts for more than 80% of all cases. Recent publications have shown mechanistic evidence for the involvement of gut bacteria in the development of both inflammatory and sporadic CRC, probably involving genotoxins and DNA-damaging superoxide radicals production, T-helper cell-dependent induction of cell proliferation and Toll-like receptor mediated induction of pro-carcinogenic pathways. However, despite a vast body of circumstantial evidence, studies thus far have not been able to identify, in rectal cancer patients, key species involved in such carcinogenic mechanisms.
HYPOTHESIS: Key bacterial species, involved in pro- or anti-carcinogenic mechanisms, may play pivotal roles in sporadic rectal cancer tumor initiation and progression.
METHODS: To aid in the discovery of species associated with sporadic rectal cancer, we compared bacterial communities of 18 early-stage rectal cancer patients and 18 individuals without rectal cancer by large-scale 16S rRNA metagenomic sequencing. Samples were collected during exploratory colonoscopy (non-cancer group) or surgery for tumor excision (rectal cancer group). All patients had stage I or II rectal cancer and were not submitted to chemo/radiotherapy before surgery. DNA was extracted and the hypervariable regions V4-V5 of the 16S rRNA gene were PCR amplified and sequenced on the Ion PGM platform. Sequence reads were filtered (*Qiime)* and clustered at 97% sequence identity (deemed operational taxonomic units - OTUs) using UPARSE. Representative sequences of each OTU were used to assign taxonomy using the RDP classifier. We obtained a total of 5,593,020 filtered sequences with a mean sequence length of 315nt ± 30nt. After filtering to include OTUs with more than 3 sequences and present in at least 25% of all samples, 1,955 OTUs remained. To investigate differences in OTU, phyla and genera abundances between both groups, raw sequence counts were normalized then log transformed.
RESULTS: We found 5/10 phyla with significant differential log abundances between both groups, with rectal cancer samples having higher *Bacteroidetes* log abundances. At the genus level, we found 64/102 genera with significant differential log abundances between both groups. Rectal cancer samples had higher log abundances of *Bacteroides*, *Ruminococcus*, *Parabacteroides* and *Roseburia* and non-cancer samples had higher log abundances of *Pseudomonas*, *Paracocccus*, *Lactobacillus* and *Bacillus*. These findings may be relevant to characterize the rectal cancer biofilm dysbiosis and to describe a microbiota heterogeneity which may be relevant for immunotherapy of this type of cancer.

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