

TITLE: Possible association of toxigenic species of *Bacteroides fragilis* and *Clostridium perfringens* with rectal cancer.

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ABSTRACT

Colorectal cancer is the third most prevalent malignant carcinoma worldwide. Evidences have shown that the intestinal resident microbiota plays a role in intestinal homeostasis playing a favorable or unfavorable role. Species of the genus *Bacteroides* and *Clostridium* are opportunistic pathogens in intestinal infections. In this study, the occurrence and molecular characterization of *Bacteroides fragilis* and *Clostridium perfringens* from fecal samples of forty (40) patients with rectal cancer and twenty (20) healthy individuals were evaluated. Patients were recruited at the Gastrointestinal Surgery Sector of the Cancer Institute of the State of Sao Paulo (ICESP) and experiments were performed at the Institute of Biomedical Science of the University of Sao Paulo (USP). Feces, blood, tumor and healthy tissue were collected. Fecal samples were cultured onto *Bacteroides fragilis* bile-esculin agar (BBE) and agar *Clostridium perfringens*. Blood samples were cultured into BHI broth and incubated in anaerobiosis. Characteristic colonies of *Bacteroides* spp. or *Clostridium* spp. were identified with the commercial kit API 20E (bioMérieux Inc, USA) or by PCR. Total bacterial DNA was extracted by the phenol-chloroform method. Similar values in the prevalence of *Clostridium* were observed. For *Bacteroides* statistically significant differences were observed ($P < 0.05$). *B. fragilis* isolated from feces or blood was non-toxigenic. A high prevalence of the *bft* gene was observed in cancer strains that in strains from healthy. *Clostridium perfringens* toxonotype D was prevalent in healthy, and the toxinotype A in cancer, but both harbored the *tpeL* gene. Deep studies are necessary to better determine the possible association of these bacteria with the rectal cancer development and patient's prognostic.

Keywords: rectal cancer, anaerobic bacteria, intestinal microbiota, *Bacteroides* spp., *Clostridium* spp.

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