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Investigating the Role of a Low-inflammatory Diet in Modulating Microbiome Biomarkers of Colorectal Cancer

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INTRODUCTION

Familial Adenomatous Polyposis (FAP) is a hereditary syndrome characterized by chronic colonic inflammation and the development of precancerous adenomas. A low-inflammatory dietary intervention helped reduce inflammation and adenoma development in individuals with FAP. Moreover, this promising strategy also reduced the differences in gene expression between normal and adenomatous tissue, increasing the expression of tumour suppressors and reducing that of potential cancer-driver genes in adenomatous tissue [1].

OBJECTIVES

By comparing the microbiome profile of subjects with FAP that followed a dietary intervention aimed to reduce inflammation in the gut, with that of colorectal cancer (CRC) patients or healthy subjects, we aimed to set up a methodological workflow to be applied in the identification of bacterial species related to the tumor and whose abundance can be modulated with diet. This workflow may contribute to the optimization or development of new strategies for CRC prevention based on diet.

METHODS

Microbiome of stools from 37 patients with stage II-III colorectal cancer who had surgery at INT (Fondazione IRCCS Istituto Nazionale dei Tumori) was quantified by shotgun sequencing. Samples were collected before the intervention (T0) and during the scheduled follow-up visits for two years (1 and 6 months after surgery, then every year) including possible time of relapse. By following the same sequencing pipeline, the fecal microbiome composition of a cohort of 120 healthy volunteers, equally distributed among vegetarian, vegan, and omnivorous diets [2] and of the FAP cohort of 27 subjects carrying a mutation in the APC gene, who underwent prophylactic total colectomy/ileorectal anastomosis and currently involved in the surveillance program at INT, were determined. FAP stool samples were referred to four different timepoints, before the beginning of the study (T0), after both 15 days (T15d) and three months of active dietary intervention (T1), and after three additional months in which the subjects followed the diet at home (T2) [3]. For all the three cohorts, taxonomic profiling was carried out by using MetaPhlAn 3 [4].

Alpha diversity was quantified using the Simpson index [5,6]. The Alpha diversity between groups was compared using a Wilcoxon rank sum test or a signed rank test for dependent groups. Bray-Curtis dissimilarity [7] was used to measure the beta diversity and Principal Coordinate Analysis (PCoA) to reveal underlying patterns by visualizing differences in beta diversity [8]. Techniques like PERMANOVA (Permutational Multivariate Analysis of Variance) and PERMDISP (Tests of homogeneity of dispersions) were employed to detect significant differences in beta diversity and dispersion between groups [9].

Differential abundance analysis (DAA) of species was performed using Wilcoxon test or signed rank test [10] to compare the groups of interest.

RESULTS

Alpha diversity quantified by the Simpson's index was significantly higher in the CRC cohort compared to the healthy subjects, both in terms of richness and evenness. Wilcoxon rank sum test revealed a statistically significant difference between the two groups ($p < 0.001$). As regards beta diversity, microbial community composition differed significantly between CRC patients and healthy subject, as revealed by

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the PERMANOVA test (p = 0.001), also due to the dispersion between the two groups (p = 0.027). DAA identified 230 differentially abundant bacterial species between CRC patient's cohort and healthy subjects. Subsequently, in order to investigate whether these bacteria were modulated by diet within the FAP population, DAA comparing taxa between samples pre and post diet was performed. This analysis revealed 3 bacterial species that were not only differentially abundant between CRC patients and healthy individuals, but also responsive to dietary intervention. This helped us identify a potentially beneficial change induced by the dietary intervention. As the abundance of all three species was higher in CRC patients compared to healthy individuals, and the diet reduced their abundance in FAP patients.

CONCLUSION

These findings suggest that bacteria may represent potential targets for diet-based modulation strategies in CRC relapsed patients. From a methodological point of view, our findings confirm that by applying a well-structured data analysis workflow to bacterial data could provide valuable insights for the management of CRC patients.

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