Molecular and microbiological characterization of distal colorectal cancer in a Brazilian population

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Introduction

Colorectal cancer (CRC) is considered a major health problem, being the third most common cancer worldwide. Risk factors for the development and progression of CRC include age, lifestyle, molecular alterations and the microbiota. Most colorectal cancers are localized on the left side of the colon and the molecular basis of sporadic colorectal cancer is widely explored in some population, yet, data on the Brazilian CRC patients is limited. This work aim to better understand the molecular alterations of the Brazilian population, characterizing the mutation and methylation profile of CRC-related genes, microsatellite instability phenotype and presence of Fusobacterium nucleatum (Fn).

Methods

We sequenced 150 cancer-related genes in 71 samples of cryopreserved CRC by Next Generation Sequencing (Illumina). The sequences was aligned against the reference genome (hg19) and somatic mutations was identified against normal tissue DNA (leukocytes). The variants found were annotated and their effects predicted. DNA methylation of seven tumor suppressor genes was evaluated by pyrosequencing and the MSI status was determined using a pentaplex PCR of mononucleotide repeat markers. The presence of Fn was evaluated by qPCR and normalized cycle threshold (Ct) values were used to quantify the amount of Fn using the 2-∆Ct method. Positive samples were classified according to the amount of bacteria found as low or high, on the basis of the median cut point amount of Fn.

Results

The mean age of the patients at diagnosis was 60 years. The majority of mutations found were missense (61.6%), followed by nonsense (22.9%) and frameshift mutations (13.9%), Figure 1 and 2 show the most frequently mutated genes. The mean mutation burden was higher in MSI-H cases (p<0.001). F nucleatum was detected in 12 (16.9%) of the colorectal carcinoma cases analyzed. The positive samples were categorized as low or high according to the median of the amount of Fn detected (Figure 3). The presence of this bacteria was associated with MSI-H (p = 0.027). SEPT9 gene showed the highest frequency of methylation, followed by ALX4, NDRG4 and BMP3 (Figure 4).

Conclusion

Our data suggests that Brazilian CRC patients exhibited a molecular profile similar to other populations, and identifies the major altered genes that could be used in future molecular cancer screening strategies and putative target therapies.