Answers to the Reviewers

We are extremely grateful to the reviewers for their suggestions and comments.

Reviewer 1

This is an excellent article and your findings will definitely add to our existing knowledge. Prognostic factors for colorectal cancer is a work in progress and a lot needs to be done in it for us to understand more about the pathology and treatment. Your article certainly adds value to it and is very well written.

Corresponding Author

We thank the reviewers 1 for the comments.

Reviewer 2

1) Why authors focused on SNPs of only VEGF-A? Authors had better explain the reason why VEGF-A is selected, not other genes, ex VEGFR, KDR or FLT1.

Corresponding Author

The following text (1st paragraph, Discussion section) of this manuscript sought to clarify why the authors have focused on the VEGF gene polymorphism in colorectal carcinoma: *The VEGF-A genetic polymorphisms in this current study were elected based on the current literature concerning the role of such SNPs in malignancies of the large intestine. VEGF-A, as a basic mediator of angiogenesis, is more likely to affect the tumor's biological behavior and phenotype (tumor size, histological grade, stage of disease, and metastatic potential). However, although several studies have demonstrated that VEGF-A polymorphisms are associated with the prognosis of CRC patients, the data have been conflicting.*

Nevertheless, we agree with the reviewer's suggestion and included in the manuscript the following text on pages 5 and 6 in the Introduction section: *The cancer targeted therapy with the use of antiangiogenic agents is aimed at inhibition of angiogenic function and tumor dissemination since neoangiogenesis stimulates the growth and invasion of adjacent tissues by tumor cells[9,12]. On the other hand, the inhibition of VEGF limits the tumor growth[9,13]. In colorectal carcinoma, VEGF levels are increased and they are related to further spread and poor prognosis[12,14]. The antiangiogenic agent bevacizumab is a humanized monoclonal immunoglobulin G antibody against recombinant VEGF activity and it is used in the treatment of metastatic colorectal cancer[13,14].*

Reviewer 2

Authors had better add the table of patients' characteristics with stage and races.

Corresponding Author

We agree the reviewer's suggestion and we included in the 9th line, Materials and Methods section, the ethnicity information. Also, we have included two tables (Tables 2 and 3), in Results section, describing the stages of colorectal carcinoma and the respective polymorphisms.

Reviewer 2

How is the expression of VEGF-A in tumor tissues?

Corresponding Author

The majority of VEGF-A polymorphism studies have been implemented on germline DNA extracted from peripheral blood because it is easily to achieve and produces large quantities of high quality DNA. However, when peripheral blood is not accessible, like in retrospective studies, archived formalin fixed paraffin-embedded tissue from resection specimens can be used. The quality of DNA isolated from fixed paraffin-embedded tissue depends on the duration of formaldehyde fixation and on the formaldehyde buffer used. Fixation can cause cross-linking and damage of DNA isolated from fixed paraffin-embedded tissue, damaging the amplification reaction and primer pattern recognition. Fixed paraffin-embedded tissue material can produce mutation artifacts, e.g., artificial C-T or G-A transitions, which should be taken into consideration when fixed paraffin-embedded tissue is the source of material for genotyping analysis (Marisi G et al. Discrepancies between VEGF -1154 G>A polymorphism analysis performed in peripheral blood samples and FFPE tissue. Int J Mol Sci. 2014;15(8):13333-43) (Reference 40). Therefore, we elected to use blood samples of patients with colorectal carcinoma because the majority of polymorphism analyses have been carried out on germline DNA extracted from peripheral blood as it is easily obtained and generates large amounts of high quality DNA. We add this comment in the 4th paragraph of the Discussion section.

Reviewer 2

Previous studies had already showed SNPs of VEGF-A and clinical findings, but those results are controversial. If authors don’t add protein expression of VEGF-A, what is novel in this article?

Corresponding Author

Although several studies have demonstrated that VEGF-A polymorphisms are associated with the prognosis of CRC patients, the data have been conflicting. We believe this study contributes to clarify the controversy concerning the eventual association between VEGF-A

polymorphisms and the prognosis of CRC patients. Moreover, the human VEGF-A gene is highly polymorphic, thus enabling wide variation in its expression between individuals from different ethnic groups. There are few studies of VEGF-A polymorphisms involving Latinos and Hispanics, and particularly Brazilians. To our knowledge, this is the only state that investigated the VEGF-A: -1498C>T and -634G>C polymorphisms in Brazilian patients.