

We thank the revisors for their comments. Our answers are:

Revisor 1:

- 1 Title. Does the title reflect the main subject/hypothesis of the manuscript? YES
- 2 Abstract. Does the abstract summarize and reflect the work described in the manuscript? YES
- 3 Key words. Do the key words reflect the focus of the manuscript? YES
- 4 Background. Does the manuscript adequately describe the background, present status and significance of the study? YES
- 5 Methods. Does the manuscript describe methods (e.g., experiments, data analysis, surveys, and clinical trials, etc.) in adequate detail? YES
- 6 Results. Are the research objectives achieved by the experiments used in this study? What are the contributions that the study has made for research progress in this field? YES This review gives us a reminder about the careful use of PPI in CRC.
- 7 Discussion. Does the manuscript interpret the findings adequately and appropriately, highlighting the key points concisely, clearly and logically? Are the findings and their applicability/relevance to the literature stated in a clear and definite manner? Is the discussion accurate and does it discuss the paper's scientific significance and/or relevance to clinical practice sufficiently? YES, YES, YES
- 8 Illustrations and tables. Are the figures, diagrams and tables sufficient, good quality and appropriately illustrative of the paper contents? Do figures require labeling with arrows, asterisks etc., better legends? YES
- 9 Biostatistics. Does the manuscript meet the requirements of biostatistics? YES
- 10 Units. Does the manuscript meet the requirements of use of SI units? YES
- 11 References. Does the manuscript cite appropriately the latest, important and authoritative references in the introduction and discussion sections? Does the author self-cite, omit, incorrectly cite and/or over-cite references? YES, NO
- 12 Quality of manuscript organization and presentation. Is the manuscript well, concisely and coherently organized and presented? Is the style, language and grammar accurate and appropriate? YES
- 13 Research methods and reporting. Authors should have prepared their manuscripts according to manuscript type and the appropriate categories, as follows: (1) CARE Checklist (2013) - Case report; (2) CONSORT 2010 Statement - Clinical Trials study, Prospective study, Randomized Controlled trial, Randomized Clinical trial; (3) PRISMA 2009 Checklist - Evidence-Based Medicine, Systematic review, Meta-Analysis; (4) STROBE Statement - Case Control study, Observational study, Retrospective Cohort study; and (5) The ARRIVE Guidelines - Basic study. Did the author prepare the manuscript according to the appropriate research methods and reporting? YES

14 Ethics statements. For all manuscripts involving human studies and/or animal experiments, author(s) must submit the related formal ethics documents that were reviewed and approved by their local ethical review committee. Did the manuscript meet the requirements of ethics? YES

R: No answer/change required. Thank you for your comments.

Revisor 2:

The manuscript entitled, “Colorectal cancer carcinogenesis: bench-to-bedside”, reports a review on genetic factors involved in CRC carcinogenesis. The authors summarized the carcinogenesis pathways and key genes that play roles in CRC development and treatment. The manuscript is informative and may draw attentions of readers who are interested in the field. The below lists some suggestions that the authors may consider.

1. “In 1990, Fearon and Vogelstein published an important paper about colorectal carcinogenesis.”, a reference should be added for this sentence.

R: Reference added.

2. “the “hypermuted” (more than 12 mutations per 106 bases) and the “non-hypermuted””, there should be a definition for the “non-hypermuted” tumors as well. What is the percentage threshold for mutations to be considered as “non-hypermuted”.

R: Definition added: “ In fact, the CRC molecular characterization done by the Cancer Genome Atlas Network found an altered WNT signalling pathway in 93% of tumours, but it also described two broadly distinct groups of tumours: the “hypermuted” (more than 12 mutations per 106 bases) and the “non-hypermuted” (less than 12 mutations per 106 bases).

3. When Figure 1 was mentioned for the first time, only part of the information provided by Figure 1 was summarized. In this paragraph, it will be better if the authors can give a full introduction of contents proposed in Figure 1.

R: Sentence changed: “Based on gene expression profiles, a classification system comprising 4 consensus molecular subtypes (CMS 1-4) was created, each having typical histologic and clinical features. In Figure 1 we can see a classification system using the consensus molecular subtypes (CMS 1-4), CIMP (CpG island methylator phenotype) and MSI (Microsatellite instability) status. The “non-hypermuted” tumours seem to correspond to group 4 and “the hypermutated” tumours to group 1 in the CRC classification proposed by Jass”

4. “Characterized by a phenotype of DNA hypermethylation at specific regulatory sites (CpG islands) in the promoter regions of genes – the CIMP.”, need a reference here.

R: Reference added.

5. The English grammar needs to be checked again, e.g. “these data seems interesting”.

R: Corrected.

Revisor 3:

Thank you for giving me a chance to review this research regarding Colorectal cancer carcinogenesis. My major comments are as follows:

1.The reference format is inconsistent. For example, reference18 does not provide doi <https://doi.org/10.1007/s11725-017-0730-2>). According to this paper, CRC was marked into four CMSs with distinguishing features: CMS1 (microsatellite instability immune, 14%) CMS2 (canonical, 37%), CMS3 (metabolic, 13%), and CMS4 (mesenchymal, 23%). There must be a problem with the proportions in Figure 1. (CMS1 vs CMS3).

R: Reference corrected. Proportions on figure 1 corrected.

2.Page 6, line 6:" characterized by a hypermutated phenotype in the absence of MSI ,"the absence of" may be inappropriate used.

R: Sentence changed: “ an example is the identification of DNA polymerase protein mutations (POLE and POLD1), that led to the description of a new molecular pathway, characterized by a hypermutated phenotype without MSI ^[13]”

3.Page 11, line 5:"KRAS exons 3 and 4 mutations (as the less common NRAS exons 2, 3 and 4 mutations) have been shown to be associated to an intrinsic resistance to anti-EGF antibodies (cetuximab and panitumumab)," we want to know whether KRAS exon 2 mutation is associated to an intrinsic resistance to anti-EGF antibodies ?

R: Information added: “KRAS exon 2 mutation is associated with an intrinsic resistance to anti-EGF antibodies. In KRAS exon 2 wild type patients, KRAS exons 3 and 4 mutations (as the less common NRAS exons 2, 3 and 4 mutations) have also been shown to be associated to an intrinsic resistance to anti-EGF antibodies (cetuximab and panitumumab), and CRC patients with these mutation have worse overall survival when they receive anti-EGF antibodies, either as monotherapy or combined with traditional chemotherapy ^[49,50] . “

Revisor 4:

This is an applicable review. With the theme of bench-to-bedside, this paper presents the current research progress of colorectal cancer. General comments:

1. Recent advances in basic research of colorectal cancer need to be supplemented, such as immune-related regulation, intestinal flora, etc.

2. Biomarkers for colorectal cancer screening need to be further introduced. More novel biomarkers can be supplemented, including cfDNA, exosomes, etc.

R: The goal of our work was to summarize data regarding colorectal cancer carcinogenesis with current applicability on a daily basis. So, themes like intestinal flora, immune-related regulation or biomarkers still under investigation or available only in expert centers were beyond our scope and were not evaluated in this revision.

Revisor 5 .

In this review, the author systematically summarizes the value of molecular and genetic features in early screening, diagnosis, therapeutic strategy and prognostic implications for CRC patients. This review covers almost all of the important CRC carcinogenesis related molecular and mutation features, including MSI, BRAF, KRAS, APC and TILs. In my opinion, it is a good review with great data integrity and scientific rigor. However, this article also has some areas desired for improvement:

1. We all know that EGFR mutation is an important tumor inducer and therapeutic target for CRC. Could the author please supplement the relevant content of EGFR in this article ?

R: Information added: “Anti-EGFR antibodies have been used for the treatment of metastatic colorectal cancer since 2004. More recently, both cetuximab and panitumumab have been approved as first line treatments for *BRAF/KRAS/NRAS* wild type patients, with a demonstrated increase in overall survival, response rate and progression-free survival. However, there are still patients with the above tumour genotype that cannot obtain these benefits or who experience rapid drug resistance and disease progression”.

2. The conclusion part is too simple. Many conclusive statements that appear in the results section should appear in the conclusions.

R: Conclusion changed:

“CRC is a heterogenous entity and its molecular and genetic subtypes have significant implications, from familial risk assessment to therapeutic choices.

Regarding the most used classification for CRC origin, there are three important oncogenic pathways: the chromosomal instability (CIN); microsatellite instability (MSI) and serrated pathways. They have different clinical and molecular/genetic characteristics. The MSI status, *BRAF*, *KRAS* and *APC* mutation status and the presence of tumour-infiltrating lymphocytes are the most studied tumour features and those more extensively correlated to clinical data. The combination of MSI status and *BRAF* mutation status can be used to help identifying patients with LS. However, tumour molecular and genetic analyses are now also known to predict response to

chemotherapy or to immune checkpoint inhibitors and to affect prognosis. Finally, DNA-based markers have already undergone clinical testing in the field of CRC screening and were shown to be useful.

Clinicians should be aware of the major known carcinogenesis pathways and most commonly mutated genes, since some clinical implications are already proven and several others are currently under investigation.