Response to reviewer 05395205

Comment 1: In Figure 1 different mutations in the pathogenesis of colorectal cancer subtypes are not clear. Which specific colorectal cancer subtypes (the authors used cancer 3 times in the figure)? Some mutations may also be noted in other cancers.
We agree that the types of CRC caused by these mutational pathways should be clarified. We have updated the figure to remove the repetitive "Cancer" labeling. In addition, we agree that it is important to note that these mutations may be found in other cancers and that these CMS are generalizations and not all CRCs follow these progressions (Lines 121-123).

Comment 2: In Figure 2, DNA mutations seem not consistent with protein changes.
We agree that the different DNA mutations noted in the figure are inconsistent with the protein changes. We provided this figure as a summary of the DNA, RNA, and protein-level alterations that have been identified in EOCRC. To clarify, we have removed the arrows from DNA to RNA to protein (Figure 2).

Comment 3: The authors mentioned decreased or increased in the table. It is not clear for readers if alterations at the DNA, RNA, and protein levels are different between early and late-onset colorectal cancer.
We agree that the labeling on the table should be clarified. In table 1, we have updated the title to specify that the table only includes DNA mutations. We also updated the name of the Prevalence column to specify that the "increased" and "decreased" labeling means that mutations to the corresponding genes have increased or decreased prevalence in EOCRC compared with LOCRC. We have also specified this in the text below the table.

Comment 4: In the section of EOCRC Proteomics, please mention which specific population and the sample size. The authors just mentioned that Holowatyj et al. found no differences in the plasma proteome of younger-onset compared with older-onset CRC using an antibody microarray platform to detect 206 proteins. However, other details are lacking.
We agree that more information should be provided about these findings. We have updated the description of this study to the following: "Another recent study by Holowatyj et al. found no significant differences (FDR q-value < 0.05) in the plasma proteome of younger-onset (n=11) compared with older-onset (n=45) CRCs using an antibody microarray platform to detect 206 inflammatory proteins. Increased sample size may shed light on interesting targets, as the authors found that the cancer-related proteins BRCA2, PTEN, WNT5B, and WNT7A, among others, had a fold change around two ($P < 0.05$) in EOCRC vs LOCRC serum" (Lines 336-341).

Comment 5: The biggest issue is that the review is simply a repetition of the literature with no attempt to synthesize or critically discuss the results presented. This is the key to a good review article. However, the article reads like a bunch of abstracts from different studies in paragraph form.
We agree that some interpretations and syntheses of the literature are missing. Due to the small number of studies that have examined many of the sub-topics discussed in this review, we wanted to provide a summary of the key findings and provide interpretation and identify gaps in knowledge, but we agree that more attention could be given to examining study design and synthesizing the literature. Therefore, we have made the following edits throughout:

- Interpretation was added in the last paragraph of the *Common molecular drivers of colorectal adenocarcinoma* subsection of the introduction (Lines 140-152).
- Interpretation was edited in the *DNA mutations associated with EOCRC* section to better connect the APC/BRAF mutation frequency in EOCRC with CMS (Lines 203-205)

- Transitions were improved and some information was condensed in the *Epigenetic modifications in EOCRC* section to better synthesize the results presented.
- Discussion of immune profiling in the *EOCRC transcriptomics* section was re-structured to better synthesize information and interpretation was added (Lines 215-217, 225-228).
- Added transitions and edited discussion and analysis of single-cell RNA-seq in EOCRC in the *EOCRC transcriptomics* section (Lines 254-275, 285-287, 303-317).
- Information in the *EOCRC proteomics* section was condensed to better synthesize results across studies (Lines 343-347).
- Transitions and interpretation were added to *EOCRC in non-western countries* section (Lines 366-372, 382-383).

Response to reviewer 05907966

Comment 1: Post-transcriptional mechanisms such as alternative polyadenylation and RNA modification should be discussed, due to their importance in carcinogenesis.
We agree that post-transcriptional mechanisms are important for colorectal carcinogenesis. We have included information on the relevance of alternative polyadenylation and post-translational methylation modifications to RNA in CRC, however, these remain unexplored in EOCRC (Lines 292-302).

Comment 2: In the section on EOCRC proteomics, post-translational modification should be discussed.
We agree that post-translational modifications should be discussed in EOCRC. We have added information to the EOCRC proteomics section to acknowledge changes to protein modifications such as glycosylation, ubiquitination, phosphorylation, and acetylation are associated with CRC but there have been limited studies examining these modifications (Lines 348-353).

Comment 3: The established biomarker and therapy for EOCRC should be discussed in depth, especially their shortcomings.
We agree that we could discuss current clinical guidelines for EOCRC in more in-depth. Currently, they are the same as with LOCRC, with the exception that EOCRCs is generally not diagnosed through routine colon cancer screenings. In addition, mutation-specific drugs are used in CRC treatment, and these mutations are less common in EOCRC; thus, EOCRC patients would be less likely to receive these drugs. We have added this information to the end of the *EOCRC biomarkers* section (Lines 454-459).