Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

The following comments suggest areas for improvement and further research to validate the study's conclusions.

1. The authors mention a lack of data on lifestyle factors, which could influence the interaction between HMGB1 SNPs and cancer risk. This limitation may affect the comprehensiveness of their analysis.

Response: Thanks for the reviewer's valuable suggestions. For lack of data on lifestyle, we could not deduce the potential interaction of *HMGB1* rs1412125T>C SNP with lifestyle. We have addressed the limitation in our manuscript. In the future, more studies should be conducted to explore these SNPs in relation to CRC development with lifestyle factors.

2. The study only investigates HMGB1 rs1412125 T>C and rs1045411 C>T SNPs, leaving out other potentially relevant SNPs in the HMGB1 gene that could also play a role in colorectal cancer risk. This narrow focus may overlook important genetic interactions.

Response: Thanks for the reviewer's valuable suggestions. In this investigation, only two *HMGB1* SNPs were included for study. However, other SNPs in *HMGB1* gene should not been ignored. And the interaction of *HMGB1* rs1412125T>C and rs1045411C>T SNPs with other *HMGB1* SNPs also should be considered. In the design stage of this study, we only included these two *HMGB1* SNPs which were of more concern in other studies before. We have addressed this limitation in our manuscript.

3. The study uses logistic regression models to adjust for confounding factors, the authors do not elaborate on the robustness of their statistical methods or any potential limitations in their analytical approach.

Response: Thanks for the reviewer's valuable suggestions. In previous studies, logistic regression models to adjust for confounding factors for case-control study. In the current study, we cited the methodology of these studies in ' ***Statistical analysis*** ' paragraph.

4. Although the power calculations indicate strong statistical power in some analyses, they mention that the power value for other comparisons was less than 0.8, which can raise concerns about the reliability of the findings in those subgroups.

Response: Thanks for the reviewer's valuable suggestions. In some subgroup, the power value for other comparisons was less than 0.8, which may be influenced by sample size and other factors. In the future, these results should be verified by more case-control studies with large sample sizes.

5. While the study includes a relatively large sample size of 1,003 CRC cases and 1,303 controls, it notes that prior studies with smaller sample sizes have yielded inconclusive results regarding the association between HMGB1 SNPs and cancer risk 3. This indicates a need for further studies to confirm the findings. **Response:** Thanks for the reviewer's very good suggestion. In the future, these findings should be verified with more studies.

6. The study is hospital-based, which may introduce selection bias. The authors acknowledge that the design could lead to limitations in how representative the sample is of the general population.

Response: Thanks for the reviewer's valuable suggestions. In the future, our findings should be verified with more well-designed case-control studies.

7. Non-significant results regarding the association of certain SNPs with CRC risk should be discussed more thoroughly to understand the implications of these findings.

Response: Thanks for the reviewer's valuable suggestions. We have discussed the association more thoroughly to understand the implications of these findings in our revised manuscript.

8. The manuscript does not sufficiently describe how control groups were selected or matched to the cases, which is important for minimizing bias.

Response: Thanks for the reviewer's carefully examination.We have added the

content in ' **MATERIALS AND METHODS----*Study subjects***'.

9. The genotyping method is stated as using the custom-SNP scan Kit, but more technical details about the process, including controls or replicates used, could enhance the reproducibility of the study.

Response: Thanks for the reviewer's carefully examination. In our manuscript, we have presented the control process (To carry out a control for genotype test, 92 samples were selected randomly. Using the same PCR method, two authors conducted the genotype test without knowing the status of participants. The findings of original genotype test were not changed.). In addition, we cited our previous study to address the technical details of the custom-SNP scan Kit as suggested （Methylenetetrahydrofolate reductase tagging polymorphisms are associated with risk of esophagogastric junction adenocarcinoma: a case-control study involving 2,740 Chinese Han subjects. *Oncotarget.* 2017;8(67):111482-111494）.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors:

1. Previous research endeavors have indicated that the elevated expression levels of the High Mobility Group Box 1 (HMGB1) protein are closely correlated with the extent of differentiation observed in patients suffering from colorectal cancer. This particular article aims to further enrich the existing body of knowledge within this specific area of study. To bolster the findings and make them more comprehensible, it is highly recommended that the research incorporates pertinent visual aids. For instance, a sequencing map that clearly illustrates the two Single Nucleotide Polymorphisms (SNPs) of the HMGB1 gene would be an invaluable addition. Such a visual representation could significantly enhance the understanding of the genetic variations and their implications in the context of colorectal cancer differentiation.

Response: Thanks for the reviewer's valuable suggestions. A custom-by-design 48-Plex SNPscan Kit (Genesky Biotechnologies Inc., Shanghai, China) was used to analyze the genotypes of *HMGB1* rs1412125T>C and rs1045411C>T SNPs. We used the same PCR method to carry out a control for genotype test. The findings of original

genotype test were not changed. **Figure 1** and **Figure 2** presented the genotypes of HMGB1 rs1412125T>C and rs1045411C>T, respectively.

2. The outcomes of the current research suggest that there is a notable association between the Single Nucleotide Polymorphism (SNP) rs1412125 of the HMGB1 gene and an elevated risk of developing colorectal cancer (CRC). In the course of conducting subgroup analyses, the results presented in this thesis indicate that this particular SNP may contribute to an increased incidence of CRC within specific subgroups, including individuals who are 61 years of age or older, non-drinkers, and those with a Body Mass Index (BMI) less than 24 kg/m^2. However, the findings of this thesis also suggest that there is no discernible association between the HMGB1 rs1412125 SNP and the occurrence of lymph node metastasis (LNM), even when considering different regions of CRC. The research does not establish any correlation between the HMGB1 gene and the presence of LNM, which further highlights the complexity and multifactorial nature of colorectal cancer development and progression.

Response: Thanks for the reviewer's positive comment.

3. In addition to the genetic factors, it is also crucial to consider the environmental and lifestyle influences that may interact with the genetic predisposition to colorectal cancer. For example, dietary habits have been shown to play a significant role in the modulation of HMGB1 expression.It has been mentioned in the discussion of this study.

Response: Thanks for the reviewer's positive comment.