

Dear Editor:

Thank you for your kind letter of “Decision on submission to Environmental Science and Pollution Research” on June 25, 2021. We revised the manuscript in accordance with the reviewers’ comments, and carefully proof-read the manuscript to minimize typographical, grammatical, and bibliographical errors. Here below is our description on revision according to the reviewers’ comments.

Part A (Peer-Review Report):

The editor’s and reviews’s comments were highly insightful and enabled us to greatly improve the quality of our manuscript. Thank you for this valuable feedback.

1. The authors should review the manuscript again for grammatical and punctuation mistakes.

Response: Thank you for carefully reading our manuscript. We have paid attention to this issue, and we have carefully modified this manuscript to the grammatical errors and punctuation mistakes. Revised portion is marked in yellow highlight in the revised manuscript.

2. As histopathological analysis is usually observer driven, the authors should mention how observation bias was accounted for in the study.

Response: We have added the how observation bias was accounted for in the revised manuscript. Please see page 5-6 of the MATERIALS AND METHODS selection of “Macroscopic evaluation of the incidence of polyps”, marked in a red colour.

3. As the authors emphasize, near organization and relatively straight collagen facilitates migration of tumor cells. The readers would be interested to know the migration pattern and proportions (% of rats that had mets) mentioned in the manuscript. Was there a specific subgroup which showed higher metastasis which was statistically significant? What are the real world implications of these findings?

Response: Growing insights from experimental studies showed that ECM collagen deposition became more obvious with the increasing collagen density, length and width, which could induce the growth of carcinoma. Collagen fibers

are the “highway” for tumor cells, helping tumor cells migrate to their destination more accurately and faster. In our study, the colonic length of cold exposure group was shorter and slightly curled with harder stiffness and a higher rate of tumor incidence and lymph node metastasis in the progression of CRC than those in any other groups. After analyzing the ECM collagen in cold exposure group, we also found the density of collagen increased significantly, the arrangement of collagen was straight, and the width and length of collagen also increased in the cold exposure CRC. We accordingly speculated that the ECM collagen of cold-exposure CRC owned more obvious collagen deposition, promoting the tumorous growth and migration, which may be one of the reasons for the high transfer ability in cold-exposure CRC. Further studies are necessary to identify collagen morphology in colonic tissues through Nonlinear microscopy techniques such as multiphoton laser-scanning microscopy (MPLSM) and second harmonic generation (SHG) provide powerful tools to image cellular autofluorescence and extracellular matrix structure in intact tissues.

Because of experimental conditions, our study only use polarized light microscopy to observe the organization of collagen in the colonic tissue, we can't image the migration pattern of the collagen and proportions(% of rats that had mets) . so as to better understand the cell-matrix interaction and how matrix organization, density, and composition affect colorectal cancer formation and progression. We're going to do use nonlinear microscopy techniques such as MPLSM and SHG to imaging collagen structure.

Relevant papers “Collagen reorganization at the tumor-stromal interface facilitates local invasion” , the investigator use both MPLSM and SHG Imaging to characterize collagen morphology in mammary tissues and found that with characteristic collagen structures such as radially aligned collagen fibers associated with tumor-cell invasion. Therefore, the real world implications of these findings was Identification and characterization of these collagen signatures sheds insight into the process of tumor cell invasion, and may serve

a diagnostic capacity for determining the invasive potential of tumors.

4. The authors should add an additional paragraph on the implications of their findings in a clinical setting. How would these findings possibly influence clinical practice? I do agree that it may be fairly early to offer comprehensive protocols for clinical practice but the readers would want to know the author's perspective on their findings and its utility. These points are valid and should be addressed by the Authors.

Response: We have added an additional paragraph on the implications of their findings in a clinical setting and How would these findings possibly influence clinical practice to address your concerns and hope that it now more informative. Please see see page 14 of the DISCUSSION selection in the revised manuscript, marked in yellow highlight.

Part B (Science editor):

1. The authors have provided non-native speaker English editing certificate by filipodia but it is not related with the current study and therefore, it should be provided.

Response: According to the comments, I have professional English language editing company to polish my manuscript. Also, I have highlighted changes in the revised version. Hopefully it will be qualified. We have also provided non-native speaker English editing certificate.

2. The signed Conflict-of-Interest Disclosure Form and Copyright License Agreement is not provided but is necessary. The Institutional Review Board Approval Form is required and provided

Response: We have provided Conflict-of-Interest Disclosure Form and Copyright License Agreement.

3. Supplementary comments: This is an experimental study with novel results. However, the points that the reviewer have emphasized should be revised by the authors.

Response: Thank you very much for your and reviewers' comments on our manuscript. After a careful revision following the comments, we submit this

revision for your review.

4. PMID and DOI numbers are not given and should be provided according to the journal guidelines

Response: We have added PMID and DOI numbers according to the journal guidelines.

5. The “Article Highlights” section is missing. Please add the “Article Highlights” section.

Response: We have added “Article Highlights” section in page 15-16 of the manuscript.

Part C (Company editor-in-chief)

The editor’s comments were highly insightful and enabled us to greatly improve the quality of our manuscript. Thank you for this valuable feedback.

1. I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office’s comments and the Criteria for Manuscript Revision by Authors. However, the quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, the author(s) must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend:
<https://www.wjnet.com/bpg/gerinfo/240>.

Response: According to the comments, I have professional English language editing company to polish my manuscript. Also, I have highlighted changes in the revised version. Hopefully it will be qualified.

Many grammatical or typographical errors have been revised and revised portion is marked in yellow highlight. All the lines and pages indicated above are in the revised manuscript.

Thank you and all the reviewers for the kind advice.

Sincerely yours,