Peer Review File

Article information: https://dx.doi.org/10.21037/tcr-23-785

Reviewer A

I congratulate the authors on providing a new dimension to mitophagy related cancer progression by identifying the possibility of immune tumor microenvironment interaction. The differential gene expression in TCGA is based on matched normal tissue from the same patient. In colon cancer, it would be the peritumoral normal looking colon. Is mitophagy gene expression changes isolated to the tumor tissue or is there a field effect on the other parts of the colon. Can the authors comment on the mitophagy gene expressions levels as it pertains to the 27 genes used the mitophagy score in non- cancer colon tissue. (colon tissue excised for non-tumor reasons). Should the differential gene expression levels be compared to normal tissue that comes from non-cancer patient samples?

Reply: Thank you for the comments. Regarding the "field effect" you mentioned, we examined this carefully and indeed did not provide specific data about mitophagy gene expression in non-cancer tissues. This is a limitation of our study, and in future studies we will endeavor to include normal colorectal tissues from non-cancer patient samples to explore a more comprehensive mitophagy gene expression profile to reveal "field effect".

In addition, you mentioned whether the expression of mitophagy genes should be compared to normal tissue from non-cancer patients. We agree with you that usually comparing cancer tissues from cancer patients with normal tissues from non-cancer patients can better reveal abnormalities of gene expression during tumorigenesis. In our study, we focused on the comparison of colorectal cancer tissues with normal colorectal tissues from corresponding patients in order to explore the changes of mitophagy gene expression within the tumor. We agree with your suggestion and encourage future studies to expand sample sources to include normal colorectal tissue from non-cancer patients to gain a more comprehensive understanding. We will add these as our limitations in the discussion section.

Changes in the text: We add some sentences about the limitations in the discussion section (see Page13, Line 338-341).

Reviewer B

The authors measured the role of mitophagy in CRC. The paper is well written.

1. Additional datasets from GEO could be considered for validation purposes.

Reply 1: Thank you for the comments. Regarding the use of additional datasets for validation, we strongly agree with this suggestion. In our study, we have used data from TCGA and GEO databases to analyze mitophagy genes in colorectal cancer. We will carefully consider your suggestion and look for suitable additional datasets in future studies, such as downloading more data of colorectal cancer from the GEO database to

further validate our findings.

2. Several recent articles from the literature are missing. Discuss the recently reported key genes and pathways in CRC. For example-

a. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC9239823/

b. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8197092/

c. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6153299/

d. https://www.nature.com/articles/s41418-021-00760-9

e. https://cancerci.biomedcentral.com/articles/10.1186/s12935-021-02065-w

Reply 2: Thank you for the comments. We have read these articles carefully and elaborated these studies in the introduction and discussion sections.

Changes in the text: See Page 4, Line 94, Reference 16; Page 4, Line 100-101, Reference 18; Page 4-5, Line 103-105, Reference 20; Page 11, Line289-290, Reference 32,33.

3. Please make the scripts and data available.

Reply 3: Thank you for the comments. We will upload the scripts and data as supplementary files.

4. Please mention the statistical methods used correctly in boxplots and wherever applicable.

Reply 4: Thank you for the comments. We add the description of statistical methods to be used in boxplots and where they are applicable in the Methods section.

Changes in the text: See Page 8, Line 195-202.