Peer-review report

Reviewer #1(Number ID: 08223545):

This study demonstrated that SLC16A8, as an oncogene, could accelerate proliferation, EMT, metastasis, angiogenesis, and glycolysis of CRC cells in the absence of oxygen. The author first confirmed the expression and prognosis of SLC16A8 in CRC and investigated the impacts of hypoxia on the proliferation, EMT, metastasis, glycolysis, and angiogenesis of CRC cells. And the author further confirmed the effect of SLC16A8 silencing on these malignant behaviors of CRC under hypoxia condition. The authors verified that SLC16A8 upregulation not only promoted anaerobic glycolysis, LDHA, and PKM2 expression in colorectal cancer cells but also suggested Warburg effect involvement under hypoxic conditions and concluded that the importance of addressing lactate efflux in cancer therapy and warrant further investigation into SLC16A8 as a potential therapeutic target. The findings emphasize the importance of addressing lactate efflux in cancer therapy and warrant further investigation into SLC16A8 as a potential therapeutic target. The connection between the target SLC16A8 and lactate efflux is not clear in the manuscript. The author needs to emphatically describe the research ideas and explain comprehensively analyze the conclusion.

1. In Fig 3C, what caused the changes in cellular morphology in 6h?

Response: Thank you for your inquiry regarding the results for the RKO cells at the 6-hour mark in Fig 3C. You may notice differences in cellular morphology in the image. This is due to the high density of RKO cells at the 6-hour time point, which leads to more densely packed cells in the field of view. This density can obscure the cell edges, making the morphology appear different compared to other groups when the image is viewed as a whole.

To address this, I have selected a better-focused image to replace the previous one. You can zoom in to see that the cellular morphology is consistent with other groups.

2. No difference could be detected in EdU staining. How did the author made the statistical analysis?

Response: Thank you for your question regarding the statistical analysis of the EdU staining results. After staining the cells with EdU and DAPI, we conducted the following steps:

1) Image Capture: We randomly selected and captured images from three different fields of view

for each group to ensure a representative sampling.

2) Cell Counting: We counted the number of EdU-positive cells and the total number of cells (based on DAPI staining) within these fields.

3) Statistical Analysis: We calculated the percentage of EdU-positive cells in each field of view and used these percentages to generate a bar graph. The statistical analysis compared the mean percentage of EdU-positive cells across different groups to determine any significant differences.

I hope this clarifies our approach to the statistical analysis of EdU staining.

3.Why the author used different kinds of cells for different test (FHC, SW480, RKO, HCT116 and LoVo cell lines)? For example, can the LoVo cell be used for transwell test (FHC, SW480, RKO, HCT116 and LoVo cell lines)? What about HUVECs?

Response:

(1) Thank you for your insightful question regarding our choice of cell lines for the experiments. The decision to use these specific cell lines was based on the results from colorectal cancer clinical samples (Fig 1), which indicated a characteristic high expression of SLC16A8 in colorectal cancer tissues.

To investigate the mechanism of action of SLC16A8 in colorectal cancer, we evaluated the expression level of SLC16A8 in four colorectal cancer cell lines (SW480, RKO, HCT116, and LoVo) and compared them to the normal fetal human colon epithelial cell line (FHC) using qPCR.

We selected colorectal cancer cell lines that showed high expression of SLC16A8 as in vitro models for our studies, to closely mimic the clinical observations and provide relevant insights into the function and impact of SLC16A8 in colorectal cancer.

(2) Thank you for your question regarding the use of LoVo cells for the transwell test and the role of HUVECs in our study.

In our research, we aimed to investigate whether hypoxia induces epithelial-mesenchymal transition (EMT) in colorectal cancer cells. To determine the malignant biological behavior of colorectal cancer cell lines RKO and LoVo under hypoxic conditions, we focused on examining their effects on endothelial cells, particularly HUVECs, rather than directly assessing the migration and invasion of colorectal cancer cells themselves.

HUVECs were chosen because they play a critical role in tumor metastasis, including processes

like angiogenesis and the interaction between tumor cells and the vascular endothelium. By co-culturing HUVECs with RKO and LoVo cells under normoxic or hypoxic conditions, we were able to assess the impact on HUVEC proliferation, migration, invasion, and angiogenesis capabilities. This approach allowed us to indirectly evaluate how hypoxia affects the interactions between colorectal cancer cells and the vascular environment, an essential aspect of metastasis.

Therefore, while LoVo cells were used in the study, the primary focus in this context was on their influence over HUVECs, which provides valuable insights into the metastatic process under hypoxic conditions.

4. The Graphical Abstract is not clear enough to help understanding the research. The schematic diagram can be made more exquisite and attractive.

Response: Thank you for your feedback regarding the Graphical Abstract. We have revised and replaced it with a more clear and visually appealing schematic diagram to enhance understanding of the research.

5. Reference citation is not enough in introduction.

Response: Thank you for your valuable feedback. We have thoroughly reviewed the introduction section and have added additional references.

6. Why the author used the nude mice bearing tumor model to investigate that effect of SLC16A8 on the growth of tumor? What about other strains of mice.

Response: Thank you for your question regarding the use of the nude mice bearing tumor model in our study on SLC16A8's effect on tumor growth.

We chose nude mice because they are an immunodeficient model, which significantly enhances both the speed and success rate of tumor establishment. This makes them an ideal and commonly used model for tumor research, as they allow for the direct study of tumor growth without the interference of a complete immune response, providing a clear view of the effects we are investigating.

While other strains of mice could potentially be used, the nude mouse model is particularly advantageous for these types of studies due to its ability to readily accept xenograft tumors, thus

allowing us to efficiently observe the biological effects of SLC16A8 on tumor growth.

Reviewer #2 (Number ID: 05774721):

The manuscript is overall well presented however, some concerns should be addressed before being considered for publication:

Major concerns 1- Although the article's format separates results from the discussion, each results section should clearly present the conclusions the authors achieve from the observed data, particularly in terms of how the results specifically affect the behavior of each biological model. These conclusions can then be revisited in the discussion.

Response: We have revised each subsection of the Results to include concise conclusions that detail how our findings impact the behavior of each biological model used in our study. These summary statements have been crafted to provide clear insights into the implications of our data. Thank you again for your insightful suggestions, which have aided in strengthening our manuscript.

2- Do the authors have approval for animal use in this experimental protocol?

Response: Thank you for your thorough review and insightful comments on our manuscript. Regarding your question about the approval for animal use, we have obtained the necessary ethical approval, and the details are provided in the "Materials and Methods" section of the manuscript.

3- How many experiments were performed to obtain the data on protein expression by western blot? None of the blots show band quantification with their corresponding standard deviations.

Response: We confirm that each group included three independent experimental replicates. We have also added quantification bar graphs with the corresponding standard deviations to the manuscript. Thanks for your suggestion.

4- Figure 7D-G is not well described in the results section. Ki67 results should be shown also as a proliferation index, not merely as an analysis of microscopy obtained images. The term "inhibition of proliferation signals" may not accurately describe the results presented.

Response:

1. We included quantitative analysis of the Ki67 proliferation index in the results section to more accurately reflect changes in tumor cell proliferation, beyond just observations from microscopy images.

2. We revised the description to avoid using the term "inhibition of proliferation signals," ensuring a more precise reflection of our findings.

We believe these changes will help to more clearly convey the implications of our experimental results and data. Thank you for your thorough review and valuable feedback on our manuscript.

Minor Concerns

5- I found several spelling and grammar errors, including in the section titles. It is important to review and correct these errors to ensure a more professional and polished presentation.

Response: We have conducted a thorough review and correction of these errors throughout the manuscript to ensure a more professional and polished presentation. Your feedback is invaluable, and we appreciate your attention to detail. Thank you again for your careful review.

6- It should be better specified that the Warburg effect refers to aerobic glucose metabolism in tumor cells.

Response: We have specifically mentioned in the introduction that the Warburg effect, also known as aerobic glycolysis, refers to the preference of tumor cells for glucose metabolism under aerobic conditions. Thank you for your suggestion.

7- In the introduction, the phrase “Compared to healthy cells, tumor cells proliferate at a rapid rate along with a higher rate of metabolic uptake” should be better explained, particularly the term "metabolic uptake."

Response: Thank you for your feedback regarding the phrase in the introduction. We have revised it to "Compared to healthy cells, tumor cells proliferate at a rapid rate along with higher metabolic requirements" to better clarify the concept. We appreciate your insightful suggestions.

8-EMT (epithelial-mesenchymal transition) and EndMT (endothelial-mesenchymal transition) should be defined when they first appear in the text.

Response: We have revised the manuscript to define EMT (epithelial-mesenchymal transition) and EndMT (endothelial-mesenchymal transition) when they first appear in the text. Your feedback has been invaluable in improving the clarity of our manuscript, and we appreciate your detailed review.

9- Is there any explanation for why transporter expression is lower in patients with stage IVA compared to stages IIIC and IV?

Response: There are several potential explanations for this phenomenon. Tumor heterogeneity might play a significant role, as different stages may exhibit distinct biological characteristics that influence SLC16A8 expression. Additionally, the dataset from the Gepia database might be subject to sampling bias or have a varying number of samples across stages, affecting the observed expression levels.

Another consideration is the role of biological feedback mechanisms that modulate transporter expression during cancer progression, potentially optimizing cancer cell survival under varied metabolic conditions. Finally, differences in treatment regimens across stages could impact SLC16A8 expression; stage IVA patients might have received varied treatments that indirectly regulate the transporter levels.

Further research is required to elucidate these differences fully. We appreciate your insightful question and are committed to continuing to explore these mechanisms.

10- In the section titled “HIF-1α promotes SLC16A8 expression and induces metabolic reprogramming in colorectal cancer cells” of the results, a brief introduction to why the HIF-1α mechanism is being studied and its link with SLC16A8 would help improve reader comprehension. Similarly, the rationale for selecting only the LoVo and RKO cell lines for hypoxia experiments must be explicitly justified.

Response: Thank you for your valuable feedback. To enhance reader comprehension, we have added a brief introduction in the results section. “HIF-1α is a crucial transcription factor that regulates cellular responses under hypoxic conditions, and its role in modulating SLC16A8 expression is pivotal for understanding the metabolic reprogramming in colorectal cancer cells. This linkage helps elucidate how hypoxic tumor microenvironments can drive the Warburg effect

and influence tumor progression."

Regarding the selection of LoVo and RKO cell lines for our hypoxia experiments, we chose these lines based on their higher expression levels of SLC16A8 compared to other colorectal cancer cell lines, as indicated by preliminary qPCR analysis. This choice allows us to effectively study the mechanistic impact of HIF-1α on SLC16A8 under hypoxic conditions where its expression and functional implications are expected to be more prominent.

11- The naming of conditions on the Blots on some figures can be redone to show more delicate and improved figures.

Response: We have reviewed and revised the labels to ensure they are more delicate across the figures. These improvements should enhance the clarity and presentation of the results. Your input is greatly appreciated.