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Reviewer 00227505

1) ***Critique: 1. The authors found that there was consistent over-expression of glycolytic enzymes and lactate dehydrogenase in keeping with the Warburg effect to facilitate rapid ATP production from glycolysis. Do the authors have any data to show the results? If so, it strengthens the manuscript.***

Unfortunately, we do not have any novel data that directly shows the glycolytic enzyme levels in pancreatic cancer. Our research was limited to Panc-1 and Mia-PaCa-2 cell lines. We indirectly deduced in our own studies that certain glycolytic enzymes played a key role in ATP production – by inhibiting hexokinase and GAPDH, we showed ATP levels were reduced. These data have been published by our group [1].

[1] James AD, Chan A, Erice Azparren O, Siriwardena AK, Bruce JI. **Glycolytic ATP fuels the Plasma Membrane Calcium Pump Critical for Pancreatic Cancer Cell Survival**. *J Biol Chem. 2013 Dec 13;288(50):36007-19.* PMID: 24158437.

2) **Critique 2. The authors state "Further characterisation of the PDAC metabolic phenotype is necessary as currently there are few clinical studies and no successful clinical trials targeting metabolic enzymes."Do the authors have any plan to conduct the clinical trial?**

At the current time, we feel there is insufficient evidence regarding the effects of metabolic inhibitors to conduct a clinical trial. The Warburg effect originally describes a simple pathological 'switch' in cancer cells from mitochrondial oxidative phosphorylation to glycolysis for ATP production. However, there is overwhelming evidence that ATP production is only one of many metabolic pathways altered by tumourgenesis. The 'metabolic phenotype' described in our review is an attempt to provide a comprehensive overview of these pathway changes. Future work in the field should add to and complete the phenotype and identify new therapeutic targets or specific combinations that could be used in the clinical setting.

3) **Critique 3. In the section of conclusion, the authors referred to the article from Ko et al. I realized the authors quoted the article since none of the article regarding pancreatic article is published. But the character between HCC and PDAC is completely different. The sentences would be needed to rewrite. The sentences would be needed to change or rewrite.**

In reporting the clinical use of metabolic inhibitors, the distinction between PDAC and HCC is, of course, important particularly as the phenotype we describe is specific to PDAC. We have added the following sentence to clarify this.

"There is, however, progress in using metabolic inhibitors in cell types other than PDAC which show a translation to in vivo treatment."

Reviewer 00074342

If I understood right, metabolic phenotype was mainly studied in cell lines. It would be very interesting to test those in real patient cohorts. Tables: could you please add hte abbreviations in the table? It would make the article easier to read.

It would be very interesting to test metabolic inhibitors in real patient cohorts. Ko and colleagues report encouraging the translation of 3-BP compounds into clinical practice. The 'metabolic phenotype' we constructed highlights the complex interactions between the metabolic pathways.

We have added common abbreviations to the tables which should make the article easier to read.

Reviewer 01557283

1) **Abstract. In the results section, the authors did not state any concrete results. The authors should express some main concrete findings and targets to treat PDAC.**

We have identified 6 potential therapeutic targets (HK, PGI, FBA, enolase, PK-M2 and LDA-A), and clarified this in the results section.

2) **Introduction. The authors should clearly state how important reviewing tumor-related metabolic enzyme is.**

The aim of the study is to provide an overview of the metabolic phenotype of PDAC. The following has been added to the introduction to better state the importance of this.

"*New potential therapeutic targets can be identified within this phenotype for further study as novel treatments for PDAC*."

3) **Results. The authors should simplify results. The results section is too long.**

The results section is now presented as 4 main sections - the hexose and triose stage of glycolysis, anaerobic fermentation and aerobic respiration (Krebs Cycle). This is more logical rather than just a list of enzymes and should be more readable. The main text of the results has been made more concise.

4) **Conclusion. The authors should state what target could be very attractive to treat PDAC.**

We have identified 6 potential therapeutic targets (HK, PGI, FBA, enolase, PK-M2 and LDA-A), and clarified this in the results section.

5) **Tables. There are many words in the tables. The authors should make an effort to simplify them, e.g., Table 1 & 3.**

Tables made more concise.

Reviewer 01212501

1) **Authors did a great job to summerize the metabolic phenotype of PDAC. It would be better that authors could suggest the differences between PDAC and other cancer in terms of metabolic phenotype. Even though author commented on "metabolic phenotype" of pancreatic cancer, these metabolic alterations may be similar to those of other cancers**

We haven't systematically compared the metabolic phenotype of PDAC to that of other cancers, although that in it self would be an interesting study. We do show some similarities of expression levels of certain glycolytic enzymes, for example, phosphofructokinase expression levels in lung and gastric cancers.

2) **Adding appropriate figures showing metabolic characteristics of pancreatic cancer will be more helpful for readers**

We have avoided adding a figure to illustrate the glycolysis pathway and Krebs cycle as it was thought to be overly complicated. We have therefore opted for a table with 'short notes' from the studies we have identified. If the editors and reviewers feel a figure would be helpful, we would be more than happy to make one.

3) **In oncologic practice of pancreatic cancer, there are, by and large, two clinical phenotype of metabolism of pancreatic cancer. One is high FDG-uptake pancreatic cancer and another is low FDG-uptake pancreatic cancer. Can authors suggeste the underlying oncologic meaning based on these review knowledge?**

High uptake of FDG may be in part due to the differing expression levels of the Glut glucose transporter proteins that correlate to tumour size and histological grading. There are no specific studies in the review that directly look at this – although it would be tempting to add this to our review, it would be purely hypothetical.

4) **Authors suggested that metabolic phenotype could be potenital target in treating pancreatic cancer. On the other hand, it would be more helpful to explain the current obstacles, such as drug specific toxicity to cancer; how can metabolite-targerted drug differentiate patient's normal enzyme from cancers' ones?**

We have identified 6 potential therapeutic targets (HK, PGI, FBA, enolase, PK-M2 and LDA-A), and clarified this in the results section. We have added a small summary to the conclusion with regards to the obstacles faced when 'blocking' glycolytic pathways rather than a targeting approach.

5) **Also, other metabolites, such as AA, Lipid.. or authors need to modify the title as " The glucose metabolic phenotype of pancreatic cancer".**

After considering the reviewer's comments, we have changed the title of our paper to "The Glucose Metabolic Phenotype of Pancreatic Cancer".

Reviewer 00069894

1) **The title 'The Metabolic Phenotype of Pancreatic Cancer' was way too common and failed to highlight the significance of the research in details. The authors tried to summary the enzymes involved in PDAC from a microscopic view, the subject of this paper should be more specific. Besides, my group has already published a paper regarding 'Metabolic Phenotypes in Pancreatic Cancer' in PLOS ONE [1] and I believe some of the research were overlapped. As a result, it is highly recommend that the authors revise the subject. The metabolic types of cancer were extremely complicated and a well-accepted conclusion was far to draw. The metabolic phenotypes of tumors were roughly classified into two categories, glucose- and glutamine-dependent metabolism. There were Warburg type, reverse Warburg type, mixed type, and null type in glucose-dependent metabolism, and canonical type, non-canonical type, mixed type, null type in glutamine-dependent metabolism. What are the authors' viewpoints referring to the relationship among the various metabolic types? In the manuscript, why would the researches simply concentrate on Warburg effect irrespective of the remaining mentioned above?**

After considering the reviewer's comments, we have changed the title of our paper to "The Glucose Metabolic Phenotype of Pancreatic Cancer". The intention of the review was to focus on glucose metabolism (Warburg type) rather than other metabolites (such as amino acid, lipids) or classifications. We also wanted to focus on studies that reported expression changes to normal tissue (so as to identify potential therapeutic targets) and where metabolic inhibitors were used to successfully manipulate cell pathways to affect the oncological potential of the cell.

2) **The metabolic enzymes in PDAC were demonstrated in cell lines. Do the authors tend to interpret the conundrum in tissues or even patients in the future?**

We have added a small paragraph on the obstacles faced when simply 'blocking' glycolysis is used as an *in vivo* treatment. In an *in vitro* cell line environment, metabolic inhibitors may be successful in reducing cell growth (etc), but would also have a non-specific effect on normal cells leading to as yet unknown toxic effects. This presents a challenge of using such broad inhibitors. A detailed metabolic phenotype is therefore useful in identifying and targeting specific oncological changes and so would theoretically have less effect on normal cells.

3) **To me, the RESULTS was too lengthy and difficult to understand. For general readers, it would be better if the professional interpretation were concise**

The results section is now presented as 4 main sections - the hexose and triose stage of glycolysis, anaerobic fermentation and aerobic respiration (Krebs Cycle). This is more logical rather than just a list of enzymes and should be more readable. The main text of the results has been made more concise.

4) **As for the CONCLUSION, the authors did not give a concrete summing-up and failed to underline the significance of the research. In some point, the abundant summary came to nothing. In what way can the research benefit the clinical application in the future?**

We have identified 6 potential therapeutic targets (HK, PGI, FBA, enolase, PK-M2 and LDA-A), and clarified this in the results section. We have added a small summary to the conclusion with regards to the obstacles faced when 'blocking' glycolytic pathways rather than a targeting approach, and also the significance of constructing a metabolic phenotype.