Review of: "The dual energetic supply of eukaryotic cells"

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This is a review article depicting the author views on a series of complex mechanisms. The Lynn Margilus' hypothesis provides the main support for an evolutive interpretation that leads to the function of the transcription factor HIF and its involvement in pathology. My comments refer to specific points that I believe require fine-tuning in view of the ultimate proposal of associating the paper with human pathophysiology.

1. In the paragraph "Energy transformation", it should be taken into account that the end product of glycolysis is pyruvate. Two molecules of ATP are formed per molecule of glucose along glycolytic reactions as the balance-net gain. The production of CO_2 and H_2O is due to OXPHOS.

2. The assertion "Mitochondria provide the cell with protection against free radicals" should be adapted to include the notion that the leakage of electrons during their transport within the mitochondrial membrane drives premature reaction with oxygen and the generation of ROS.

3. Given the relevance assigned to the HIF1α subunit of HIF, a comment on the mechanism involved in the stabilization of the protein should be provided. It is relevant how mitochondrial activity influences dioxygenase (prolyl hydroxylase) activity through the availability of nucleocytosolic 2-oxoglutarate, succinate, and fumarate.

4. The narrative spreads the notion that glycolysis and OXPHOS are autonomous mechanisms. Pyruvate transport into the mitochondria is important for proper performance of the tricarboxylic acid cycle. It is mainstream its role in the formation of citrate both for downstream cycle activity and suppling the nucleocytosolic pool via tricarboxylate carrier protein. After ATP citrate lyase cleavage, citrate yields acetyl-CoA and oxaloacetate. Oxaloacetate reduction to malate generates NAD^+ in the cytosol and underpins glycolysis. The sole resort to glutaminolysis and β-oxidation to maintain tricarboxylic acid cycle activity is not a safe option if a lasting homeostasis is expected.

5. The assertion that "the ATP produced by the mitochondria significantly exceeds the capacity of SET-AG, resulting in the shutdown of its activity" should be fine-tuned. If I understood well, SET-OP refers to OXPHOS, and this starts from glycolysis products. Acetyl-CoA is the product of the activity of pyruvate dehydrogenase complex, and oxaloacetate can be the product of the anaplerotic reaction catalyzed by pyruvate carboxylase. Being most receptive to author's view, it can

only be stated that a high production of mitochondrial ATP, reduces the need for glycolytic ATP.

6. Glycolysis cannot be considered solely as a source of ATP. The first step of glycolysis yields glucose-6-phosphate that can be shunted to the pentose-phosphate pathway (PPP) to yield NADPH and ribose-5-phosphate for nucleotide and enzyme cofactors synthesis, namely NAD^+ and CoA. A further connection with glycolysis occurs at the sixth step through the reaction of glyceraldehide-3-phosphate with fructose-6-phosphate catalysed by trasketolase.

7. Glycolysis has another offshoot at the seventh step of glycolysis. 3-Glycerophosphate can be used for *de novo* serine synthesis and one-carbon metabolism. This route seems important for *de novo* purine biosynthesis and cell proliferation. By the way, this reduces the contribution of glycolysis to ATP production, since this depletes the supply of phosphoenolpyruvate to pyruvate kinase, while the synthesis of serine yields inorganic phosphate by phosphoserine phosphatase.

8. Key assertions in the text need bibliographic support. For instance, the calculation of CO_2 molecules produced from ADP-PU in anoxia. In this connection, most current studies are performed in hypoxia in atmosphere with 1% O_2. This makes it necessary the reference to setting conditions.

9. The reference to nitrogenase should be fine-tuned since these are microbial enzymes. To the best of my knowledge, human beings only contain these types of enzymes in gut microbiota.

10. The abbreviation AA is introduced without a previous explanation. I assume the author prefers the initials of ascorbic acid to refer to vitamin C.

11. The sections: “Vitamin C and ATP are the initiators of energy transformation” and “Structure of SET-AG and SET-OP” are difficult to be understood by a general audience. The absence of detailed captions to the figures is an additional drawback.

12. The paragraph stating: “In the serum of intravenous AA-treated patients with cancer, the level of ADP increases. At the same time, the uric acid decreases, which may be because the high serum AA level activates the ADP-PU units of SET-AG of all cells, without ADP-ATP transformation” is not accompanied by bibliographic references that help

understand the medical significance of the assertion.

13. The significance of the section devoted to “Ribose” in the context of the review is not clear since the main input relates to the nutritional effect of ribose supply on glucose homeostasis and its influence on plasma insulin concentration. Since the purpose of the manuscript is the energetic supply of eukariotic cells, it could better focus on the production of ribose 5-phosphate by ribokinase (ATP:d-ribose 5-phosphotransferase). A process directly involved in ATP synthesis that bypasses the slower production of ribose 5-phosphate in the pentose- phosphate pathway.

14. The sentence in the abstract regarding “the same structures help the development of cancer and the increase of its malignancy” is not developed in the main text. This is especially important because glycolysis in the presence of 0_2 has been considered a unique property of malignant cells, and recent research has disclosed that a metabolic rewiring supporting aerobic glycolysis is a key mechanism of defence against infection utilized by immune system cells.

15. References should be updated to include most recent research.