Reviewer's report

Title: Symbiotic energy metabolism based on lactate shuttle can sustain prostate cancer progression

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Reviewer: Luc Pellerin

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In this study, the authors have investigated the ability of prostatic tumor cells to modify not only their own metabolic profile but also the one of fibroblasts in order to take advantage of lactate supply from these neighboring cells to promote their proliferation. They show that when different tumor cell lines are cultured in low glucose medium, lactate has a mitogenic effect. They suggest that such an effect might be related to an enhancement in expression of the monocarboxylate transporter MCT1 and of the cytochrome c oxidase induced by lactate, most likely via a reduction of the active form of the AMPK. In parallel, they demonstrate that fibroblasts cultured in presence of prostate tumor cell conditioned medium become more glycolytic and express more monocarboxylate transporter MCT4, by a mechanism that involves the transcription factor HIF-1#.

Inversely, tumor cell lines cultured in presence of conditioned fibroblast conditioned medium proliferated better, an effect prevented by activation of AMPK. A similar effect was observed in vivo when both tumor cells and conditioned fibroblasts were injected together. To support their claims, they also looked at the expression of MCT1 and MCT4 in sections from human patients and found a distribution consistent with their hypothesis in prostate tumors. This study provide a series of evidence both in vitro and in vivo supporting an interesting concept. It could be improved substantially and give it a greater impact if the authors could pay attention to the following remarks:

Major Compulsory Revisions

1. The authors chose to characterize the metabolic profile of four tumor cell lines but then experiments are done by selecting some of them, depending of the experiment, without clear justification. The demonstration would gain in clarity if ALL experiments were done with the same cell lines.

2. In Figure 1B, why not showing the level of expression of MCT1 as well ?

3. In Figure 1C, why not showing immunohistochemistry in all four cell lines ?

4. In Figure 1E, please provide statistical analysis.

5. In Figure 2B, why showing the results only for the LNCaP cells ?

6. In Figure 2C, does this correspond to the level of expression of MCT1 in cells cultured in low glucose and in presence of lactate ? If yes, there must be a quantitative assessment and a comparison with levels in standard culture
conditions.

7. In Figure 3A, why choosing this time the tumor cell line WPE1? Moreover, a quantitative assessment of western blots together with statistics to demonstrate a significant effect would be necessary. Do the author imply a causal link between the change in MCT1 expression and the reduction in the activation of AMPK? If yes, they should show it using the inhibitor metformin.

8. In Figure 4, why choosing to use the cell line PC3 in this case? The blot in Figure 4B should be repeated and quantitated. It is not so clear that MCT4 expression is enhanced by the conditioned medium. What is the mechanism by which the PC3 conditioned media induces HIF-1α? It is obviously not an oxygen level-dependent mechanism so what are the other options (see Semenza 2009 Physiology 24:97-106)? Could it be tested with appropriate inhibitors in this case? This point should be at least discussed.

Discretionary Revisions

1. In figure 6H, it is unclear why the authors are showing these immunostainings in the present context. Please argue or remove.

**Level of interest:** An article of importance in its field

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests