Reviewer’s report

Title: A lactate shuttle system between tumour and stromal cells is associated with poor prognosis in prostate cancer

Version: 1 Date: 7 January 2014

Reviewer: Céline Pinheiro

Reviewer’s report:

In this manuscript by Pétega-Gomes N. et al, the authors evaluated the expression of several key metabolism-related proteins in the stroma and cancer cells of 480 prostate tumours. Expression of MCT1, MCT4, GLUT1, GLUT12, LDHV, PDK1, CAIX, AMACR, ACOX-3 and DBP was assessed by immunohistochemistry and correlated with prognostic variables. The authors provide evidence for a metabolic symbiosis between cancer-associated fibroblasts (CAFs) and cancer cells in prostate cancer. This is an important study, however, the authors should consider the following concerns:

Major Compulsory Revisions

1. The question and rationale of the work posed by the authors is either not clear or not completely answered as the expression of some of the markers (LDHV, AMACR, ACOX-3 and DBP) is not at all explored in the Discussion section. Please discuss the relevance of the data found for these markers and how they fit in the hypothesis.

2. Both the Introduction and Discussion sections lack important information by other authors. Please describe in more detail the metabolic symbiosis between CAFs and cancer cells, by including in the Introduction section information related to other studies, such as the studies in prostate (already included in the Discussion), lung (Cancer Biol Ther. 2007 Sep;6(9):1476-9) and colorectal cancers (Cancer Res. 2006 Jan 15;66(2):632-7). Include these 2 last articles also in the Discussion.

3. Some information included in the manuscript is not supported by references: the regulation of MCT1 in the Introduction section, GLUT expression in cancer cells previously observed by the authors in the Discussion section and prognostic value of perineural invasion in the Discussion section.

4. The Results section may be improved if the data obtained is more extensively described. PDK1 expression frequency in tumour cells and stroma is not quantitatively compared while the expression frequencies of the markers exclusively found in cancer cells is not further described: are they high frequencies, low frequencies?

5. The second paragraph of the Results section describes an analysis without any result description or figure/table. Please clarify this analysis by including the result description concerning this analysis in the same paragraph. Also, this
paragraph refers “the same proteins”, however, the proteins analysed are only 3, instead of the 10 initially studied. Please clarify this issue in the Results section as others expressions suggest analysis of the 10 proteins while the figures show only part of them (ex. first part of fourth paragraph and description of Figure 3).

6. The third paragraph of the Results section is essentially the legend to Figure 2. Please remove it from the Results section.

7. Please be cautious when stating that “no expression/staining” is found in some cases (Results, fourth paragraph, line 6 and line 10). The cut-off used in the present study, where only moderate and strong final scores were considered positive, should not create the confusion that negative cases show no protein expression.

8. The Discussion section is poorly explored. The results obtained can be further discussed, based on results by other groups. For instance, the Reverse Warburg effect is mentioned in the abstract but left behind in the rest of the manuscript. The Discussion can be much more improved if a discussion, based on Reverse Warburg effect studies by Lisanti’s group, is included. Similarly, as mentioned before, studies on the metabolic symbiosis between cancer cells and CAFs by Koukouraki’s group should be included in this section. Additionally, the expression frequencies of these proteins in cancer cells should be compared with studies by others.

Minor Essential Revisions

1. The manuscript shows some English incoherence and other small typing errors such as: “tumour” appears both as “tumour” and “tumor”, the Results section shows a mixture of verbal conjugations (past and present), “rim” instead of “kidney” in Table 1, p (for p value) must be in italic, lack of commas, etc. Please review.

2. Is the last sentence of Introduction’s 1st paragraph in the correct place? Should it not be at the end of the Introduction, following the main aim of the work?

3. The section Patient sample selection should begin by stating the type, number and origin of the samples. Also, please list in this section the clinic-pathological information used (transfer the sentence “Preoperative serum total PSA,...” from the Results section to the Material and Methods section).

4. Please confirm if the version of SPSS used was 17.0, as the last version is 21.0.

5. The 3rd sentence of the Results section is a repetition of what was stated in the 2nd sentence. Please review.

6. p values in Table 2 that do not reach significance should not be presented in bold.

7. p=0.052 is not significant, therefore, the association of MCT1 and MCT4 co-expression, in cancer cells and CAFs respectively, with biochemical recurrence after surgery does not exist.
8. In the Discussion section, paragraph 5, which proteins show changes along malignant transformation?

9. The Conclusion is a summary of the last paragraph of the Discussion. Replace the Conclusion by the last paragraph of the Discussion.

10. Provide a more detailed legend for Figure 1, including the meaning of “*”.

Discretionary Revisions

1. Please clarify why there are only 203 non-neoplastic samples, if the 408 patients were only included if both normal and tumour tissues were available.

2. The abbreviations BAFs and PAFs are introduced in the Results section, but no longer used in the rest of the results description. The continued use of these abbreviations may help to understand sentences that are in the present form somewhat confusing, for example: fourth paragraph of the Results section.

3. Figure 3 includes representative immunohistochemical reactions of only part of the proteins studied. Among the proteins that were only expressed in cancer cells, why only MCT1 is represented in Figure 3? Why the other proteins are not also included in Figure 3? Also, Figure 3 should be the first Figure of Results section.

4. Why use 2 CAFs in Figure 4? An image with only one tumour cell and one CAF would be clearer. Also, this Figure would be more adequate in the Discussion section, to illustrate the hypothesis.

5. Was the expression of the different proteins in cancer cells associated with the clinico-pathological data? Such data would greatly improve the manuscript.

**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