Author's response to reviews

Title: Lactate transporters and vascular factors in HPV-induced squamous cell carcinoma of the uterine cervix

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Author's response to reviews:

Dear Dr
Dafne Solera, Executive Editor,
BioMed Central

Thank you for the opportunity to submit the revised format of the manuscript Lactate transporters and vascular factors in HPV-induced squamous cell carcinoma of the uterine cervix, after carefully edition following the Reviewers’ suggestion.

Sincerely yours

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São Paulo, 20 June 2014

Response to Reviewer’s
Reviewer: Jerome Doyen

Reviewer’s report:

Major Compulsory Revisions

1- detailed characteristics of population are missing (median age, number stage I, II, III, TNM classification, etc ...); it has to be added either in the text or within a dedicated table.

As suggested by the reviewer, the characteristics of the population were included in the Methods Section.

2- it is not clear whether authors have already reported the same results in a previous publication (reference 28) for MCT1, 2 and 4; did the authors use exactly the same patients? In this case authors should put the results for MCTs in the introduction or discussion paragraph.

The series used in the present study is different from the one used in the previous publication. The sentence “using a different cervical cancer series” was included in the Discussion section to eliminate any confusion.

3- HPV status is missing in all patients’ samples (either cervicitis, dysplasia and cancer samples); it is indirectly done routinely with the p16 immunostaining and has to be reported as well in this study. (Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. Sano T, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Am J Pathol. 1998 Dec;153(6):1741-8). It would be very interesting to try to correlate p16 protein staining with tumor progression and metabolic phenotype; this could mimic the data seen in vitro.

The idea of the Reviewer is certainly nice but unfortunately, this is a retrospective series with limited material to perform HPV identification.

4- could authors also see stromal MCT4 staining in patient samples? It was described as an important characteristic in several tumors, with maybe a prognostic impact due to stromal and not only tumoral staining. (Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. Fiaschi T, Marini A, Giannoni E, Taddei ML, Gandellini P, De Donatis A, Lanciotti M, Serni S, Cirri P, Chiarugi P. Cancer Res. 2012 Oct 1;72(19):5130-40. doi: 10.1158/0008-5472.CAN-12-1949. Epub 2012 Jul 31)

MCT4 and the other proteins were only analyzed in cancer cells since the vast majority of the cases were predominantly represented in the TMA by malignant tissue with scarce stromal tissue surrounding the affect cells.

5- why did authors not analyze VEGF proteins in non-malignant tissues and in keratinocytes?

VEGF proteins were only analyzed in malignant human samples since the vast majority of the cases were predominantly represented by malignant tissue with
scarce stromal tissue surrounding the affect cells. Normal keratinocytes express VEGF proteins by stimulation.

6- results of staining in keratinocytes are difficult to interpret with the photos chosen by authors; in order to better quantify it, authors should perform western blot to rigorously demonstrate small quantitative changes.

The purpose of 3D culture was to evaluate the expression of the proteins considering the histology, e.g. evaluate the expression in the basal layer, the lower layers and the upper layers. If we perform WB, we will loose the 3D cell distribution.

Minor Essential Revisions:
7- what authors wanted to write line 19 page 7 instead of "e"
We replaced the “e” by “and”.

Discretionary Revisions:
8- why authors choose 48 years as cut-off for age?
Forty-eight years is the mean value for age and was chosen as cut-off since the age of the patients follows a normal distribution.

9- authors should mention how they did 3D culture.
An adequate reference was added in the Material and Methods Section (, as previously described [19]) to refer to 3D culture experiments.

10- photos for MCT4 staining in keratinocytes in vitro does not seem to show MCT4 staining; could author choose other photos?
The photos selected are representative of more than one reaction. MCT4 immunoexpression is absent from PHK pLXSN but appears, although with low intensity, in PHK infected with HPV oncoproteins (especially in PHK pLXSN E6E7).

11- authors should remove HIF-1 alpha throughout all the conclusion since no experiments did analyse this protein (they should only mention hypoxia instead).
All mentions to HIF-1 alpha during the Discussion Section are related to data obtained from other studies and the adequate reference is provided. In any circumstances we refer to HIF-1 alpha suggesting that we analysed this protein; rather, we use references related to HIF-1 alpha and hypoxia to support our results. In the Conclusion Section, HIF-1 alpha is always mentioned in parallel with hypoxia conditions.

12- authors did not discuss why MCT1 expression was not correlated with basigin expression although it is its main chaperon protein.
The sentence “Additionally, as hypothesised in other studies [27, 32-34], other chaperones not yet identified should be involved in MCT trafficking to the plasma membrane.” was added in the Discussion Section.
13- authors should add in discussion that larger sample are needed to rigorously confirm these results and in order to perform multivariate analysis and correlation with survival.

As suggested by the reviewer, the sentence “Further studies, with larger and best characterized squamous cervical cancer series, are required to rigorously confirm these results, as well as evaluate possible associations with survival.” was included in the end of the Discussion Section.

14- the following articles are missing in the introduction or discussion:
- articles dealing with previous reports on glycolytic proteins in cervical cancer,


- article demonstrating clinical benefit of anti-VEGF therapy in cervical cancer:
  Improved survival with bevacizumab in advanced cervical cancer.

As suggested by the reviewer, the majority of the above-mentioned references were included in the manuscript.

Reviewer: Peter Vaupel

Reviewer’s report:

1. Major compulsory revisions:

In this manuscript the authors state that HIF -1 alpha, GLUT 1 and CA IX are "hypoxia markers" or "intrinsic markers of hypoxia" in cancers of the uterine cervix, although there is clear evidence that all markers mentioned several authors have repeatedly shown that the expression of these markers (proteins) does not correlate with hypoxia in this cancer entity (e.g., Mayer et al., Cancer Res. 2004, Clin. Cancer Res., 2005). This should be mentioned in the Background and Discussion section of this manuscript.
Although the above-mentioned studies show no quantitative correlation between HIF-1 alpha and GLUT1, GLUT1 is regularly used to identify hypoxic regions (e.g., Mayer et al., Int J Oncol. 2011; Markowska et al., Eur J Gynaecol Oncol. 2007) or is associated intimately associated with hypoxia (e.g., Airley et al., Int J Cancer 2003; Airley et al., Clin Cancer Res. 2001). Therefore, the authors would like to maintain the use of the term “hypoxia markers”.

However, to improve the quality of the discussion, the above-mentioned references were included in the Discussion Section, along with the sentence “It is important to mention that, as described in few studies [30, 31], both HIF-1# and GLUT1 expression may not be quantitatively correlated to hypoxia in cervical cancer, however, GLUT1 is shown to be associated with hypoxia in this type of tumor and, as in the present study, is regularly used to identify hypoxic regions [6, 8, 32, 33].”.

I am not sure whether or not "metabolic markers", a term also used throughout the MS is an adequate description of these proteins. Certainly, they are all "intrinsic markers of tumor progression" as described by Mayer et al. So far, neither HIF-1 alpha nor its target genes can be judged as "pure" hypoxia marker proteins.

The term “metabolic markers” was applied to MCTs, the chaperone, GLUT1 and CAIX, to distinguish from the VEGF family (vascular markers), as those proteins are directly involved in the metabolic reprogramming of cancer cells. Even if the markers are not “pure” hypoxia marker proteins, they are clearly associated with the metabolic adaptations in cancer; therefore, we consider “metabolic markers” an appropriate term for GLUT1 and CAIX. Actually, other articles (e.g., Kim et al. 2013) also use the term “metabolic markers” to refer to GLUT1 and CAIX.

It is recommended that the authors may focus on the role of HPV in the upregulation of these proteins (according to the title of the MS).

The authors understand that, although some discussion is made about the possible role of hypoxia in the pattern of expression, the main focus of the manuscript is the participation of HPV in the upregulation of the different proteins analysed, as depicted by the experimental approaches used and the results presented.

In the 2nd paragraph of the Background section, the pathogenesis of tumor hypoxia needs some refinement: oxygen diffusion limitations are - inter alia- the consequences of both exacerbated cell proliferation together with inadequate and chaotic angiogenesis (see Bayer et al., Int J Radiat. Oncol. Biol Phys).

As suggested by the reviewer, the Introduction Section was improved and the reference included.

2. Minor revisions:
At the end of the IHC evaluation- section: ALG is not a (co-)author of this MS. ALG was replaced by AL-F.