Author’s response to reviews

Title: GPC3 reduces cell proliferation in renal carcinoma cell lines

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Author’s response to reviews: see over
To:

Dr. Dafne Solera
Executive Editor of BMC Cancer

Dear Editor,

Thank you for reviewing the manuscript “GPC3 reduces cell proliferation in renal carcinoma cell lines” (MS: 9249555461320455).

We have revised the manuscript based on the comments made by the reviewers and now we are resubmitting the new manuscript for your consideration. We also have the written English revised by a specialized company in language editing (Elsevier). The answers to the reviewers’ comments and the corresponding changes that we made on the manuscript are presented below (all changes in the manuscript are highlighted).

We would like to take this opportunity to express our thanks to the reviewers who identified areas of our manuscript that needed corrections or modification. We would also like to thank you for allowing us to resubmit a revised copy of the manuscript.
Reviewer number: 1

Reviewer’s report

Major concerns are as follows:

1. The authors showed about the result of two kinds of cell line, ACHN and 786-O. There is the difference in effect by transfection of GPC3 between these two cell lines. The authors should show the result of another cell lines.

   We analyzed the GPC3 expression in three primary (CaKi-2, A-498 and 786-O) and two metastatic clear cell renal cell carcinoma cell lines (CaKi-1 and ACHN) in order to determine the pattern of GPC3 expression in these types of renal carcinoma cell lines. As all cell lines presented GPC3 mRNA suppression, we chose two representative cell lines to progress with functional studies, one primary renal carcinoma cell line (786-O) and one metastatic renal carcinoma cell line (ACHN), with the aim to verify the GPC3 function in a primary and a metastatic clear cell renal carcinoma cell line. Furthermore, these experiments are expensive and require much time to carry out with five cell lines.

   The effect of GPC3 transfection in 786-O and ACHN was similar. In both cell lines, GPC3 overexpression arrests cells to G1 phase and consequently reduced the cell proliferation rate in clear cell renal cell carcinoma cell lines. These results suggest that GPC3 acts as a tumor suppressor in primary and metastatic clear cell renal cell carcinoma cell line.

   The requested information was placed in the Abstract and Materials and Methods parts of the manuscript file (It is highlighted in the text, lines 56-60; 65; 131-133).
2. The authors used the rabbit polyclonal anti-GPC3 (ABCAM) in this immunohistochemistry experiment. 1G12 monoclonal anti-GPC3 (BioMosaics) is most common.

The referee is correct when states that larger number of publications on the study of GPC3 related to cancer development is concern about anti-GPC3 monoclonal antibody 1G12 [1-5]. On the other hand, literature reports experimental evidences that rabbit polyclonal anti-GPC3 is also suitable for this type of application, as described by Luo et al. [6], Lin et al. [7] and Nakatsura et al. [8]. However, we have chosen the utilization of rabbit polyclonal anti-GPC3 in this work because our objective was to verify the presence or absence of the GPC3 protein after transfection with GPC3 expression vector. Thus, densitometric analyses have corroborated the fact that this polyclonal antibody is appropriate for this type of experiment. Our immunocytochemistry experiment can be considered specific because we use a control (cells transfected with empty vector) in which it is possible to verify lower protein expression than in cells transfected with GPC3 expression vector. In our work, the empty expression vector presents this purpose to contrast with the vector expressing GPC3. Furthermore, we performed a step in the immunocytochemistry protocol to avoid non-specific background.

Due to the reasons described above, we have built the foundation of knowledge required to test and assure the successful use of these polyclonal antibody on renal study.

Level of interest: An article whose findings are important to those with closely related research interests
Reviewer number: 2

Reviewer’s report

The authors present very interesting results in expression of GPC3 in renal cell carcinoma and the growth-inhibitory effects of GPC3 in renal cell carcinoma cell lines. The results are well described and presented and support the conclusions of the authors.

Minor essential revisions:

1. In Fig. 1A Comparisons are made to the “normal renal tissue”. Since kidney histology is heterogeneous, which component of the normal tissue was the comparison made? Proximal, distal renal tubules, loops of Henle, etc.?

   The normal renal tissue used for the comparison belongs to cortex. In this region is present the Bowman’s capsule, proximal convoluted tubule and distal convoluted tubule.

   We added the information about kidney histology from normal renal tissue samples in the Materials and Methods part of the manuscript (Highlighted in the line 170).
2. In a previous manuscript by Okon, Pol J Pathol. 2008;59(1):15-20, GPC3 was found over-expressed in chromophobe renal cell carcinomas. The authors should address this in their discussion and comment whether they also observed this in their studies, as a point of distinction between regular (clear cell) and chromophobe renal cell carcinomas.

   Okon et al. [9] have shown a point of distinction between GPC3 expression in chromophobe carcinoma and clear cell renal cell carcinoma, GPC3 expression was upregulated of chromophobe carcinoma and downregulated of clear cell carcinoma. In our study, we didn’t analyze the GPC3 expression in chromophobe carcinoma, however our data of GPC3 expression in clear cell renal cell carcinoma samples and cell lines agree with Okon et al. [9].

   The requested information was placed in the Discussion part of the manuscript file (It is highlighted in the text, lines 312-315).

3. The authors should also briefly discuss expression of GPC3 in other types of cancer and stress that GPC3 is highly over-expressed in hepatocellular carcinoma, in contrast to the renal cell CA.

   We added the demanded information about GPC3 expression in other types of cancer in the Discussion part of the manuscript (Highlighted in lines 298-308; 312-322).

   Level of interest: An article of outstanding merit and interest in its field

   Quality of written English: Needs some language corrections before being published
The written English was revised by a specialized company in language editing (Elsevier).

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

Declaration of competing interests: I do not have any competing interests.

References


Best regards,

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