Author's response to reviews

Title: Therapeutic implications of an enriched cancer stem-like cell population in a human osteosarcoma cell line

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ANSWER TO THE REVIEWERS:

Editorial requests

Ethics
Experimental research that is reported in the manuscript must have been performed with the approval of an appropriate ethics committee. A statement to this effect must appear in the Methods section of the manuscript, including the name of the body which gave approval, with a reference number where appropriate.

Answer: A statement explaining the animal care and handling and the approval by the ethics committee with reference number was included in a separate section.

Animal Care
Six-week-old male Balb/c nude mice were obtained from Charles River Laboratories and housed under pathogen-free conditions in individual ventilated cages. Sterile food and water were provided at libitum. The animal experiments were performed according to the local and international guidelines on animal experimentation and were approved by the Institutional Ethics Committee of the Faculty of Medicine of University of Coimbra for animal care and use (Approval ID:38-CE-2011).

Competing interests
Please include a 'Competing interests' section between the Conclusions and Authors' contributions. If there are none to declare, please write 'The authors declare that they have no competing interests'.

Answer: A Competing Interests statement “The authors declare that they have no competing interests.” was included before the section “Authors Contributions”, according to the Editorial requests.

Reviewer’s report
“In this study, the authors investigate the role of CSCs populations from human osteosarcoma cell line in the responsiveness to conventional therapies. This manuscript is well written, elegant and very interesting. The hypotheses are confirmed by the results obtained and methods used are effective in carrying out the experiments. The results are clear and the discussion explains and argues effectively the data obtained. But there is one
consideration to be clarified. The authors analyze MSCs markers both on sarcospheres and MNNG/HOS cells. They state that there is no significant differential expression of MSCs markers between sarcospheres and MNNG/HOS cells. If sarcospheres are cancer stem cells, most probably MSCs marker expression on sarcospheres should be higher of that expressed on MNNG/HOS cells. On the basis of this consideration, the authors should explain why they find no differences in expression of MSCs markers.”

Answer: When we planned this experiment we were expecting to find a higher expression of the MSC surface markers in sarcospheres compared with parental MNNG/HOS cells and that these markers could be used to identify this subpopulation of CSC in osteosarcoma, but unfortunately this was not observed and there is still lacking a specific marker that can identify unequivocally osteosarcoma CSC. In our opinion this immunophenotypic overlap between adherent cells and sarcospheres is due to the mesenchymal origin of osteosarcoma. There is substantial evidence that osteosarcoma arise from a mesenchymal stem cell in a consequence of impaired differentiation into osteoblasts caused by genetic and epigenetic alterations. Therefore it is conceivable that even non-stem osteosarcoma cells retain some properties of mesenchymal stem cells, including the surface markers. This is explained in the discussion section in page 14, as written below:

“However, the expression pattern of MSC-related antigens cannot be used as a specific marker of stem-like cells in osteosarcoma, since parental MNNG/HOS cells exhibited an immunophenotype similar to that of sarcospheres. This is in line with the evidences that osteosarcoma originates from a primitive MSC in a consequence of impaired differentiation into osteoblasts that undergo malignant transformation. Therefore it is conceivable that more differentiated parental MNNG/HOS cells retain some properties of MSC, including the expression of cell surface markers and that the overlap of immunophenotype markers is related with the stage of differentiation of MSCs at the time of the mutation [24]. Moreover, after culturing in specific differentiation conditions, MNNG/HOS cells differentiated towards osteoblasts, which indicate these cells still have some propensity of the original lineage.”