Reviewer’s report

Title: Tumor Cells With Low Proteasome Subunit Expression Predict Overall Survival in Head and Neck Cancer Patients

Version: 1 Date: 22 October 2013

Reviewer: Claire Rodriguez-Lafrasse

Reviewer’s report:

In this paper, Lagadec et al. demonstrate, based on the expression of a low proteasome function, that HNSCC cell lines contain a small subpopulation of cells with characteristics of cancer stem cells (radioresistance, high self-renewal capacity, in vivo tumorigenicity). Furthermore, in a cohort of 82 HNSCC patients they show that a weak expression of the proteasome subunit PSMD1 predicts unfavorable outcome after radiotherapy. This work is in continuation of their previous contributions in breast cancer and glioblastoma. This is a new development and gives new insight into the physiopathology and response to treatment of HNSCC cancers and is thus worthy of publication.

Major Compulsory Revisions:

Three major points require clarification:

1. Table 2 (clinical information) is missing

2. In their previous work (Lagadec et al., Breast Canc Res, 2010), the authors compared the expression of low proteasome function (ZsGreen-cODC+ cells) with classical markers of CSC (CD24-/low/CD44high) in a “cancer initiating cell” subpopulation of breast cancer cells and concluded that “not all CD24-/low/CD44high cells were positive for ZsGreen-cODC, indicating that ZsGreen-cODC+ cells constituted a sub-population of the CD24-/low/CD44high population”. In HNSCC, it is also fundamental to compare the expression of low proteasome function with that of the classically admitted markers of CSC (side population (Sun et al., 2010), CD44high(Facompre, 2012), ALDHhigh (Chen et al., 2010)…). Do the authors consider that ZsGreen-cODC+ cells are a part of, different from, or the totality of the cancer stem cell sub-population?

3. One strength of this paper is to confirm the results obtained in cell lines on biopsies of HNSCC patients. In the latter, they establish Kaplan-Meier curves for survival and locoregional tumor control according to three levels of PMSD1 expression. Although the response of the different cell lines to fractionated irradiation was studied in terms of ZsGreen-cODC+ and tumorsphere formation, a link is missing between the low proteasome expression and radioresistance. Clonogenic cell survival performed on the low and high ZsGreen-cODC sub-population of the 6 cell lines should demonstrate if a relationship exists between low proteasome function and radioresistance.

Minor essential Revisions:
- The abstract is not sufficiently clear about the objectives of the paper
- Fig 2b and 2c: maintain the same iconography for SCC12 and SCC6
- Since all the experiments cannot been performed on all the cell lines (example fig2B,C,D), the choice of SCC6 and SCC12 as reference cell lines requires justification.
- The experimental protocol which enables fluorescence in cells grown as tumorspheres to be quantified is not detailed in the material & methods section.
- Error in typography of Fig 5e (there are 2 Fig 5d panels)
- If a comparison is made between the number of cells in the windows of fluorescence measurement of the FACS profiles (fig 2e) and the mean results presented in fig 2b for ZsGreen-cODC+ and ZsGreen-cODC- populations, the results appear very different for SCC17b and at a lower level for SCC6 and FaDu. Could the authors justify it?
- The experimental protocol in the legend for fig 2c,d is not clear. If I understand well from the text, cells were seeded at a clonal density for the formation of tumorspheres, 72 hours after 5 x 3 Gy irradiation of monolayers or tumorsphere cultures. We could have expected an increase in the population forming spheres from the non-irradiated (0 Gy) tumorsphere compared with cells grown as monolayers and thus containing fewer tumorsphere-initiating cells. This point requires discussion.
- Results in fig 2b are surprising (high variation in the % of ZsGreen-cODC+ cells in non-irradiated cells). Yet this experiment is essential to demonstrate the relationship between low proteasome expression, radioresistance and tumorsphere formation. It is difficult to conclude from the conflicting results in the two cell lines and the analysis of a third line should enable a conclusion to be reached.
- The clinical results clearly demonstrate a statistically significant relationship between PMSD1 expression and survival but are not significant when PMSD1 expression is compared with locoregional recurrence. The text in the result and discussion section should be modified so as not to give these two criteria an equivalent value.

**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' below.