Reviewer's report

Title: Self-renewal and chemotherapy resistance of p75NTR positive cells in esophageal squamous cell carcinomas

Version: 5 Date: 28 October 2008

Reviewer: Gabriela Dontu

Reviewer's report:

The authors answered the major criticisms. The following minor revisions are recommended before publication. Recommended revisions are listed in the order they appear in the manuscript:

1. In the Methods section, “Animal preparation and xenograft …” indicate the cell line that was injected.
2. In the Results section, “Expression of p75NTR in ESCC specimens”:
   Indicate in the text, in the first two paragraphs the picture panels that are discussed NEE, WDC etc.
   The statistical analysis for correlation with histoclinical parameters should be uniform, using the same test for all parameters. The Chi-2 test (as performed by reviewer, instead of Cochran-Mantel-Haenszel Statistics) indicates borderline statistical significance with poorly differentiated cancers (p=0.054) and strong correlation with well differentiated cancers (p=0.002). It is not clear why the other method used by the authors shows different correlation.
   Even if the p75NTR positivity does not correlate with undifferentiated cancers, the localization in poor vs. medium vs. well differentiated cancers and the patterns of immunostaining shown by the other markers make a very strong case for association of p75NTR with the stem/progenitor cells in these cancers. It is also relevant that the same is true for the normal epithelium. These aspects should be discussed in more detail in the text (results and discussion). The statistical analysis should be reviewed by a statistician to confirm or invalidate the association with tumor grade.
3. In the Results section, last paragraph, delete “suggesting asymmetric division occurred during culture”. The assay did not test for symmetry of division, the statement is speculative.
4. In the Result section, “Sphere forming capacity of p75NTR”. The text and figures discussed in the text should be revised.
   It appears that erroneous percentages of p75NTR pos cells are given. Figure 4A, gates and percentages of p75NTR should be indicated in the flow charts. The percentages indicated in the figure legend appear to be wrong. They do not reflect the data shown in the flow cytometry analysis and they do not represent
enrichment compared to control conditions (shown in Figure 2). These should be corrected in the text and figure legend. Percentages should be mentioned for all cell lines in the text, in the spheres versus standard adherent conditions. The gates that generated these percentage should be indicated on the flow charts.

Figure 4 H, the figure legend does not reflect the labels of the flow charts. Re-label the last two panels involucrin and cytokeratin 13, instead of p75NTR.

5. In the Results section, “Xenograft tumorigenicity assay”:

Indicate the cell line used.

If tumors generated in these animals were saved, perform flow-cytometry, immunohistochemistry or both to test which tumors recapitulated parental tumor heterogeneity with respect to p75NTR expression in particular (other markers used in in vitro assays if desired).

If the tumors generated by p75NTR pos cells differ from those generated by p75NTR neg cells, in that they have both p75NTR pos and neg cells or they have a larger repertoire of markers in general and they resemble the parental tumor, one could argue that the p75NTRneg cells are likely progenitor cells, with ability to initiate tumors in the experimental system used, but possibly with a lower proliferation potential compared to p75NTR pos cells, which grew tumors when implanted in smaller numbers. This concept would gain important support if the p75NTR neg cells have a more limited differentiation potential than p75NTR pos cells in vivo as well as in vitro.

6. In the Discussion section

First sentence, replace “proliferate” with “proliferation”.

In the “Self renewal capacity of p75NTR” cells eliminate line 4: “i.e asymmetric division. The self-renewal….experiments. Same sub-section, second paragraph: Sphere formation in serum-free medium is not a property of self-renewal. Sphere formation in serum free medium is an in vitro surrogate assay for self renewal and was used in testing brain CSC etc…..

The last paragraph should have a subtitle to respect the structure of the Discussion section. Eliminate the sentence: This result was in contrast to the generally of the CSC hypothesis. Discuss the results incorporating the comments in 5. and the results of the tumor analysis suggested in 5.

Eliminate the sentence” these data implicated that Matrigel…..

The data suggest that adding Matrigel to the cells injected in vivo improves the success of xenotransplantation. Add comments on the bias that the experimental system may introduce. Cells are implanted meta-topically, which may account for tumorigenicity of both p75NTR pos and negative, due to a more permissive environment (sub-cutaneous) compared to environment of origin.

7. In Conclusions: eliminate “(4) p75NTR pos cells manifested high
tumorigenicity as compared with p75NTR neg cells. p75NTR pos cells manifested slightly increased tumorigenicity as compared with p75NTR neg cells, generating tumors when implanted in numbers of 500 cells/injection, whereas p75NTR neg generated tumors only when implanted in numbers equal or higher than 2000. These results suggest that p75NTR neg cells may be progenitors with high proliferation potential.

The manuscript would be considerably improved if experiments suggested in criticism 5) are performed, but publication should not depend on it.

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

**Quality of written English:** Needs some language corrections before being published

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:**

I declare that I have no competing interests.