Reviewer's report

Title: Gene expression down-regulation in prostate tumor-associated stromal cells involves organ-specific genes

Version: 1 Date: 17 April 2009

Reviewer: audrey player

Reviewer's report:

MAJOR REVISIONS:

ABSTRACT Section:
1. In the Abstract, the authors note that "Benign tissues" were examined. Please be specific, as "normal" tissues are designated throughout the text. Results from analysis of 1 vs. the other, can be very different.

METHODS Section-
"Sorting" results appear to be based on sorting of 2 prostate samples. I understand the difficulty in obtaining clinical samples and amounts needed for these experiments, but I feel that more samples should be examined, as validation of the current results.
Pathology of bladder samples should be included in the "Tissue Specimen" section.
Western Blot- should include control gene used for the experiments.
MACs isolation procedure- CD13 antibody source should be included? CD49+ also?
Gene expression microarray- Please designate how many Affymetrix genechips were used for this study

RESULTS Section:
1. "Gene expression validation"- GAPDH and ACTIN genes used as control? Please clarify, as only GAPDH mentioned in the Methods, and only ACTIN results reported in experimental results.
2. "Gene expression in CP vs NP" section
CD49+ first mentioned in the Results section. Please describe in the Methods.
3. "Down-regulation of organ-specific..."
As a suggestion... divide this into sections, with the first being "Identification of potential organ-associated candidates".... or something similar.
Maybe the authors should be more direct in stating the objective of these (ie,
bladder vs prostate) experiments. As an example, the experiments were performed to identify markers/genes that are likely associated with prostate compared to bladder..." This statement was made, but because this is a very important point in their approach, I feel that it needs to be emphasized for the reader.

The authors state that CNTN1, SPOCK3 and MAOB function in development—can you include "as determined by KEGG"? If not mistaken, this is a very important observation in your study and should likely be emphasized.

I am not sure if the current experiments identify organ-specific genes, as much as genes likely differently expressed between the bladder and prostate. I don't feel that the authors can identify the 3 genes associated with development as organ-specific genes, even though they demonstrate differential levels under the study conditions. If more samples were included in the study and validated by IHC and/or functional data (or the like), then, I would agree such a statement could be made. Title and subsequent statements in the text are based on analysis of a limited number of samples, and KEGG.

Line 274- CD49/PELO- please explain?

4. CXC section- are the differential expression values determined based on comparison of 'raw signal intensities' from microarray (or other) source; or CP/NP as determined by the HTself program? What was the RT-PCR control gene used for these comparisons?

FIGURE Section:
Figure 1A- CD49+ included here? Also, at some point, shouldn't the author validate CD13+? The authors mention that all "sorts" were validated by FACS. Is this true for all, not just CD90+?

Figure 1B- the signal intensities correspond to what/which exactly? It's unclear. There are 3 samples and 2 intensities. Raw, unadjusted values?

Figure 4- Please report ACTIN control levels for all of the samples examined?

Table 1 shows decrease in ACTIN in CP compared to NP, but no difference when used as control in Figure 4 RT-PCR results. Please explain.

Written Legend for Figure 4:
Figure 4- re-label (in text C not mentioned). Current format is confusing. ACTIN not GAPDH as control? Please report ACTIN control for all samples examined in the figure, not just 1 sample pair? Signal intensities correspond to which? Similar question for other figures where intensities are designated.

MINOR REVISIONS:
1. The present address corresponds to which of the authors?
2. The authors note that 27 prostate samples were used for this study. Not
entirely accounted for?

3. The clinical sample designations (ie, 08-021, etc...) are a bit hard to follow. Suggestion =TS1, TS2, etc.. (Suggestion only; not critical).

4. Methods - Gene Expression section- the authors write "Samples showing no evidence of RNA degradation were used". Would suggest that RNA integrity numbers be given, as their statement is very vague.

5. Suggest that a FACs- Section to be added after the MACs instead of as part of the "Tissue Section"?

6. For the section beginning, "Comparison to the whole transcriptome datasets"- since the PENK gene is not represented til figure 3, it's a bit confusing in its current written format.

7. Figure 1 Legend is mislabeled. Data includes Western and Affymetrix results, not only Western.

8. Legend for figure 4 is a bit confusing in its current written format. In the text / manuscript, Figure 4C is not mentioned.

9. Legend for Figure 5 and Table 1 not found.

10. Reference 29 is incomplete

**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