Author's response to reviews

**Title:** Snail Transcription Factor Negatively Regulates Maspin Tumor Suppressor in Human Prostate Cancer Cells

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**Author's response to reviews:** see over
**Title:** Snail Transcription Factor Negatively Regulates Maspin Tumor Suppressor in Human Prostate Cancer Cells  

**Version:** 1  
**Date:** 20 May 2012  
**Reviewer:** Ming Zhang  

**Reviewer’s report:**

The manuscript by Neal et al. describes a potentially important finding that snail transcription factor regulates maspin expression in prostate cancer cells. It is a very interesting study. Most of the data presented are convincing and consistent with the negative regulation of maspin expression by snail transcription factor. The manuscript is well written and is acceptable after the following revisions.

1. Data presented in Figure 4 are confusing. First, the difference of luciferase activity between neo and snail expressing cells is very small. It is not clear whether it is significant by two-tail statistical analysis. Second, it is not clear whether the difference of luciferase activity significant between control and snail silenced cells, since no * was marked for these cells. Third, the location of snail binding E box is labeled at a different place. Is this a different E-box site than that was shown in Figure 5?

First, we did find that although the difference was small it was statistically significant between 22Rv1 Neo and Snail-high, and secondly as well between C4-2 NS and C4-2 E8 (*p<0.5). We have reflected this in Figure 4. Thirdly, we realized there was an error in labeling and have corrected it so that the correct E-boxes are depicted in both Figure 4 and Figure 5.

2. The ChIP data is the strength of this paper. However, one control is needed to clearly confirm that snail binding to the maspin promoter site. Snail IP needs to use another region (non-specific control) for ChIP-PCR. Reviewer noticed that both IgG and snail antibody have enriched signals in the IP products from 22Rv1 snail 30 extracts. Thus, it is necessary to confirm that snail antibody is not amplifying signals non-specifically. Alternatively, the authors should either do EMSA or luciferase assay with mutated snail-binding site to confirm that the site is specific for snail binding.

The reviewer comment was well taken and we repeated the PCR following ChIP using primers to the maspin intron that should not be bound by any of the proteins we were testing. Indeed we found that only the input showed up, while there was no signal with IgG, RNA pol II or Snail antibodies. This shows that Snail antibody is not amplifying signals non-specifically. We have reflected this data within Methods section page 10 lines 7-9; Results section page 13 lines 22-23; Figure 5B; and Figure 5 legend page 24 lines 9-10.
Reviewer's report
Title: Snail Transcription Factor Negatively Regulates Maspin Tumor Suppressor in Human Prostate Cancer Cells
Version: 1 Date: 3 June 2012
Reviewer: Manu Platt

Reviewer's report:
This manuscript investigates the link between the transcription factor Snail and Maspin, a serine protease inhibitor as potential mechanism for prostate cancer tumor progression either through migration, invasion, or EMT. Overall these researchers have performed complete set of experiments to support their hypothesis that Snail can negatively regulate maspin expression by directly repressing maspin promoter activity, leading to increased cell migration and invasion. However there are some suggestions that could improve this study.

Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)
1. Some of the nomenclature for the stably transfected cells Neo 10, Snail 10, Snail 30 are confusing in that, without close inspection, it appears to be dosages, but they are just numbers of transfectants. Perhaps renaming them as Snaillow or Snailhi to be clear.

We have renamed the clones to reflect Snail expression levels so instead of using numbers we have changed it to Snail-low (for low Snail-expressing clones), Snail-medium (for medium Snail-expressing clones) and Snail-high (for high Snail-expressing clones). These changes are reflected in Figures 2, 4 and 5, and Supplemental Figure 1.

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)
1. Overall, clarity regarding the rationale for the use of the different types of prostate cancer cell lines would help the readers understand why some experiments were done with 22Rv1 cells vs. C4-2 vs. DU-145 cells, etc.

This point was well taken and we have included more justification for the cell types utilized. We have stated that we used 22Rv1 cells to represent a cell line that does not express detectable Snail to overexpress Snail and examine maspin expression (page 11 lines 16-18). We have stated that we used C4-2 and DU145 to represent higher Snail-expressing cell lines in order to knock Snail down and examine what happens to maspin expression (page 12 lines 9-11).

2. There are also some abbreviations that are not explained that would add clarity to the manuscript.

We have made sure to elaborate on any abbreviations utilized in the article, especially the ones within the introduction that we missed before.
Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)

1. In figure 1, the RT-PCR is not as clear, but the Western blot data is. A clearer image of the RT-PCR gel should be included that supports the authors' points that Snail is up in the prostate cancer cells since from the included figure, only C4-2 and 22rv1 seem to have a signal.

We have re-grown cells and repeated the PCR and western blot analyses to make sure we have representative data that reflects what is seen in subsequent data. After 3 repeats, this revealed to us that 22Rv1 cells did not have detectable levels of Snail as seen in Figure 1 and 2, and stated in Results section page 11 line 8.

2. For figure 2, a representative picture of the cells post migration or post invasion would add credibility to the figure as it is not especially clear how these numbers were quantified.

We have included a representative picture of the cells post migration and invasion for clarity in Figure 2. We did state in the Methods that cells stained with crystal violet were solubilized with Sorenson solution which then allows for the color to be quantified with an Elisa plate reader by measuring optical density (OD 590 nm).

3. Figure 5, the representative gel shown in figure 5B should match the quantification shown below in 5C or some explanation should be provided as to why, because as it appears now, leads to questions of the validity of the results in either 5B or 5C.

We have gone back to make sure we chose the representative gel for Figure 5B that more clearly matched the quantification shown in Figure 5C.

4. Statistical significance of each of these assays should be performed as well and indicated with asterisks or not.

We have carefully gone through the figures to ensure that we include all the statistics and asterisks that were erroneously omitted previously. This has been included in Figures 2, 4, Supplemental Figure 1. We have indicated within the Results section that in Figure 3 the migration was decreased with P value of 0.071 for DU145 knockdown cells on page 12 line 14 and P value of 0.072 for C4-2 cells with Snail knockdown on page 12 line 17. This has also reflected within the figure legends.