Reviewer's report

Title: Expression profiles of ANXA1 in human gastrointestinal cancers and downregulation of ANXA1 in gastric cancer

Version: 1 Date: 31 December 2013

Reviewer: shengzhi wang

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Gao et al. performed comprehensive examination of the expression of ANXA1 in different gastrointestinal cancers. They found that while ANXA1 is widely expressed in the normal gastrointestinal tissues, it is down-regulated in some cancer tissues but up-regulated in others. Importantly, Gao et al. found that forced expression of ANXA1 in gastric cancer cells leads to inhibition of cell proliferation, migration and clone formation. Interestingly, ANXA1 expression is found negatively correlated with COX-2 expression in experimentally manipulated gastric cancer cells as well as gastric cancer tissues, suggesting COX-2 pathway might mediate the tumor-suppressor function of ANXA1.

This study provides a systematic interrogation and clarification of ANXA1 expression in various gastrointestinal cancers and established the potential tumor-suppressor role of ANXA1 at least in gastric cancers. This work will be an important contribution to the field of ANXA1 cancer biology.

The manuscript is well written and the conclusion is mainly supported by the data, however, I have concerns over some parts of the manuscripts as listed below.

Major Compulsory Revisions:

1. Figure 5C and 5D: I assume pcDNA3.1 vector transfection should not alter cell proliferation compared to un-transfected cells, however, if the two figures (5C and 5D) are superimposed together, one will find pcDNA3.1 transfected N87 cells are actually having higher proliferation rate compared to pcDNA3.1 transfected AGS cells. This contradicts the observations made in Figure 4D, where N87 cells having lower proliferation rate compared with AGS cells. Please explain.

2. Second paragraph, page 11: The authors claimed that “these data suggested tumor suppressor function of ANXA1 to inhibit proliferation partly through regulating the production of COX-2.”. This is not convincing to me if the evidence only includes the negative correlation of ANXA1 and COX-2 in vitro and in vivo. Further experiments are needed for this claim, for example, by showing that overexpression both ANXA1 and COX-2 simultaneously abolish the effect of inhibition of cell proliferation by ANXA1 alone.

Minor Essential Revisions:


1. Methods, Statistical analysis section: It’s not very clear what the “in vitro”, “in vivo” or “within group correlations” data are. I suggest explicitly specify for those experiments listed in the figures which statistical tests are used. As a standard way to report statistical tests result, please specify what kind of test is used in the figure legend rather than simply report the p value.

2. Figure 1B, figure 3A, figure 4A: The authors said that the y axis is “fold of changes….. divided by log”, what does it mean by “divided by log”? Does it mean the fold of change in log scale?

3. Figure 1c and figure 4B: What are the two bands observed in the blots? Are the 42KD bands the beta-actin? And the 37 KD bands ANXA1? These needs to be stated in the figure legend and result description.

4. Figure 4C: What are subcellular locations of ANXA1 based immunofluorescence staining in the two cell types? Are there any difference between them? Please also show an image for GES-1 cell staining so we can compare the immunofluorescence difference with mRNA and protein expression result as shown in figure 4A and 4B.

5. Figure 4D: According to the description in the method section, there are three repetitions for this proliferation measurement, please show the standard deviations of different repetitions as error bars for each time points. Looks to me this could be modeled as a repeated measure experiment and please perform the statistical test (for example, repeated measure ANOVA) to check whether there is overall difference of the proliferation rate between two cell types. In addition, post hoc analysis needs to be done.

6. Figure 4E: Are the invasion cell numbers between the two cell types significantly different? Please perform a statistical test and describe the test result.

7. Figure 5A and paragraph 3 in page 10: “Enforced expression of ANXA1 in AGS cells induced markedly changes of morphology of AGS cells and ANXA1 translocated from the cytosol to the plasma membrane”: It is not very clear to me based on the figure what the morphological changes are? Is the bright green patch in ANXA1 transfection image a single cell or a cluster of cells? The membrane translocation is also difficult to distinguish. Please show an enlarged and distinguishable image. Does the same membrane translocation also occurred when the transfection is done in N87 cells? What is the biological implications or significance for this membrane translocation?

8. Figure 5B: In theory, we should expect a much stronger fluorescence signal when we do overexpression of ANXA1. It is hard to see that comparing ANXA1 transfected cells with pcDNA3.1 transfected cells even though the western blot did show mild increase.

9. Figure 5C and 5D: what is the statistical test is used to get the p value here as marked by the asterisk? Again this is a repeated measure experiment. Please report the details of statistical analysis result in the figure legends.
10. Figure 6A: the authors mentioned “silencing of ANXA1 by ANXA1-shRNA promoted cell viability in N87 cells”. Have authors examined whether silencing ANXA1 using shRNA affects clone formation and cell migration compared to scramble shRNA in N87 cells?

11. Figure 6D: Are the first two columns are for ANXA1 and the last two columns for COX-2? Please also state it clearly in the figure legend.

Discretionary Revisions:

1. Figure 2, third panel, tumor image: Please label which part is “well-differentiated squamous cell carcinomas” and which part is “weak or not in poorly differentiated carcinomas” as described in first paragraph, page 9. The same for cholangiocarcinoma and gastric carcinoma.

2. Figure 6B: have the authors examined whether Cyclin D1 expression is boosted in ANXA1 knocked-down N87 cells? The authors did not make any points by showing Cyclin D1 data here. If not relevant, there is no need to show.

Level of interest: An article of outstanding merit and interest 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