Reviewer's report

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

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Reviewer's report:

The manuscript by Gao and colleagues explores expression profile of ANXA1 in human gastrointestinal tumors and possible correlation between ANXA1 and Cox2. It is interesting. However, a number of issues need to be addressed.

Major points

1. The title is “Expression profiles of ANXA1 in human gastrointestinal cancers and downregulation of ANXA1 in gastric cancer”. As the authors not only discuss the downregulation of ANXA1 in gastric cancer but also in other gastrointestinal cancers. I would suggest them to change the title.

2. Fig.1 c: There are 2 bands, which band is ANXA1 band? They also need to add the actin blots as loading controls. And from this figure, I cannot draw a same conclusion as them, “ANXA1 protein expression was down-regulated in CHO, ESC and GC.” I think the expression levels are totally different between sample1 and sample2.

3. Fig. 2, Fig. 3 B-H: The quality of the images is not good. The authors should show the high quality high magnification images.

4. Fig.2 3rd panel. The authors conclude that “Immunohistochemistry for ANXA1 revealed high expression level of this protein in well-differentiated squamous cell carcinomas, but weak or not in poorly differentiated carcinomas (Fig.2 third panel).” Where is the evidence?

5. Fig.3H, the authors propose “immunohistochemistry for ANXA1 in liver metastases displayed a significant reduction of this protein in tumor cells compared with surrounding benign liver cells……. ANXA1 is a differentiation marker” However, this does not make sense as liver metastases and surrounding benign liver tissue are totally different. The tumor cells are from gastrointestinal tissues, and Fig. 1 already shows that ANXA1 has higher expression level in liver cells than that of gastrointestinal tissues.

6. Fig.4 B: There are 2 bands, which band is ANXA1 band? Also Actin blots are needed.

7. Fig. 4 D, E: N87 and AGS are different cell lines, so this does not make sense to compare their proliferation and invasion.

8. Fig. 5A, B: The authors suggest “ANXA1 translocated from the cytosol to the plasma membrane” However, I think ANXA1 is still in the cytosol according to their images.
9. Why do the ANXA1 western blots here have only one band?

10. Fig. 5 C-H: The authors need to check the transfection efficiency first, and the efficiency must be very high. Otherwise, they can’t use these cells to do such experiments.

11. Fig. 6: The authors conclude that “tumor suppressor function of ANXA1 to inhibit proliferation partly through regulating the production of COX-2.” I do have some problems with this conclusion. If the authors really want to show that COX2 is the downstream effector. They need to get COX2 knocking down and overexpression data, other than only correlation between ANXA1 and COX2.

Minor points

1. Page 13: The authors mention “selected two anti-ANXA1 antibodies after antibody specificity confirmation by Western blotting.” Where is the 2nd antibody’s data?

2. Page 14: “ANXA1 expression is “tumor-specific”.” What does this sentence mean? It is very confusing.

**Level of interest:** An article of importance in its field

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

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests