Reviewer's report

Title: Extracellular Matrix Signatures of Human Primary Metastatic Colon Cancers and their Metastases to Liver.

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Reviewer: Xiaoguang Fang

Reviewer's report:

The manuscript entitled “Extracellular Matrix Signatures of Human Primary Metastatic Colon Cancers and their Metastases to Liver” is novel and interesting. Authors identified a number of protein highly expressed in the live metastasis colon cancer by using mass spectrum analysis. The identification of these proteins would lay a foundation for the colon cancer treatment. For publication, the following concerns should be addressed.

Major concerns:

As the author mentioned, there are variation among different clinical dataset. There are also variation and only a small overlapping between the proteins author identified and the clinical database. Therefore, it is still questionable whether the proteins (HPX, SPP1 and COMP, etc) authors identified actually play roles in the liver metastasis in vivo. I recommend authors to make some supplemental experiment confirm their conclusion. Authors could knockdown the candidate gene by shRNA or ectopic expression gene in the colon cancer cell line, then inject these cell lines into nude mice. In the final, account the liver metastasis node number and make Statistics comparison. Then based on this result, authors could make the related conclusion.

Minor concerns:

1. Please describe clearly what are the steps 1, 2, 3, 4 in figure1A? In figure legend or method part, there is no description about the procedure.

2. Align the number 1, 2, 3, 4 with each blot band. Furthermore, in the last blot of Figure 1A, the Actin is not aligned with GAPDH. Please provide new figure with clear indication.

3. It seems that author selects actin and GAPDH as the marker for the extraction from cytoplasm, Pan-histones as the marker for the extraction from nucleus. Please explain why there is no any marker in step 4 and ECM-rich Fraction of the normal colon extract. And the level of GAPDH is inconsistent with the level of actin in metastasis extract. There is no actin in the ECM-rich fraction of the normal liver extract, and GAPDH is missing in step 4 and ECM-rich Fraction. The level of Pan-Histones is inconsistent in each step, which may be caused by inappropriate protein concentration. Therefore, please make the experiment again and provide convincing blot data.
4. In the first paragraph of the result part, the author describe like “….Figure 1A shows the efficiency of the sequential extraction protocol leading to significant enrichment of collagen I …”. Since we know that the molecular weight of Collagen I is 138KD, why the band of Collagen I is shifting so much. Please make sure that you used the correct antibody or your samples are not degraded before running the gel. Other issue is that the word “significant” should be cautious in this sentence, because there is no statistical comparison in the related figure.

5. In the first paragraph, authors describe like this “….Concomitant depletion of intracellular proteins (actin, GAPDH, histones) in the final ECM-enriched samples…. Why there are actin and GAPDH in the sample of colon tumor ECM-rich Fraction.

In the second paragraph of the result part, authors described as follow “reproducibility for normal colon and colon tumor samples from patient 2 (Additional File 2). " But in the related figure, “Additional File 3” was labeled. Please correct this error.

6. In the result part, authors described their data as follow “….identified subsets of tumor-specific proteins: 37 proteins were characteristic of the colon tumor matrisome, 7 proteins were characteristic of the metastasis matrisome and 23 proteins were characteristic of both primary tumors and metastases….”. From their mass spectrum comparison, they identified 37 proteins are related with colon tumor formation, 7 proteins are related with metastasis, and 23 protein are related with both of them. After analysis in the clinical dataset, only three proteins (HPX, SPP1 and COMP) are closely associated with metastasis, only four genes (MMP1, MMP2, MMP11, and LEFTY1) are associated with colon cancer formation. Therefore, only a little of protein ID author identified are associated with clinical survival. Most of proteins authors identified have nothing to do with the clinical survival. How do author explain this based on their MS data.

7. From the Mass spectrum result author identified, a lot of known proteins related with liver metastasis in other studies are not appeared in the authors’ results. For example, L1CAM (1), Cadherin-17 (2), periostin (3), TMPRSS4 (4)

References:

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**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.