Peer Review File

Article information: https://dx.doi.org/10.21037/jgo-23-247

Reviewer A

Shu et al. used ProteomeXchange and TCGA datasets to analyze the relationship between the expression of EPRS gene and prognosis, and validated it in vitro experiments. Their research suggests that EPRS may be a potential prognostic marker for liver cancer, and high expression of EPRS inhibits the occurrence and development of liver cancer. This is a verification of dry wet combination, with complete logic, but there are still some issues that need to be addressed:
1. In the methodology section, line 135, DAVID tools and STRING databases, as well as Cytoscape and GEPIA tools, authors should add reference citations.
Reply: Thank you for your comment. We added references for DAVID and STRING dataset (see page 5, line 135 and 138).

2. The author should add a reference to "Sangerbox" in line 169 of methodology （https://doi.org/10.1002/imt2.36”. In addition, it is also necessary to indicate the download time, as the database is constantly updated.
Reply: Thank you for your comment. We added reference for sangerbox as well as time in line 169 page 6.

3. What is the relationship between EPRS and clinical features?
Reply: Thank you for your comment. In this study, we tend to understand the relationship between EPRS and disease prognosis at the molecular level. At present, there is no correlation evidence that EPRS is associated with any clinical characteristics

4. What are the potential regulatory pathways for EPRS?
Reply: Thank you for your comment. According to research, EPRS could promote liver fibrosis, which maybe promote the occurrence of liver cancer. In other cancer, EPRS could mediated-WNT/GSK-3β/β-catenin cascades.

Reviewer B

Hepatocellular carcinoma (HCC) has a high incidence, and current treatments are ineffective. In the manuscript “Identification of glutamyl-prolyl-tRNA synthetase as a new therapeutic target in hepatocellular carcinoma via a novel bioinformatic approach”, authors explored potential diagnostic and prognostic biomarkers for HCC by conducting bioinformatics analysis on genomic and proteomic data.
Couple questions are required to be answered before it will be accepted.
(1) The methods of abstract were too simple. Please supplement.

Reply: Thank you for your comment. In method of Abstract, we added more information (see page 3 line 61-65).

(2) The glutamyl-prolyl-tRNA synthetase was the crucial topic in the paper. Please make a brief introduction about the functions of EPRS in the introduction.
Reply: Thank you for your comment. In introduction, we described glutamyl-prolyl-tRNA synthetase as showed in page4, line116-122.

(3) It was better to add related reference (DOI: 10.21037/jgo-22-303) about the potential prognostic biomarkers for hepatocellular carcinoma.
Reply: Thank you for your comment. We added the reference in introduction in line4 page106.

(4) In the figure 7, why the EPRS-1 and EPRS-2 were both showed?
Reply: Thank you for your comment. glutamyl-prolyl-tRNA synthetase had two forms, so we tested for both.

(5) The downregulation or upregulation EPRS using lentiviral vectors in HepG2 cells showed that EPRS promoted cell proliferation and migration, without effecting apoptosis. But, the result figures were missing.
Reply: Thank you for your comment. We checked and confirmed didn't conduct the experiment, and we deleted this part. Thank you.

(6) It was better to test the roles of EPRS in HCC.
Reply: Thank you for your comment. We used basic experiment showed that EPRS was higher expression in HCC.

(7) It was showed that the EPRS could be prognostic biomarkers for HCC. It was necessary to test the correlation between EPRS and prognosis of HCC.
Reply: Thank you for your comment. In figure 5B, we test the correlation between EPRS and prognosis of HCC, high EPRS expression indicated worse prognosis of HCC.

(8) Why to test ACLY and HSPA4 in the study? Please state in the discussion.
Reply: Thank you for your comment. In figure 5A, only EPRS, ACLY and HSPA4 were higher expression in HCC. And we described it in discussion part.

Reviewer C

1. Please define ALL abbreviations shown in figure 1 in its figure legends.
Reply: Thank you for your comment. Confirmed modification.

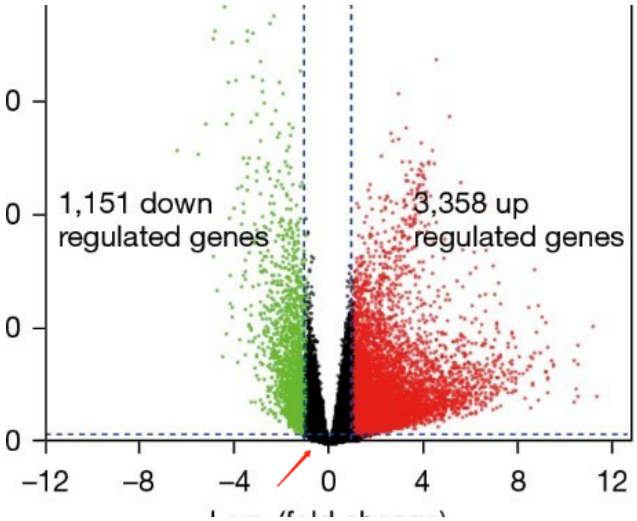
651

652 Figure 1 Flow chart. HCC(hepatocellular carcinoma),TCGA(The Cancer Genome

653 Atlas), KEGG(Kyoto Encyclopedia of Genes and Genomes).

654

2. Figure 2: Please also define those black dots either inside the figure or in figure legends.



Reply: Thank you for your comment. We have added an explanation in the legend.

656 Figure 2 Data processing and differential expression analysis. Volcano plots of proteins

657 (A) and genes (B) in HCC tissues and adjacent normal tissues, The black dots in the

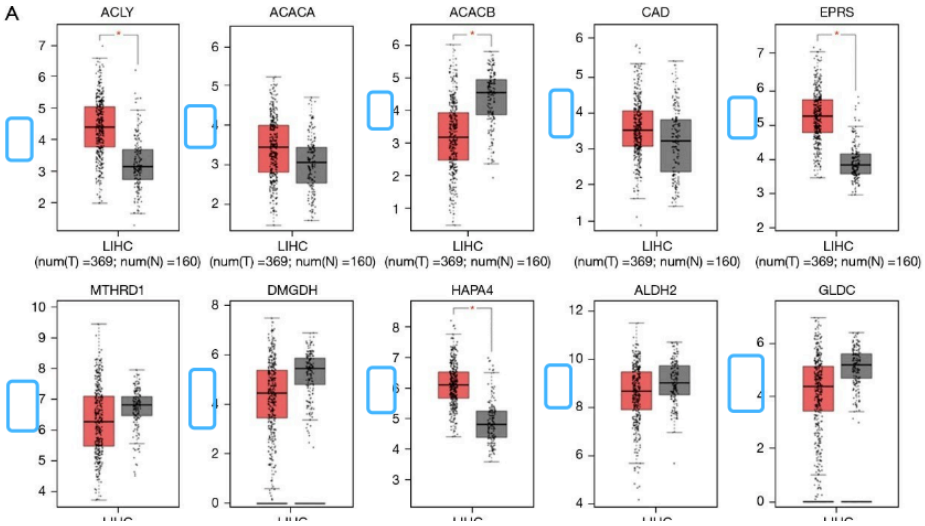
658 figure represent genes or proteins that do not show significant differences. Common

659 upregulated (C) and downregulated (D) proteins or genes. The red circles represent

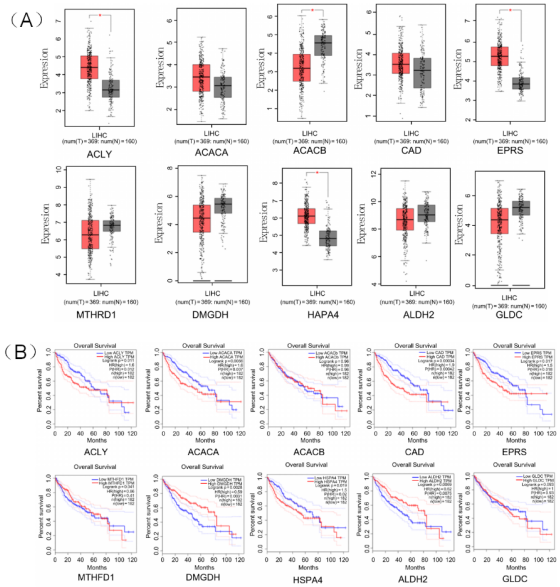
660 genes, and the blue circles represent proteins. HCC, hepatocellular carcinoma.

3. Figure 5

a. Please provide descriptions of Y-axis for below figures.



Reply: Thank you for your comment. Confirmed modification.



b. Please define "*" in figure legends.

Reply: Thank you for your comment. Confirmed modification.

680 prognosis. The ten genes with the highest connectivity in the PPI network were
681 considered as candidate genes. (A) mRNA-expression levels of the indicated 10 genes
682 in HCC and adjacent normal tissues, based on GEPIA, Where '*' represents p<0.05;
683 (B) Kaplan–Meier Plotter analysis of the same 10 genes. P<0.05 was considered to

4. Images in Figure 6 are from HPA, please provide the websites that directly link to those images in figure 6 legends.

Reply: Thank you for your comment. Confirmed modification.

689 Figure 6 EPRS, ACLY, and HSPA4 proteins were higher in HCC tissues (patient ID:
690 2766) than in normal liver tissues using Hematoxylin-Eosin (HE) staining, based on
691 information deposited in the HPA database. (A) Liver tissue with normal EPRS
692 expression was obtained from a 55-year-old male (patient ID: 2429 ,
693 https://www.proteinatlas.org/ENSG00000136628-EPRS1/tissue/liver#img); (B) liver
694 tissue with normal ACLY levels was obtained from a 30-year-old female (patient ID:
695 3222, https://www.proteinatlas.org/ENSG00000131473-ACLY/tissue/liver#img); (C)
696 liver tissue with normal HSPA4 expression was collected from a 54-year-old female
697 (patient ID: 2429 , https://www.proteinatlas.org/ENSG00000170606-
698 HSPA4/tissue/liver#). HCC, hepatocellular carcinoma; EPRS, glutamyl-prolyl-tRNA
699 synthetase; ACLY, ATP citrate lyase; HSPA4, heat shock protein family A member 4.
700 Magnification X200.↵

5. There's no "*" in your provided Figure 7, please check the legends.

(P=0.0484). C = tumor tissue, N = adjacent normal tissue. *P<0.05. EPRS, glutamyl-prolyl-tRNA synthetase; mRNA, messenger RNA; HCC, hepatocellular carcinoma.

Reply: Thank you for your comment. Confirmed modification.

Figure 7 EPRS expression between fresh HCC tissues and adjacent non-tumor tissues. (A) EPRS mRNA was significantly upregulated (P = 0.0401) in HCC tissues (cancer) compared with adjacent non-tumor tissues (normal). (B) EPRS protein-expression levels between HCC and adjacent normal tissues. The results indicated that EPRS was overexpressed in HCC tissues compared with that in adjacent normal tissues (P=0.0484). C = tumor tissue, N = adjacent normal tissue. ~~*P<0.05~~. EPRS, glutamyl-prolyl-tRNA synthetase; mRNA, messenger RNA; HCC, hepatocellular carcinoma.