Reviewer's report

Title: Epidermal growth factor induces HCCR expression via PI3K/Akt/mTOR signaling in PANC-1 pancreatic cancer cells

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Reviewer: Steven Hochwald

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Major Compulsory Revisions

Figure 2A: HCCR-1 expression is the same in each lane. Where is the empty vector? Where is the data showing overexpression of HCCR-1.

Figure 2B: The authors indicate that HCCR-1 enhances the growth of Panc-1 cells. The figure is missing error bars. No statistics is performed here demonstrating a significant increase in growth.

Figure 2D: No error bars or stats

Figure 2E: No stats

Figure 2F: Discrepancy between the scoring system used and the results reported. In the methods the authors indicate that they used the Allred 8-unit system for proportion score and intensity score. However, the data is reported as HCCR-1 positive and negative. There is no such score in the system described in their methods.

Figure 2G: The authors indicate that strong expression of HCCR-1 is detected in pancreatic carcinomas compared to normal tissue. Only one lane is normal pancreas. More results for normal tissue must be shown to support this statement.

Figure 2 legend has spelling mistakes where Panc-1 is listed as PNAC1 cells.

Results from Figure 3 suggest that PI3K/mTOR signaling affecting Akt only indirectly regulates HCCR-1 levels. According to figure 3B, It takes about 16 hours after EGF treatment to see an increase in HCCR-1 protein levels. However, EGF stimulates phosphorylation of Akt after 2 minutes. What is the time after EGF stimulation in figures 3C and 3D in which HCCR-1 proteins expression is determined after EGF stimulation? How long are cells treated with LY294002 or rapamycin in figures 3C and 3D?

In figure 4A, AKT overexpression may influence EGFR/p-EGFR expression as well as other important tyrosine kinases (e.g. IGF-1R/p-IGF-1R). The authors should show the expression of these kinases to indicate whether changes in HCCR-1 are due to alterations in signaling from other kinases.

Figure 4B should have statistical analysis of the relative luciferase activity in the different groups.
**Level of interest:** An article of limited interest

**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