Reviewer’s report

Title: A panel of protein kinase high expression is associated with postoperative recurrence in cholangiocarcinoma

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Reviewer: Chirag Nepal

Reviewer's report:

Padthaisong S et al have analyzed 12 protein kinases through immunohistochemistry in 190 iCCA patients and identified EGFR, HER4 and EphA3 were associated with tumor recurrence status, recurrence-free survival and overall survival. The cohort size is large enough to draw statistical conclusion, and results looks convincing. The authors should strengthen these findings in another cohort, perhaps from TCGA and ICGC RNA-seq data, and show these observations are reproducible. The authors should improve the presentation of results by converting tables into suggested figures, so the biological message is clear.

Major Comments

The authors briefly mention some of their previous work on why they choose only these 12 proteins kinases. The authors could improve the reasoning on why these 12 proteins were selected, and state what are the evidence (at DNA and RNA level) on these genes from recent large-scale multi-omics papers in CCA, such as (PMID: 29360550, 29278425, 28297679, 26258846, 28667006). These papers have revealed different driver genes based on mutations, RNA expression, copy number and methylation. The authors should cite these papers and state whether any of these 12 genes (based on exome and RNA-seq data) were reported as putative driver, what is the expected frequency of mutations. The authors should try to put these genes into larger context.

The authors made Table 1 to summarize the "Patients characteristics" section, which is not appealing and hides the crucial information about individual patient. The table should be converted into heatmap and show information about every variable (tumor-location, sex, age, histology, primary tumor location etc) for all 190 patients. The authors could take inspiration from Figure 1A (top section) from PMID: 28667006 or any of the above-mentioned papers. The authors could use two color code to represent tumor location (intrahepatic or extrahepatic) and align other features using different color codes with labels. It will show information about individual patient and if any two features are co-occurring it likely will be obvious in the Figure 1 and makes the message clear.

Also add information about post-operative recurrence, locoregional recurrence and distant recurrence from table 2 into the new Figure 1. The message will be easy to follow for readers.
The authors divide protein expression into high and low expression. Please mention what criteria (just one line) was used in result section to divide protein expression into high and low expression. The authors state different percentages for high expression of protein, which is uninformative to read, so please make a heatmap with 12 row (as protein) and 190 columns as expression level. The authors could use actual expression levels or binary (1: high expressed and 0:low expressed) and make the heatmap. It will give an unbiased information on which proteins are being highly co-expressed. This should be Figure 1A and the current Figure1 should be Figure 1B.

The authors mention that EGFR, HER4 and EphA3 was significantly associated with early recurrence. Are these the same patients? How many patients overlap. Is the recurrence even more significant if the authors select only a subset of patients where all three proteins are highly expressed?

The authors analyzed correlation of proteins and presented in the table. Plot the correlation values using correlograms. Include all 12 proteins in correlation analysis.

The authors showed high expression of EGFR, HER4 or EphA3 have a shorter RFS, then how the RFS would look if you select only patients that have high expression of all 3 proteins, i.e, high expression of combination of all 3 proteins. Importantly, the authors should reanalyze TCGA data and reproduce the finding. If TCGA patients with high expression of EGFR, HER4 and EphA3 are selected, will it have a shorter RFS.

Minor comments

Improve legend of current Figure 1.

Figure 3 has multiple panel but lacks sub panel information, both in Figure and in text.

In line 224, the sentence begins with "The previous results..." which could be changed to "The above results...."

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.
Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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