Reviewer’s report

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

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Reviewer: Massimiliano Cadamuro

Reviewer's report:

In this manuscript, Padthaisong and colleagues evaluated the potential usefulness of a panel of protein kinases and other parameters as marker of outcome in CCA recurrence.

The main results obtained by the Authors are that the expression of three markers, EphA3, EGFR, and HER4 are independent prognostic factor to predict CCA outcome (RFS and OS) following surgical intervention.

The experimental plan is adequate to the purpose of the manuscript as well as for the statistical analysis and data obtained on postoperative specimens are novel and potentially of interest. Despite this, some data could be added and some results could be more extensively explained to strengthen this yet interesting paper.

Major concerns:

1. Authors could show, at least in a supplemental figure, the immunohistochemical expression and evaluation of all the antibodies they used and not only of the ones in Fig. 1 and 2.

2. In CCA β-catenin could be expressed in the correct membranous position, or absent or in the nucleus to act as a transcription factor (Abraham SC, Hum Pathol. 2003; Settakorn, J Clin Pathol. 2005; Gu MJ, Hepatogastroenterology. 2012). To better evaluate the significance of this protein, Authors could evaluate its expression in the different compartment and correlate this with outcome.

3. Data regarding the lacking of significance of VEGFR3 and VEGF-C are quite surprising for the Reviewer; several manuscripts highlighted the importance of those growth factor in CCA invasiveness and metastatization (Park BK, Am J Clin Oncol. 2006; Aishima S, Mod Pathol. 2008; You Z, Genet Mol Res. 2015; Zhao R, Oncol Lett. 2015; Cadamuro M, J Hepatol 2019): could the Authors briefly comment these differences? Moreover, the lack of effectiveness on VEGFR3/VEGF-C axis could be explained by a reduced expansion of the lymphatic plexus and an increased recruitment of blood vessels in fluke-induced CCA? to answer the question, Authors could evaluate blood microvessel density (MVD) and lymphatic microvessel density (LMVD) using specific markers (CD31/CD34

Minor concerns:

1. Please use VEGFR3, instead of VEGFR.

2. In the Abstract is written (VEGF-C), please correct as VEGF-C or vascular endothelial growth factor-C (VEGF-C).

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.

No

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 recommend additional statistical review

Quality of written English  
Please indicate the quality of language in the manuscript:

Not suitable for publication unless extensively edited

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