Peer Review File

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Reviewer A

Comment 1: The authors repeatedly mention that they associated "mRNA for HIF1" but in the Table showing the included studies, it is mentioned that IHC (immunohistochemistry) was employed? So which is it? This is very important as HIF1a is *not* regulated by oxygen tension at the transcriptional level and only the presence of nuclear HIF1a protein is indicative of its activity (also explained in line 42).

Reply 1: The detection of hypoxia-inducible factor 1-alpha was done by immunohistochemistry in the included studies. The immunohistochemistry detects protein expression, not mRNA. We made a mistake in the writing. Thank you for your comment.

Changes in the text: In the whole manuscript, we changed "expression of mRNA for HIF-1α" to "expression of HIF-1α" and highlighted in yellow; except in the "database validation" part because, the detection method in that part was by RTPCR not IHC.

Comment 2: In light of the above, I wonder how appropriate the inclusion of the GEPIA mRNA data is. Querying gene sets that associate with HIF1a activity is likely more informative.

Reply 2: We did our meta-analysis based on protein results (IHC); then we need to verify also at mRNA level the differential expression of HIF1A in PDAC and normal pancreas despite the fact that, there is somehow an intermediate correlation (medium consistency) between mRNA levels of HIF-1α and protein levels in PDAC (Protein atlas). For the purpose, GEPIA database is a famous webserver used for gene profiling and integrative analysis, and cited in high quality papers (doi: 10.1007/s00432-022-03928-z, doi: 10.18632/aging.204039, doi: 10.1038/s41598-022-09889-0). So, we used GEPIA database to confirm that there is differential expression of HIF1A between PDAC and normal pancreas. High expression of HIF1A in PAAD than in normal pancreas emphasizes the role of HIF1A in PAAD development and progress.

Change in text: see Page 9 – 10, line 213 – 223

Comment 3: High HIF1a associates with advance tumor stage. Therefore, its association with other clinical variables (N status e.g.) is not surprising. The authors should therefore test the prognostic power of HIF1a in a multivariate model that includes the relevant variables.

Reply 3: The association of high HIF-1α with advanced tumor stage and positive LNM is not surprising but still meaningful, especially in pancreatic cancer because no meta-analysis to date have been published considering association of HIF-1α expression with clinico-pathological parameters in PDAC. Therefore, having considered overall survival (OS) as the major parameter reflecting prognosis, we performed univariate meta-analysis (HR for OS and RR for clinico-pathological parameters). We included clinico-pathological parameters to increase the perspective on the role of HIF-1α as a predictive marker in diagnosis of PDAC.

Change in text: See page 10, line 205 – 208

Comment 4: Line 6; I think that the association of high HIF1a with poor outcome is rather well-accepted despite a small number of papers showing otherwise.

Reply 4: Regarding the overall survival, we conducted a meta-analysis of 648 patients (356 high and 292 low) included in 10 studies. Of these 10 studies, just 01 (Leppanen et al, 2018) reported association between weak expression of nuclear HIF-1α and poor prognosis. The reasons of this contradictory result may be retrospective nature of the study, relatively small sample size, and low number of T4 (clinical stage IV) cases. There are also contrary reports, including association of weak HIF-1alpha expression and poor prognosis in squamous cell carcinoma (Fillies et al, 2005 and Santos et al, 2012) and breast cancer (Vleugel et al, 2005). Accordingly, we understand that the role of HIF-1α might be more complex than previously thought and the use of this marker as a hypoxia-related prognostic factor should be addressed with caution.

Change in text: see page 2, line 27 – 29; see page 9, line 189-190

Comment 5: Results sections are very short. I think they could benefit from merging.

Reply 5: We merged results according to outcomes: clinicopathological parameters and overall survival.

Change in text: see page 7, line 145 – 161, and page 8, line 163 – 173.

Comment 6: It would be nice if the authors could find a way to combine Figures 3–7, perhaps by reporting the summary statistics of the meta-analysis.

Reply 6: we combined figures according to outcome, and now we have figure 3 (outcome: Clinicopathological parameters), figure 4 (outcome: Overall Survival)

Change in text: see figures 2 and 3

Reviewer B

This is a short educative paper. I would like to accept this paper but I would like you to correct/refer to some important points.

Comment 1. Title: Word positive should be removed. You can see from figure 2 that even normal tissue expressing HIF1a in many cases. HIF1a per se is a necessary signal that tissue need to compensate for low oxygen levels (which is good).

Reply 1: we removed the world "positive" in the whole manuscript. Thank for your comment.

Comment 2. You should state somewhere that there is somehow a correlation between mRNA levels of HIF1a and protein levels. On human protein atlas for instance there is an intermediate correlation (medium consistency) or RNA and protein levels in PDAC.

Reply 2: we totally agree

Change in text: see page 10, line 215 – 217

Comment 3. State please that the role HIF1a is complex and a therapeutic intervention should be considered with caution.

Reply 3: we totally agree

Change in text: see page 2, Line 27 – 29

Comment 4. Nomenclature: HIF1A is the gene. Protein: Hypoxia-inducible factor 1-alpha. Please correct.

Reply 4: we totally agree

Change in text: changed in the whole manuscript as you can see in page 11, line 234