Peer Review File

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Reviewer A

Comment 1: it is not clear from the short introductory text why precisely FHL2 was analyzed and not one of the thousand and one other proteins associated with tumor growth. Please argue.

Reply: First of all, thank you for your comments, the four-and-a-half LIM-only (FHL) subfamily of proteins belongs to the LIM-only family of proteins. and share a high degree of homology throughout the amino acid sequence.(1) These proteins are identified by their characteristic four cysteine LIM homologous structural domains. Five members are classified as subfamily FHL, namely FHL1, FHL2, FHL3, FHL4, and activator of human testicular CREM (ACT). FHL2 is one of the most examined members of the family. FHL2 expression is present in different tissues and organs and has been reported to be a key player affecting a variety of cancers such as breast, gastrointestinal (GI), liver, and prostate cancers. The expression profile of FHL2 appears to have a significant functional role in the carcinogenesis of these cancers, which are mediated by different types of transcription factors, including tumour suppressors and inducers.

FHL2 exhibits different expression patterns in a cell-specific manner, and its expression pattern shows significant differences between different types of cancer. For example, fhl2 is predicted to be a potential tumour suppressor gene as it is down-regulated in prostate cancer, rhabdomyosarcoma, and hepatocellular carcinoma; in contrast, higher expression of FHL2 is observed in breast, ovarian, lung, and colon and human melanoma, implying oncogenic properties of FHL2 in these cancers(2). However, according to the current study, there is no study on the potential prognostic impact and biological function of FHL2 in pan-cancer. Although the function of FHL2 has been reported in cancers such as non-small cell lung cancer, glioma, and ovarian cancer, and FHL2 has shown some potential as a target gene therapy, the discussion of the function of FHL2 has been limited to epigenetic and signaling pathway alterations, so we hope to combine bioinformatics approaches to provide a relatively comprehensive analysis of the function of FHL2.

Reference:

1. Fimia GM, De Cesare D, Sassone-Corsi P. A family of LIM-only transcriptional coactivators: tissue-specific expression and selective activation of CREB and CREM. Mol Cell Biol. 2000;20:8613–22.

2. Cao CY, Mok SW, Cheng VW, Tsui SK. The FHL2 regulation in the transcriptional circuitry of human cancers. Gene. 2015;572(1):1-7. doi:10.1016/j.gene.2015.07.043

Comment 2. M&M section, title " Distribution of FHL2 expression in molecular subtypes and immune subtypes"… Subtypes of what?

Reply: First of all, thank you for your comments, we used the Tumor Immune

System Interactions and Drug Bank (TISIDB) database to analyse the various molecular subtypes into which patients with different types of cancer can be classified based on FHL2 (Figure 2). On the other hand, the tumor immune microenvironment plays an important role in the development of tumors. By using the TISIDB database, we found that based on the association of FHL2 expression with immune function, we were able to classify patients into six immune subtypes across a wide range of cancers (Figure 5a). Therefore, we used the TISIDB database to classify tumours into different molecular and immunological subtypes based on the expression of FHL2 for a deeper understanding of the tumour microenvironment and prognosis.

Comment 3. the figure legends are too briefly described. I believe a broader explanation would be helpful to the general readership. For example, a list of abbreviations for numerous tumors analyzed, although scattered throughout the text, would be helpful here. Also, a list with the full names of the major genes/proteins associated with FHL2 expression in tumors, such as VIM, CDH2, SNAI1, SNAI2, MMP2, MMP3, MMP9, MMP11, ZEB1, TWIST1, and TWIST2 shown in Fig. 11a would facilitate reading

Reply: Thank you very much for your comments, based on your suggestions, we have described the figure legends in detail and included a list of terminology abbreviations to help with reading

Comment4. It would also be helpful to have, for example, brief descriptions for molecular, and immunological subtypes of tumors as well as for mRNA modification gene subtypes, etc.

Reply: Thank you very much for your comments, Molecular subtypes, immunological subtypes, and mRNA modifying gene subtypes describe the different characteristics of tumours. Molecular subtype analysis of tumours demonstrated the correlation of FHL2 expression with different molecular subtypes of tumours, such as Basal, Her2, LumA, LumB, and Normal in breast cancer, which predict different prognoses. In addition, immunosubtyping analysis demonstrated the association of FHL2 expression levels with different immunosubtypes of tumours, such as significant differences in FHL2 expression levels between C1, C2, C3, C4, and C6 immunosubtypes of breast cancer. Finally, mRNA modification gene subtypes refer to different types of mRNA post-transcriptional modifications, such as m1A, m5C, and m6A. The relationship between FHL2 expression and mRNA post-transcriptional modification types was analysed. (Figure 2 and 5)

Reviewer B

Comment 1. The title of the manuscript looks not appropriate as a representative of collected results.

Reply1: First of all, thank you for your comments. We do agree with you, so we have changed the title to "Comprehensive pan-cancer analysis of FHL2 and

suggesting an association with poor prognosis in lung adenocarcinoma" to more appropriately represent our findings.

Comment 2. Some figure legends are missing, which hampers the understanding of the messages from results.
Reply2: First of all, thank you for your comments. We do agree with you, therefore we describe some of the figure legends in more detail to facilitate reading, such as legends of figure2, 4, and 8.

Comment 3. In contrast to the goals and conclusive remarks presented in the manuscript, the presented data looks still preliminary and seems to require more additional supporting evidences, reaching to the conclusions that authors have proposed in the Abstract and Discussion section:
1) Abstract: "Conclusion: Our comprehensive bioinformatics analysis identified FHL2 as a potential prognostic marker in cancers."
This is too general statement. Specifically which can be concluded as a potential prognostic marker in cancers through this bioinformatics analysis?
2) The conclusive remarks are not likely to be strongly supported by scientific evidences:
Results: line 145 , "These results suggested that FHL2 may play an important role in tumorigenesis and tumor progression."
Line 161, "The results showed a positive correlation between FHL2 and mRNA modification genes in various cancers, but no significant association in UVM."
Line 166, "Taken together, these studies indicated that FHL2 was significantly associated with the tumor microenvironment and may be involved in post-transcriptional modification."
Line 172, "However, FHL2 expression was significantly correlated with the pathological stage and TNM stage(Fig. 7i-l).": Actually the results do not show a significant correlation with the pathological stage and TNM stage between M0 and M1.
Line 187, "These results suggested that FHL2 expression may be associated with tumor initiation and progression."
Line 199, "Taken together, we can conclude that these results suggest a strong association between FHL2 and EMT …"
Discussion line 217, "In contrast, FHL2 is low expressed in THCA and has a better prognosis, which indicated that FHL2 plays a tumor suppressor role in thyroid cancer. To sum up, FHL2 can not only promote cancer but also inhibit cancer."
Line 222, "The positive correlation between FHL2 in chemokines (or receptors) and immunomodulators also suggest that FHL2 may play a crucial role in the immune direction in many cancers. These results indicate that FHL2 can be used as a meaningful diagnostic biomarker of pan cancers and participate in immune regulation."
Line 259, "…elevated FHL2 expression was significantly related to poor prognosis and advanced pathological stages, which revealed the practicality and

feasibility of the use of FHL2 for overall LUAD prognosis."
Line 269, "Here, we found that mutated FHL2 resulted in a poor prognosis, indicating a breakthrough in the study of FHL2 function."
Line 274, "…FHL2 is positively related to most of the EMT genes in LUAD. Meanwhile, the single-cell sequencing further demonstrates that FHL2 plays an important role in metastasis, angiogenesis, inflammation, and hypoxia…"
Line 279, "Therefore, our bioinformatics analysis suggests that FHL2 may be a potential prognostic marker in a wide range of cancers."
Conclusion: line 282, "In the present study, we comprehensively analyzed the localization of FHL2 in pan-cancer, discovering that its expression was correlated with clinical prognosis, tumor staging, CAFs infiltration, mRNA modification, andother dimensions, which was helpful to understanding the role of FHL2 in tumorigenesis and progression from numerous perspectives. In addition, we further studied the role of FHL2 in lung adenocarcinoma, exploring the expression, prognosis, and mutations of FHL2. The functional study of FHL2 indicated that FHL2 plays an important role in EMT, especially through TGF- β signal pathway. To sum up, FHL2 served as potential prognostic biomarkers as well as therapeutic targets in diverse cancers."
Reply: Indeed, the case you pointed out is a problem in our presentation, and we have made detailed changes to our concluding statements. However, since bioinformatics analyses aim to use algorithms to compute gene expression data and produce results that can guide biological questions at the data level, but are not supported by the results of sufficient scientific experiments, we cannot be too decisive in making conclusions. However, the aim of our study is to use bioinformatics to project the relationship between FHL2 expression and prognosis and immune infiltration in various cancers based on existing data to guide the direction of future experiments, and therefore a large number of experiments have not yet been designed to support the results. In the future, we will further focus on experimental validation of our bioinformatics analysis results to further clarify the genetic characteristics of FHL2 in various cancers.

Comment 4. Introduction: General descriptions about cancer and lung cancer seem not to be necessary. Rather, the advantages and profits of bioinformatics analysis in cancer prognosis may be more informative for readers.
Reply4: First of all, thank you for your comments. We have removed the redundant part of the general description of cancer and lung cancer based on your suggestion and added a description of the advantages of bioinformatics in the prognostic analysis of cancer in the introduction section. Based on the rapid development of biological databases, we have been able to access a large number of samples of bioinformatics data on cancer and paracancerous tissues in publicly available databases. This allows for results from bioinformatics analyses with larger sample sizes and enriched with a wider range of cancer types to be broad or representative. It is also possible to find other important researchable directions beyond the previously reported results.

Comment 5. Typos:
line 54, functions(6, p. 2).
Line 188, the title should be bold letters: “Significant correlation between FHL2 and EMT”
Line 242, CAFs(32, p. 2).
Reply5: Thank you for your comments, we have modified the above issues according to your comments.