Peer Review File

Article Information: http://dx.doi.org/10.21037/jgo-20-120

Review Comments:

This study, according the abstract, has the aim to determine the association between TEFM and tumor progression and prognosis in HCC. Furthermore, in the conclusions they speculate on prediction of survival based on levels. This is a translational objective that requires understanding of clinical aspects of HCC. It goes beyond a report on the levels in different cell lines and tissues.

I have the impression that the manuscript balances between basic biological observations and translational application. Especially this second aspect is not well reported.

Translational: can the authors present the current practice of HCC diagnosis in the clinic, which techniques are used, role of imaging etc. Are tissue biopsies used as a method for routine screening, can this technique be used frequently in the same individual, are risks involved, hence TEFM determination by mRNA or protein expression as first diagnosis? One niche are the patients that undergo tumor resection, have these patients been treated by chemo, radiation etc prior to resection and are those suitable for TEFM determination? Does this study tells us anything about TEFM levels and recurrence after resection?

Reply: Thank you for your suggestions. The purpose of this study was to determine the association of TEFM with tumor progression and prognosis in HCC patients. All patients were diagnosed for the first time and there was no history of chemotherapy or radiotherapy before surgery, and we think those are suitable for TEFM determination. However, this study don't tell us about TEFM levels and recurrence after resection.

Comment 1: Using TEFM mRNA levels for survival prediction raises a question how to expand this beyond a single patient cohort. Line 197 they used a TEFMhigh vs TEFMlow subgroup, the cut-off is determined arbitrarily. In most of the studies in literature use a training and a validation set. Determine the optimal cutoff in the training and validate this in a second group of patients. When important the p value will be lower than 0.04

If the translational aspect is not the focus of this study but more focussed/limited to the association. This information is valuable and warrants reporting, then more basic aspects of the gene and its association with other processes in cancer can be evaluated.

Reply: Thanks for your suggestions. TEFM high and low groupings are obtained with reference to related bioinformatics articles. To analyze the association between TEFM and clinicopathological parameters, patients were divided into two subgroups

(TEFM^{low} and TEFM^{high}) according to the median value of TEFM expression levels. Accordingly, the mRNA expression level of TEFM was high in 82 cases and low in 83 cases. In deed, the translational aspect is not the focus of this study but more focussed to the association. We found that the mRNA expression level of TEFM was significantly correlated with sex, AFP level, and vascular invasion ($P<0.05$).

Comment 2: In general, at multiple occasions the English languages needs to be improves (e.g. line 51/52, and line 93, 97).

Reply: Thanks for your suggestions. We have modified these language.
Changes in the text:
Line 51/52: In addition, HCC is usually at an advanced stage after a definite diagnosis, which delays treatment and leads to a poor prognosis.
Line 93: All specimens were confirmed by the pathology department, and were stored in a -80°C ultra-low temperature refrigerator for frozen storage 30 minutes after surgical resection.
Line 97: All patients were diagnosed for the first time and there was no history of chemotherapy or radiotherapy before surgery. Fresh tumor tissue samples were cut from the tumor, and their corresponding adjacent normal tissues were obtained from the tumor edge ⩾3cm.

Comment 3: The abstract has to be revised, it is to long on methods.
The background on the gene/protein (lines 61-81) are not balanced to the focus of the article, a bit long. TEFM is reported in the past in NCBI databases as C17Orf42, expression data is available through that link but not include here, there could be valuable supportive data.
The use of the L-O2 cell line is problematic see https://web.expasy.org/cellosaurus/CVCL_6926
This cell line is reported as not a good control for normal liver: Hela+fetal.

Reply: Thanks for your suggestions, It is really helpful to us. We have revised the abstract of the manuscript, especially the part of methods.
TEFM is reported in the past in NCBI databases as C17Orf42, we have checked its refenrence. All the recent refenrence are reported as TEFM.
L-O2 cell line is reported as not a good control for normal liver: Hela+fetal.
In the reviesed manuscript, we used THLE-3 cells as the control, and done the Western blot again to detect the expression of TEFM in different liver cancer cells and normal liver cell.

Comment 4: Line 106, can they explain 1% antibiotics.
Reply: 1% antibiotics (100 mg/L streptomycin, 100 U/mL penicillin; Hyclone; GE Healthcare Life Sciences, Logan UT, USA)
Changes in the text: see Page 4, line 107: 1% antibiotics (100 mg/L streptomycin, 100 U/mL penicillin; Hyclone; GE Healthcare Life Sciences, Logan UT, USA)

Comment 5: The figure 1 and the text in line 242 appear to be in conflict.
Reply: Thanks for your suggestions. We have modified this part of the manuscript
Changes in the text: Western blotting results demonstrated that the protein expression of TEFM was highest in Hep3B cells and lowest in QGY-7701 cells.

Comment 6: Line 290/291, what are the current markers or gene signatures for HCC and how do they perform compared to TEFM,
Reply: We selected HCC marker genes such as vascular endothelial growth factor (VEGF), tumor protein p53 (TP53), ras association domain family member (1RASSF1), cyclin dependent kinase inhibitor 2A (CDKN2A), golgi membrane protein 1 (GOLM1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), catenin beta 1 (CTNNB1), marker of proliferation Ki-67 (MIKI67), and AXIN1, according to reference 21and 22.
[21] Grandhi MS, Kim AK, Ronnekleiv-Kelly SM, et al. Hepatocellular carcinoma: from diagnosis to treatment. Surg Oncol 2016;25: 74-85.
[22] Banaudha KK,Verma M. Epigenetic biomarkers in liver cancer. Methods Mol Biol 2015;1238: 65-76.

Comment 7: Line 295: predictive for what?
How does TEFM perform compared to the use of BCLC-staging criteria?
Reply: Thanks for your suggestions. We have modified this part of the manuscript. Predictive for HCC prognosis.
Changes in the text: In line 295, to further confirm the effect of TEFM alone and among the other predictive factors of HCC prognosis.
The level 3 TCGA data (lihc_tcga_rna_seq_v2_mrna) were obtained at from the TCGA database. Meanwhile, clinical information, OS and DFS data were downloaded for analysis. The clinical information don't include the BCLC-staging of the samples. BCLC-staging data are not available from the TCGA dataset, so we don't know how does TEFM perform compared to the use of BCLC-staging criteria.

Comment 8: Lines 316-321: I would like to have an explanation why these markers were selected, many studies have evaluated gene expression in HCC tissue, molecular subclasses have been reported and genes mechanistically or associated with HCC stage or progression have been reported. TEFM looks like it fits there at some place, this has to be discussed in detail in the paper.
Reply: We searched HCC marker genes which used in the clinical diagnosis from the reference. The results of gene correlation analysis showed that the expression level of the TEFM gene in HCC was significantly positively correlated with the expression levels of the HCC marker genes, such as VEGF, TP53, etc. All these HCC biomarker genes are associated with HCC carcinogenesis. However, we don't know the molecular mechanism of TEFM in carcinogenesis and development. We speculated that TEFM gene may be function as an oncogene in the HCC cells and tissues.
Changes in the text: In the Disscussion part, we add some sentences to discuss the

potential role of TEFM in tumorigenesis and development. TEFM gene may be function as an oncogene in the HCC cells and tissues.

Comment 9: Line 349: the authors claim a vital role for TEFM, can they explain this within the framework of a mechanism?
Reply: Thank you for your adivise. The molecular mechanism of TEFM in tumorigenesis and development have not been reported. In our experiment, we found that TEFM may regulate mitochondrial gene expression and oxidative phosphorylation activity, and further change the type of cell metabolism. This hypothesis needs to be confirmed by more cellular and molecular biology experiments.
Comment 10: I might have missed it, line 355 they are speaking about breast cancer, explain?
Reply: Thanks for your suggestions. We have modified this typos.
Changes in the text: In line 355: This is the typos. It should be HCC samples

With the analysis of TGCA data for HCC, did the authors find information of the role of TEFM in other types of cancer?
Figure 1: problem whether L-O2 is a correct control and secondly see remark results section regarding HepG2/Hep3B.
Reply: Thanks for your suggestions. We have done the experiments again to detect the expression of TEFM in different liver cancer cells and normal liver cell. In this time, we use THLE-3 cells as the control, and the result confirmed that the protein expression of TEFM was significantly upregulated in diverse HCC cell lines, including HepG2, SMMC-7721, BEL-7402, Hep3B, QGY-7701, and SK-Hep1, compared with THLE-3 cells.

Fig 3A: the distribution of mRNA levels in cancers and normals is very variable, it does not suggest to be a useful marker.
Can they explain/discuss the suggestion from fig3B that TEFM mRNA goes down in stage IV?
Reply: Thanks for your suggestions. The data of TEFM mRNA expression levels in HCC tissues and noncancerous liver tissues of HCC were queried from TCGA and Genotype-Tissue Expression (GTEx) datasets by GEPIA (http://gepia.cancer-pku.cn/). The TEFM mRNA expression levels between the multiple pathological stages in HCC were analyzed by GEPIA. The distribution of TEFM mRNA levels in cancers and normals is very variable, and all the mRNA expression data were downloaded from TCGA database. The mRNA expression data of TEFM is authentic. Next, we used RT-PCR and Western blot to determined the mRNA and protein levels in HCC samples, and both the mRNA and protein expression level of TEFM is not very variable, and suggested that TEFM may be a useful marker for HCC, and it may be useful in HCC diagnosis and theraphy. However, we cannot explain why the TEFM mRNA goes down in stage IV of HCC, it should be confirmed by more cellular and molecular biology experiments.

Figure 4: See comments above on high *vs.* low and training and validation cohort.
Reply: In Figure 4, to analyze the association between TEFM and clinicopathological parameters, patients were divided into two subgroups ($TEFM^{low}$ and $TEFM^{high}$) according to the median value of TEFM expression levels. Accordingly, the mRNA expression level of TEFM was high in 82 cases and low in 83 cases.