Reviewer-1

Reviewer Name: J Kleeff

Review Date: 2019-08-24 17:43

Specific Comments To Authors: The manuscript by LuLu Sun and colleagues analyses miR-30c in pancreatic cancer. To this end, expression levels of miR-30c and the target twinfilin 1 (TWF1) were determined in human pancreatic cancer by quantitative real-time PCR and immunoblot analysis. It is shown that miR-30c expression is decreased in pancreatic cancer and that miR-30c supressed pancreatic cancer cell proliferation. Further, TWF1 is a direct target of miR-30c and miR-30c negatively correlates with TWF1. This is a potentially interesting analysis, and the experimental procedures are -in general- sound and valid. There are some concerns that should be addressed: 1.The rationale to analyse miR-30c is not clear. Why did the authors chose this microRNA? 2. The authors should provide data on the localisation of miR-30c in pancreatic cancer tissues. 3. The normal human pancreatic ductal epithelial (HPDE) cell line is not an optimal control for pancreatic cancer cells. This should be discussed. 4. It is quite surprising, that there was only one target gene appearing in all three online bioinformatics tools (TargetScan, miRDB and miRTarBase), with 55, 849, and 521 predicted genes. How can this be explained? How accurate are these online tools?

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments To Authors (File):

Reviewer-2

Reviewer Name: Anonymous

Review Date: 2019-08-20 10:33

Specific Comments To Authors: I would like to commend the authors for presenting this research data. Clearly, there is still an open discussion about the mechanisms of carcinogenesis in pancreatic cancer, and the role of microRNA in particular. Specifically, the role of microRNA-30 in pancreatic cancer was not researched and described, so this paper has a certain degree of novelty. Surely, like many other previously described molecular pathways, microRNAs are not the sole players but the current research adds significant piece of information to the global picture of the complex network of altered regualtion in pancreatic cancer. Overall, little criticism could be expressed regarding this study. The manuscript is concise and well-structured. The aim and the goals of the study, methodology and results sections are comprehensive and clear. This is a nice example of the translational research combining in vitro cell culture experiments, use of animal model and inclusion of some human patient data.

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Accept (General priority)

Specific Comments To Authors (File):

Response to reviewers

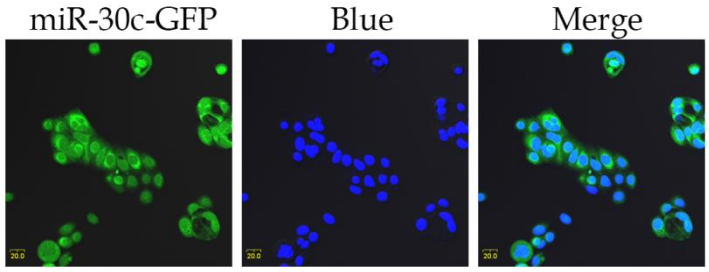
Reviewer#1

1.1 Our research group screened differently expressed miRNAs between pancreatic cancer cells and the normal human pancreatic ductal epithelial (HPDE) cell, miR-30c is one of the different expression miRNAs but not the one with the most significance. So we decided to study one miRNA first and test whether it has clinical significance and function. The research on the miRNA with the most significance is still in progress. And we did not mention the screening experiment, which we planned to put that

result in our next article in progress. Thank you for your reviewing on our work.

1.2 miR-30c is a mature miRNA,

The routine mechanism of a mature miRNA is to inhibit the target gene expression in the cytoplasm. The location of a mature miRNA is associated and predicted its function. We believe your suggestion is innovative and feasible, maybe the localisation of miR-30c in pancreatic cancer is different from other cancers, which suggest a different regulation mechanism of miR-30c exist. However, we do have difficulties. Our lab could not synthesize miRNA probe and did not carry out the FISH experiment routinely. We negotiated with other labs and they told us that they could help us but it would take two months at least to get a satisfying result. Considering the heterogeneity of pancreatic cancer and other non-tumorous tissues, we think we could use a GFP-labled miR-30c-GFP mimics to discover the location of miR-30c in pancreatic cancer cell line BxPC-3 preliminarily. If a mechanism for miR-30c nuclear import exists in pancreatic cancer, we can see the GFP in the nuclear. As we can see, we found mature miR-30c mainly located in the cytoplasm of BxPC-3 cells after transfection for 48 h. Maybe no extra mechanism of miR-30c exist in pancreatic cancer, at least in pancreatic cancer cell line BxPC-3. We admire the innovation and preciseness of the reviewers of WJG magazine，but we hope to publish our results earlier for the researchers worldwide to know. As you all know miR-30c is a hot miRNA, which was already published in lung cancer and breast cancer.



BxPC-3

1.3 As we all know, heterogeneity is one of the hallmarks of cancer. Normal pancreas tissue is an optimal control for pancreatic cancer cells, but right now normal pancreas tissues are not available. The expression of miR-30c in pancreatic cancer is definite, as the public GEO datasets and our RT-qPCR experiments all supported this conclusion. The main purpose of using HPDE cell as a control is just to compare the different expression levels of miR-30c between different pancreatic cancer cells for us to decide which pancreatic cancer cell lines were suitable for further experiments. According to your advice, we have revised our manuscript (line 195-197) and added information (line 318-319) in the DISCUSSION part as you presented, which were all marked in red. Thank you for your reviewing on our work.

1.4 There five genes (TWF1, RAD23B, S100PBP, MIA3 and VPS33A) in commen among the three bioinformatics tools. As an actin-binding protein, TWF1 regulates diverse aspects of actin dynamics, whose function attracted us firstly. Meanwhile, TWF1 was reported to be an oncogene in breast cancer and lung cancer (reference 13, 14, 15). Therefore we focused on TWF1. Accordingly, we have revised the Fig 3A and manuscript.

Reviewer#2

Thank you for your reviewing on our work.