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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 83410

Title: Molecular profiling reveals potential targets in cholangiocarcinoma

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 02461627
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: United Arab Emirates

Author's Country/Territory: United States

Manuscript submission date: 2023-01-21

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-01-22 05:36

Reviewer performed review: 2023-01-30 13:35

Review time: 8 Days and 7 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation

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Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [Y] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No scientific significance
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The manuscript entitled "Molecular profiling reveals potential targets in cholangiocarcinoma" and authored by Liu and colleagues through mRNA and protein profiling, identified very high similarity between CCA tumors derived from the P53null/KRasG12D mice and human CCA tumors, thus providing a potential preclinical CCA model for investigating the CCA tumorigenesis. In addition, data presented in this study suggested that Notch1 and cell cycle associated pathways could be potential therapeutic targets in CCA patients. Given the natural product origin of both small molecule inhibitors (Arcyriaflavin and Flavopiridol) it is recommended to add a short paragraph to introduce GENERAL health-promoting benefits of natural products. The following studies address such shortcoming: http://dx.doi.org/10.4236/jdm.2011.13006, PMID: 17151319, PMID: 32460808, https://doi.org/10.4236/ajps.2018.96091, https://doi.org/10.1186/s41936-020-00177-9, PMID: 33255507, PMID: 22812448. Other than the raised comments and pending adequate revision, the present study could make a good read for the journal subscribers. Other comments • Proofreading is required. • Uncropped gels for all blots should be made available as supplementary data. • All

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western blots must be quantified and properly analyzed. • Figure 2 lacks proper statistical analysis • Figure 4D image and its legend should have labels to clarify relevant lesions • What exactly the Y-axes are scaled to? • A conclusion figure illustrating how cell cycle and notch pathways contribute to CCA should be added. • References should be enriched with more diversified investigations. Results from the following studies could serve this purpose: https://doi.org/10.1186/s41936-022-00321-7, PMID: 36432184, PMID: 35740022.

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Reviewer's code: 05687852
Position: Peer Reviewer
Academic degree: MD, PhD

Professional title: Doctor, Professor

Reviewer's Country/Territory: Taiwan

Author's Country/Territory: United States

Manuscript submission date: 2023-01-21

Reviewer chosen by: AI Technique

Reviewer accepted review: 2023-01-25 15:38

Reviewer performed review: 2023-01-31 11:49

Review time: 5 Days and 20 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[Y] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No novelty
Creativity or innovation of this manuscript	[Y] Grade A: Excellent [ ] Grade B: Good [ ] Grade C: Fair [ ] Grade D: No creativity or innovation

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Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

The authors utilized the transcriptomic profiling of Cholangiocarcinoma (CCA) to explore the oncogenic driver events, analyzed the expression of the relevant genes, and identified the feasibility of target therapies for tumorigenic lesions. Albeit, I consider these findings to provide new insight into cancer-related fields, I still have some suggestions. 1, Most figures are highly professional, however, the authors should guide the readers to the meaning of the images appropriately; otherwise, it is likely to cause misunderstandings. Therefore, I suggest that the author consider revising these figure legends again. 2, In Fig2A suggested that several cell cycles associated genes were up-regulated in CCA cancer lines, including PCNA, E2F1, CDK2, CDK4, and CDK6. Since the authors gave a general answer on gene expression, is there any evidence of different roles in cancer phenotypes of these genes? Please perform pertinent bioinformatic analyses and provide examples of studies investigating miRNA alteration or DNA methylation (https://biit.cs.ut.ee/methsurv/) (PMID: 29264942, 34834441, 35740947). 3, So far, the tumor infiltrates immune cells and is vital for patient survival. However, it is worth validating their data correlated with immune cells by using the

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"TIMER" (http://timer.cistrome.org) analysis tool (PMID: 32442275, 34329194, 35454940). 4, Since Connectivity Map (CMap) can be used to discover the mechanism of action of small molecules, functionally annotate genetic variants of disease genes, and inform clinical trials. It would be fascinating if these data could be correlated with other clinical databases. Therefore, I suggest the authors can validate their data via CMap or proteinatlas, and discuss these methodologies and literature as well as the validated data for cancer recurrence or metastasis in the manuscript (PMID: 25613900, 29195078, 32064155) 5, The author should use other statistical analyses such as ANOVA to calculate the P-value for three or more groups of data, and please update the “Statistical Analysis” of the Method during further revision. For example, please add the correct P-value for Figure 2. Same as Figure 3, please also perform statistical analysis for these data. 6, There are few typo issues for the authors to pay attention to; please also unify the writing of scientific terms. “Italic, capital”? For example, Jag1, Jag2 in Page 6, and Italic form of JAG1/JAG2 in page 7. 7, The font is too small for some of the current figures, meanwhile, the manuscript also needs English proofreading.

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Reviewer's code: 06475363
Position: Peer Reviewer
Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: United States

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Review time: 9 Days and 15 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Novelty of this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] Grade C: Fair [ ] Grade D: No novelty
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Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[Y] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In this study, the author explores the driving factors of carcinogenesis according to the transcriptome analysis of CCA in the public database, analyzes the expression of related genes, and determines the feasibility of targeted therapy in terms of these carcinogenic factors. First of all, it is retrieved from the transcriptome database of TCGA that cell cycle and Notch-related pathways are activated in CAA, and then verified in cell lines, model mice, EBI and GEO databases. In the meanwhile, it is also proved that the growth of CAA cells can be inhibited by inhibiting cell cycle and Notch-related pathways, suggesting that cell cycle and Notch can serve as biological indicators for the occurrence, development and treatment of CAA. This paper focuses on the activation of cell cycle and Notch in CAA as well as the possibility of treatment, and the author also gives detailed proof in the paper. Besides, this paper is logically clear and has relatively full contents. However, there are still some shortcomings in this paper. For instance, there have already been numerous studies in CAA on the driving factors of cancer mentioned in this paper, i.e. "cell cycle and Notch-related pathways", which is less innovative, so the author is requested to give relevant descriptions. The concrete questions are as below:

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1. Previous studies have reported the role of cell cycle and Notch in CAA, which the author has found these two indicators via the database and also verified them in this paper. The author is requested to supplement the previous studies as well as the innovation of this paper in the discussion. 2. In the Preface, the author states that "it is in urgent need to develop early detection markers and effective treatment methods for CCA patients". Do the cell cycle and Notch, which are the focus of this paper, change at the early phase of tumor formation? The author is requested to supplement relevant content in the discussion. 3. The internal reference GAPDH was chosen for the detection of cell line mRNA in Figure 2, while different internal references, i.e. α-Tubulin and GAPDH were chosen for the protein detection of cell line in Figure 3 and mouse tissue in Figure 4. The author is requested to explain the reasons and provide the original pictures of WB. 4. Do the P53null/KRasG12D gene editing mice used in Figure 4 spontaneously form tumors, and what is the age of the chosen diseased mice? The author is requested to supplement this content. 5. There is no normal cell line control in the experiment of detecting the cytotoxicity of inhibitors of cell cycle and Notch pathways to CAA in Figure 5. Notch is associated with cell proliferation, and cell proliferation can be apparently inhibited by inhibiting cell cycle or Notch, so it is requisite to distinguish the differences between these inhibitors and normal cells. 6. The author searched for the key biological indicators associated with CAA by means of bioinformatics, and then focused on cell cycle as well as Notch gene, by contrast, P53null/KRasG12D gene editing mice was used in the mouse model. It was proved that this model is similar to human CAA and is a potential pre-clinical CCA model. It seems that the changes of P53 and KRas precede the changes of cell cycle and Notch, then why not P53 and KRas are chosen as the biological indicators of CAA directly for the purpose of treatment?