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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 85245

Title: DARPP-32 promotes colorectal cancer growth by activating the PI3K/AKT pathway

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 04049611
Position: Peer Reviewer
Academic degree:

Professional title:

Reviewer's Country/Territory: Reviewer_Country

Author's Country/Territory: China

Manuscript submission date: 2023-04-19

Reviewer chosen by: Geng-Long Liu

Reviewer accepted review: 2023-05-24 05:22

Reviewer performed review: 2023-06-01 06:17

Review time: 8 Days

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish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

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Scientific significance of the conclusion in this manuscript	[ ] Grade A: Excellent [ ] Grade B: Good [Y] 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

Major comments: 1. Previous study demonstrated that PP-1 directly dephosphorylates AKT to modulate its activation (Cell Death & Differentiation. 2010, 17(9): 1448-62). Moreover, the authors described that DARPP-32 would act as a PP-1 inhibitor when the Thr34 residue of DARPP-32 is phosphorylated by PKA. Please conduct some experiments to check the phosphorylation level of DARPP-32 at Thr34 residue and the status of the PP-1 activity in CRC cells with DARPP-32 overexpression. 2. The authors implied that overexpression of DARPP-32 could promote cell proliferation, migration, and invasion of CRCs, whose phenomena may be induced by DARPP-32-mediated enhancement in the activation of the PI3K/AKT pathway. For clarifying the significance of PI3K/AKT activation in DARPP-32-mediated several phenomena in CRC, PI3K or AKT blockers should be applied in the present study. 3. GAPDH expression was significantly up-regulated in human colorectal carcinoma tissues (J Bioenerg Biomembr. 2012, 44(1):117-25), and over-expression of β-tubulin is associated with poorer outcomes in colorectal cancer (Cells. 2019, 8(1):25). These articles implied that the expression levels of GAPDH and β-tubulin may be altered in CRC, and thus these molecules seem

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inappropriate to be used as the internal controls in the present study. 4. The authors should explain the rationale why SW480 rather than other CRC cells with higher DARPP-32 were chosen for the RNA-seq study. Can SW480 cells be a typical representative of CRC? 5. Cancer cells must have some genetic mutations and altered signaling transduction, and these changes may disturb our observation of the intracellular roles of DARPP-32. The experiments regarding the over-expression of DARPP-32 should be re-conducted in normal cells, such as NCM460, rather than CRC cells if the experimental purpose is to understand the carcinogenic possibility of over-expressed DARPP-32 in normal cells. 6. In Figure 1G. Please check the correctness of the data or the label of the y-axis because in general the relative expression level of the control group should be defined as 1. 7. In Figures 2 and 4. The cytotoxic effect induced by silencing DARPP-32 should also be evaluated in normal cells (NCM460) to understand whether the drug targeted on DARPP-32 will cause significant cytotoxicity in normal cells. Minor Comments: 1. Please check the contextual correlation of the sentence "Epidermal growth factor receptor (EGFR) mutant in non-small cell lung cancer" (Lines 105-106). 2. The experimental results or conclusions of the present study should not be described in the Introduction section. 3. Please provide the clinical demographics of the tissue donors and the approval number of the IRB study. 4. Please carefully check typing or grammatical errors as well as confirm the correctness of the style (uppercase or lowercase) of the words or terms. For example, "CO2", "10%SDS-PAGE.After", "We thank Professors …", "molecular pharmacological in …", "… approved this animal experiments" 5. Abbreviations should be defined at the first mention and then present consistently. For example, TAM, siDARPP-32, "KI-67 vs. Ki-67", OE 6. Please provide the mRNA accession number for the genes detected in the qPCR assay. 7. Please provide the sequences of siRNA2 and siRNA3 as well as the working concentration of all siRNA. Is there a negative control siRNA applied? 8. Please leave a blank space between the

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value and its unit. For example, "100ul 50μM" (Line 171). Besides, the volume unit should be corrected as "μl" rather than "ul". 9. Please provide detailed information (e.g. catalog number, company, city, and country) of commercial kits and antibodies. 10. Please describe the procedure of data conversion of immunoblotting and qPCR assays in the Materials and Methods section. 11. Please provide sequencing depth in the RNA-seq experiment. Besides, it's a wrong description that the quality and integrity of total RNA product were determined by using a NanoDrop spectrophotometer (Lines 204-205). 12. Please provide the gender of BALB/c naked mice (Line 230). 13. "The student's test" is an incorrect name (Line 240). Besides, other statistical analyses should be described in the Materials and Methods section. 14. Please define what is "normal CRC samples" (Lines 247-248). 15. Some references miss information regarding volume, issue, page number, or article number. 16. In Figure 1. What do the red and gray bars mean? What do the abbreviations, COAD and READ, mean? Is there any substantive difference between Figure 1A and 1B for the purpose of the experiment? It's a redundant description "****P<0.0001" (Line 503). 17. In Figure 2. Is there any lentivirus or siRNA treatment in the NC group? 18. The expression level of DARPP-32 protein was significantly higher in HCT166 cells than that in normal cells (Figure 1H). However, no DARPP-32-positive cells can be apparently observed in the NC group (Figure 3C). Why? 19. In Figure 2, the cell viability was significantly reduced by about 30% after siRNA intervention. However, there seems to be no significant change in cell density after siRNA intervention for 48 hr (Figure 5C). Why? 20. In Figure 6. Please explain why PI3K/AKT pathway rather than MAPK pathway was chosen for further examination in the expression correlation between these proteins and DARPP-32. Moreover, the qualities of some immunoblotting images of PI3K and p-PI3K proteins need to be largely improved. Please replace them.

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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastrointestinal Oncology*

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Title: DARPP-32 promotes colorectal cancer growth by activating the PI3K/AKT pathway

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 00607640
Position: Editor-in-Chief
Academic degree: PhD

Professional title: Professor

Reviewer's Country/Territory: Taiwan

Author's Country/Territory: China

Manuscript submission date: 2023-04-19

Reviewer chosen by: Geng-Long Liu

Reviewer accepted review: 2023-06-16 03:24

Reviewer performed review: 2023-06-20 01:40

Review time: 3 Days and 22 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
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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) [Y] Minor revision [ ] Major revision [ ] Rejection
Re-review	[ ] Yes [Y] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous
	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

In this paper, the authors examined the role of dopamine and cyclic adenosine monophosphate-regulated phosphoprotein (DARPP-32) on the colorectal cancer progression and the potential underlying mechanisms. The paper is interesting. However, some minor concerns are for your consideration. 1. Abbreviations used should be with its full name when it firstly appears. 2. Fig 6E, statistics is recommended. 3. An editing of English language is recommended.

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RE-REVIEW REPORT OF REVISED MANUSCRIPT

Name of journal: *World Journal of Gastrointestinal Oncology*

Manuscript NO: 85245

Title: DARPP-32 promotes colorectal cancer growth by activating the PI3K/AKT pathway

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Peer-review model: Single blind

Reviewer's code: 04049611

Position: Peer Reviewer

Academic degree:

Professional title:

Reviewer's Country/Territory: Reviewer_Country

Author's Country/Territory: China

Manuscript submission date: 2023-04-19

Reviewer chosen by: Xin-Liang Qu

Reviewer accepted review: 2023-06-30 11:14

Reviewer performed review: 2023-07-03 10:28

Review time: 2 Days and 23 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
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) [Y] Minor revision [ ] Major revision [ ] Rejection
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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statements	Conflicts-of-Interest: [ ] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

Minor comments: 1. One submitted table contains some non-English characters. Please check it. 2. The Introduction section should only include the present study's background (relevant research) and experimental purposes. Please omit the last paragraph regarding experimental findings and the conclusion of the present study in the Introduction section. Besides, please also clearly describe the experimental purposes in the Introduction section. 3. Please integrate the responses for the significant comments or concerns into the Discussion section and point out the possible limitations regarding the statement "DARPP-32 may be a potential therapeutic target for CRC" in the experimental condition without normal cells included. 4. According to the description regarding the quantification of qPCR in the Materials and Methods section, DARPP-32 relative expression levels were calculated according to the 2-ΔΔCt method. However, the authors stated that we did not normalize the expression levels of the control group (NCM460 group) to a value of 1. Why? 5. Please provide the sequence of the negative control siRNA and mention the working concentration of siRNA in the Materials and Methods section. 6. Why were female mice chosen for the in vivo study? Is it possible that animal menstrual cycles or sex differences interfere with experiments? 7. Reference #27 still needs the page number.