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PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 75936

Title: Anoctamin 5 regulates the cell cycle and affects prognosis in gastric cancer

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 05469117
Position: Editorial Board
Academic degree: PhD
Professional title: Adjunct Professor, Chief Physician, Deputy Director
Reviewer's Country/Territory: China
Author's Country/Territory: Japan

Manuscript submission date: 2022-02-28
Reviewer chosen by: AI Technique
Reviewer accepted review: 2022-03-22 16:21
Reviewer performed review: 2022-03-22 19:07
Review time: 2 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Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish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
Re-review	[Y] Yes [ ] No
Peer-reviewer	Peer-Review: [Y] Anonymous [ ] Onymous

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

SPECIFIC COMMENTS TO AUTHORS

Thank you for giving me a chance to review this research regarding the role of Anoctamin 5 (ANO5) in the regulation of tumor progression and the clinicopathological significance of its expression in gastric cancer (GC). The results obtained indicate that ANO5 plays a significant role in the cell cycle of GC cells, especially in the progression of G1/S checkpoint, and a high expression level of ANO5 was identified as a poor prognostic factor in GC patients. The present study demonstrated that ANO5 has potential as a poor prognostic biomarker and a novel therapeutic target for GC. This paper is a well-written ,My major comments are as follows: 1.On page 10, in the last paragraph"Tumor staging was conducted according to the International Union Against Cancer (UICC)/TNM Classification of Malignant Tumors (8th edition) [39]. "The reference 39 here is marked incorrectly. It should be 38.Please check the correctness of other references. 2.In Table 3,why is the 5-year overall survival rates the lowest (73.9 %) When the cutoff value is 1.3?

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Reviewer's code: 06152876
Position: Peer Reviewer
Academic degree: MD
Professional title: Doctor
Reviewer's Country/Territory: China
Author's Country/Territory: Japan

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Reviewer performed review: 2022-03-31 08:25
Review time: 1 Day and 4 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
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SPECIFIC COMMENTS TO AUTHORS

This study explored the role of ANO5 in GC progression by conducting retrospective clinical study, in vitro functional assays as well as a brief mechanism investigation. IHC staining indicated the prognostic role of ANO5 in GC patient while functional assays indicated its oncogenic role in GC progression. Further microarray analysis suggested that ANO5 regulated the cell cycle progression by mediating the expression of cyclin-associated genes. Personally I find this manuscript both interesting and informative. However, several points have to be addressed before acceptance. 1. Regarding functional assays, there are a few concerns: 1). For the knockdown-based functional assay, it is more common to use two pairs of siRNA instead of one; 2). to evaluate cell proliferation, I'm wondering if you could introduce CCK-8 to evaluate cell proliferation in the long run? 3) ANO5 is dramatically down-regulated in MKN7. Have you ever considered performing overexpression-based functional assays? 2. Have you ever checked the online database (GEO, TCGA and etc.) or used tools (such as GEPIA2) to validate the significance of ANO5 in GC? If ANO5 is shown to be oncogenic in GC, these findings help consolidate the observations reported in the present study. 3. As both MKN45 and NUGC4 cell lines were used in the functional assays. I'm wondering why you didn't include MKN45 into the microarray analysis. As we could see in the mechanism studies, down-regulation of ANO5 significantly reduced not only phosphorylation level of Rb but also the expression level of normal Rb in MKN45 cell line, which was different from NUGC4 (Figure 4C). This indicates that there might be some inherent difference between these two cell lines when their ANO5 expression is ectopically dysregulated. 4. The resolution of figure 4A is too low to read. 5. Legend of figure 4D, "The down-regulation of ANO5 suppressed the phosphorylation of the

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JNK protein in NUGC4 and MKN45 cells. " I think it should be "increase". 6. As you have focused on CDK2 and JNK phosphorylation and proposed that ANO5 regulated the cell cycle via the up-regulation of p21 through activating JNK cascade, have you ever considered conducting further validation studies by introducing reverse approaches?

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Reviewer's code: 05177597
Position: Editorial Board
Academic degree: MD, PhD

Professional title: Associate Professor, Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: Japan

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Reviewer accepted review: 2022-03-31 08:12

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Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] 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
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SPECIFIC COMMENTS TO AUTHORS

In this study, the author performed a comprehensive discovery of Anoctamin 5 (ANO5) in the regulation of tumor progression and the clinicopathological significance of its expression in gastric cancer. However, there are several major issues to be addressed. 1. In the selection of gastric cancer cell lines, the author selected five cell lines, but only two of them were used in the study (part of the study was only applied to one cell line), the author did not show or discuss the experimental results of other cell lines in the discussion 2. The description of the experimental results on"ANO5 regulates proliferation and cell cycle in GC cells"and "ANO5 inhibits apoptosis in GC cells"seems repetitive and could be shortened without affecting content. 3. As for the"Effects of low-chloride conditions", the author's experimental results can only prove the relevance between ANO5 and chloride, but can not demonstrate causality. 4. FIG. 6a shows"Non-cancerous gastric epithelia were immunohistochemically stained using an anti-ANO5 antibody",while FIG. 6b shows"Primary human GC samples were immunohistochemically stained using an anti-ANO5 antibody". However, about the immunohistochemical results, FIG. 6A is deeper than FIG. 6b, which can not proved that ANO5 was predominant in the cell membranes and cytoplasm of GC tissue 5. The criteria for staining intensity score for the expression of ANO5 is not specified the specific location of gastric cancer tissue