

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 42009

Title: Glucocorticoid receptor regulates expression of microRNA-22 and downstream signal pathway in apoptosis of pancreatic acinar cells

Reviewer's code: 00646291

Reviewer's country: United Kingdom

Science editor: Ruo-Yu Ma

Date sent for review: 2018-09-05

Date reviewed: 2018-09-12

Review time: 7 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input checked="" type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language	(High priority)	<input checked="" type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input checked="" type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Major revision	<input checked="" type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

Delete the word gene from the title of the article so that it reads "Glucocorticoid receptor regulates expression of miR-22 and downstream signal pathway in apoptosis of AR42J cells" Please delete the word overexpression in the sentence "Nr3c1 overexpression

plasmid". This sentence could be rephrased as "Nr3c1 plasmid encoding the glucocorticoid receptor". "As revealed by qRT-PCR assay, the expression of miR-22 was 78.25 ± 6.61 times higher in the miR-22 mimic group relative to the miRNA NC group, companied with an obviously increased acinar cell apoptosis rate (32.53 ± 1.15 vs 18.07 ± 0.89 , $P = 0.0006$). Please replace the word companied with the word accompanied. "The upregulation of miR-22 could depressed its target gene, ErbB3, and the phosphorylation of PI3K and Akt." Please replace the word depressed with the word suppress. "Downregulate the expression of GR could upregulate the expression of miR-22, which further promoted the apoptosis of AR42J cells." Please replace the word downregulate with the word downregulation. "Downregulate the expression of GR could upregulate the expression of miR-22 and further promote the Cae-induced apoptosis of AR42J by targeting ErbB3 and further suppressing the PI3K/Akt signaling pathway." Please rephrase the sentence so it reads "Downregulation of GR gene expression could upregulate the expression of miR-22 and further promote the Cae-induced apoptosis of AR42J by targeting ErbB3 and further suppressing the PI3K/Akt signaling pathway." The description of the figure 2D should be more detailed. In the figure 2F Western blot analysis of the levels of p-PI3K, p-Akt and apoptosis-associated proteins should be performed with the miR-22 mimic transfection alone in the absence of Cae. The description of the figure 3D should be more detailed. The experiments assessing the role of GR in the regulation of the miR-22 expression should be performed in the presence and absence of dexamethasone. Western-blot analysis shown in the figure 5E should include total PI3K and Akt levels, as well as the levels of the proteins shown in cells that were not treated with Cae and in cells treated with dexamethasone. Replace the word data in the figure legends of the figures 4 and 5 with the word data.

INITIAL REVIEW OF THE MANUSCRIPT



**Baishideng
Publishing
Group**

7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

<https://www.wjgnet.com>

Google Search:

☐ The same title

☐ Duplicate publication

☐ Plagiarism

☐ No

BPG Search:

☐ The same title

☐ Duplicate publication

☐ Plagiarism

☐ No

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 42009

Title: Glucocorticoid receptor regulates expression of microRNA-22 and downstream signal pathway in apoptosis of pancreatic acinar cells

Reviewer's code: 03459514

Reviewer's country: Spain

Science editor: Ruo-Yu Ma

Date sent for review: 2018-09-30

Date reviewed: 2018-10-14

Review time: 14 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input type="checkbox"/> Grade B: Very good	<input type="checkbox"/> Grade B: Minor language	(High priority)	<input checked="" type="checkbox"/> Anonymous
<input checked="" type="checkbox"/> Grade C: Good	polishing	<input type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input checked="" type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input checked="" type="checkbox"/> Major revision	<input type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input checked="" type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

Name of Journal: World Journal of Gastroenterology Manuscript Type: BASIC STUDY

Ms. No.: WJG 42009 Title: Glucocorticoid receptor gene regulates expression of miR-22 and downstream signal pathway in apoptosis of AR42J cells. Authors: NA. In this

work the authors describe the effects of microRNA-22 (miR-22) on caerulein-evoked apoptosis in rat pancreatic acinar cells. Elements regulating the expression of miR-22 are assayed. The authors suggest that expression of miR-22 promote the caerulein-induced apoptosis of AR42J. Involvement of PI3K/Akt signaling pathway is pointed out. The topic of research is interesting in relation with therapeutical approaches for pancreatitis. There are some points that have called my attention. Here please find my comments. 1. English style and grammar. There are spelling and grammatical errors through the manuscript. Some expressions, as for example “best method of death for cells” should be rewritten. 2. Pancreatic AR42J cells are an amphycrine type of cell. Prior to performing exocrine studies the cells must be differentiated by incubation in the presence of dexamethasone. It is not mentioned in the manuscript whether the authors carried out this procedure prior to the stimulations. 3. miR-22 primer sequence is missing. This information must be given in the manuscript. 4. It is not clear to the reader why determination of amylase release is carried out. It is not clear which relationship, if any, exists between the increase in amylase release and the pro-apoptotic effects of caerulein. 5. Results section: The paragraph “AR42J cells were transfected with the miR-22 mimic as described. The expression of miR-22 was significantly elevated in the cells transfected with the mimic compared with the miRNA NC cells (Figure 2A). As shown in Figure 2B and 2C, the mRNA and protein expression levels of ErbB3, the target gene of miR-22, were significantly lower in cells overexpressing miR-22 than in the control cells” is complicated to understand. mRNA and protein expression levels of ErbB3, the target gene of miR-22, were significantly lower in cells overexpressing miR-22: is this right? The information given seems controversial in the way the information is written. One would expect an increase in the mRNA and protein expression levels of ErbB3 with upregulation of miR22. 6. Separately miR22 and Cae induce apoptosis. And the same is observed with their combination. It is not clear why the authors use the combination of

both to study cell death and the relevance of these observations to get a conclusion. A comparison between the effects of miR22 alone and that of miR22 plus Cae should be carried out in order to see if the effect is additive. 7. It would give important information to study the effect of Cae on Nr3c1 expression, bearing in mind the action of Nr3c1 on miR22 expression. Moreover, the relationship between miR22 and Cae needs to be better explained. Specially, because the authors state that the upregulation of miR-22 promoted Cae-induced apoptosis of AR42J cells. 8. In the results section the authors mention that the transcription factor Nr3c1 regulates the expression of miR-22. However, later on it is stated that ErbB3 is the target gene of miR-22, and that it is repressed. This information leads the reader to misunderstanding and needs clarification. 9. A figure summarizing the major findings of the manuscript would help the readers get a clear idea about the effects of miR22 on AR42J cells, the pathway activated and its relationship with Cae.

INITIAL REVIEW OF THE MANUSCRIPT

Google Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ No

BPG Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ No

PEER-REVIEW REPORT

Name of journal: World Journal of Gastroenterology

Manuscript NO: 42009

Title: Glucocorticoid receptor regulates expression of microRNA-22 and downstream signal pathway in apoptosis of pancreatic acinar cells

Reviewer's code: 03257477

Reviewer's country: Mexico

Science editor: Ruo-Yu Ma

Date sent for review: 2018-10-10

Date reviewed: 2018-10-17

Review time: 7 Days

SCIENTIFIC QUALITY	LANGUAGE QUALITY	CONCLUSION	PEER-REVIEWER STATEMENTS
<input type="checkbox"/> Grade A: Excellent	<input type="checkbox"/> Grade A: Priority publishing	<input type="checkbox"/> Accept	Peer-Review:
<input checked="" type="checkbox"/> Grade B: Very good	<input checked="" type="checkbox"/> Grade B: Minor language	(High priority)	<input checked="" type="checkbox"/> Anonymous
<input type="checkbox"/> Grade C: Good	polishing	<input checked="" type="checkbox"/> Accept	<input type="checkbox"/> Onymous
<input type="checkbox"/> Grade D: Fair	<input type="checkbox"/> Grade C: A great deal of	(General priority)	Peer-reviewer's expertise on the
<input type="checkbox"/> Grade E: Do not	language polishing	<input type="checkbox"/> Minor revision	topic of the manuscript:
publish	<input type="checkbox"/> Grade D: Rejection	<input type="checkbox"/> Major revision	<input checked="" type="checkbox"/> Advanced
		<input type="checkbox"/> Rejection	<input type="checkbox"/> General
			<input type="checkbox"/> No expertise
			Conflicts-of-Interest:
			<input type="checkbox"/> Yes
			<input checked="" type="checkbox"/> No

SPECIFIC COMMENTS TO AUTHORS

The objectives of the study were achieved by the experiments used. The main contributions of the study were the findings regarding the regulation of miR-22 expression by a glucocorticoid receptor and the mechanistic effects of miR-22 over

apoptosis in the rat pancreatic acinar cell line (AR42J). However, some points should be considered to improve the manuscript. The title is inappropriate because a gene is not responsible for the regulation of the mRNA expression. The correct form is the product of the expression could be the RNA or the protein. It would be better to delete the word gene. The abstract summarizes the work. Key words reflect the focus of the manuscript. Methods are described adequately. Regarding the results, the objectives of the study were achieved by the experiments used. The main contributions of the study were the findings regarding the regulation of miR-22 expression by a glucocorticoid receptor and the mechanistic effects of miR-22 over apoptosis in the rat pancreatic acinar cell line (AR42J). In general, the manuscript interprets the findings almost adequately and appropriately, highlighting the key points clearly and logically. The findings are relevant to the literature and discuss the relevance of the findings. However, there are some points to consider for improving the manuscript, mainly the discussion section.

1. Analysis of phosphorylated proteins by W-blot, specifically PI3K and Akt, requires the normalization of densitometry with total proteins besides a constitutive protein.
2. Discuss deeply how the fact of overexpressing the Nr3c1 gene in absence of the ligand is enough to diminish the transcriptional activity of miR-22. This glucocorticoid receptor has a cytoplasmic location and, upon ligand binding, is transported into the nucleus.
3. Besides authors' previous work, other studies have demonstrated the effect of miR-22 over apoptosis and its mechanistic effects (v.g. Pant (2017) Butyrate induces ROS-mediated apoptosis by modulating miR-22/SIRT-1 pathway in hepatic cancer cells. *Redox Biology*; Lv (2018) MiR-22-3p Regulates Cell Proliferation and Inhibits Cell Apoptosis through Targeting the eIF4EBP3 Gene in Human Cervical Squamous Carcinoma Cells. *Int J Med Sci*). These studies must be mentioned in the discussion and it would be desired to put in the recent literature context the novel results of the present work.
4. The effects over PI3K, Akt and apoptosis markers with si-Nr3c1 without Cae are

necessary to see the effect without inducing apoptosis with Cae. 5. The measurements of amylase are little discussed. For example, what is the power of these quantifications? How authors explain differences in amylase concentrations between treatment with Cae and missing of differences in the presence of si-Nr3c1 or miR-22 mimic. 6. Illustrations are of good quality and adequately illustrative of the paper contents. 7. Biostatistics. The manuscript meets the requirements of biostatistics. 8. The manuscript cites appropriately important references. However, some recent references regarding effects of mi-R22 over apoptosis must be added. Some suggestions are given above. Minor modifications. In fig 5, it is not evident the effect induced by Cae over the apoptosis markers, at the same level as in the fig. 1. It is suggested adding the lane without Cae treatment to compare the level of these markers without stimulation by Cae. As well, it is not justified the treatment with Cae to see the effect of the Nr3c1 silencing over basal apoptosis. Is it significant the effect of the si-Nr3c1 as with Cae? In the figure legend of figure 2, **P < 0.01 is not necessary. In the phrase "Then, The mimic of miR-22, Nr3c1 overexpression plasmid..." correct the capital letter. Could depress instead could depressed In fig. 4 and fig. 5, replace Data instead of Date. Rephrase "Luciferase reporter analysis and site-directed mutagenesis indicated that the binding site (GACAGCCATGTACA) of the glucocorticoid receptor (GR), which is encoded by the Nr3c1 gene" because is not clear.

INITIAL REVIEW OF THE MANUSCRIPT

Google Search:

- ☐ The same title
- ☐ Duplicate publication
- ☐ Plagiarism
- ☐ No



**Baishideng
Publishing
Group**

7901 Stoneridge Drive, Suite 501,
Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242

Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com

<https://www.wjgnet.com>

BPG Search:

[] The same title

[] Duplicate publication

[] Plagiarism

[Y] No