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HIGH IMPACT --- PEER REVIEW REPORT

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JOB DETAILS

MANUSCRIPT TITLE:	Expression Patterns of Galectins-1, -3, and -7 are Prognostic for Overall Survival in Ovarian Cancer
DATE:	02/02/2024

PEER REVIEWER'S COMMENTS

Note from the Peer Reviewer, John Doe

Dear Author,

My name is John Doe, and it has been a pleasure reviewing your manuscript. I have a PhD and a comprehensive understanding of molecular biology and other areas such as microbiology and immunology, biochemistry, and structural biology. I have 5 years of peer review experience, and I have reviewed for *International Journal of Biological Macromolecules* (impact factor: 6.95), *Journal of Molecular Biology* (impact factor: 5.47), and *FEBS Letters* (impact factor: 4.12), among others.

I have thoroughly reviewed your manuscript with a focus on areas critical to journal peer review and provided suggestions to strengthen the presentation of your research and highlight its impact and novelty. This report also includes feedback on the language quality, structure, and submission readiness of your manuscript from the Senior Science Editor and Managing Editor that I hope you will find useful.

Peer review is a subjective process with different experts often having different opinions. While this diversity of opinion is a strength of the peer review process, we understand the challenges involved in addressing differing expert views and assure you of our support through the publication process.

Best wishes for the submission of your manuscript!

Summary

- **Is the paper ready for submission in its current form?**
 - No; please check my comments in this report and in the manuscript.
- **Major issues – Likely to cause journal rejection**
 - The reporting of the results has several weaknesses with respect to the structure of the section and the description of the findings. I also noted some ambiguities in data presentation in the figures and tables. In addition, analysis related to paired expression of the studied galectins is incomplete.
 - One of the aims stated for the study is to investigate whether the expression levels of different galectins are correlated in ovarian cancer” because “there is a critical need for a comprehensive study of various galectins in a representative ovarian cancer panel.” However, you have not evaluated the correlation between combinations of different galectins and the outcome (survival). This aspect of the study needs to be clarified.
 - The number of patients does not seem to be justified by power analysis, and it is unclear whether the sample size was sufficient to achieve statistical significance. A power analysis should be performed, and whether it confirmed that the results of the study were statistically conclusive should be indicated.
 - There seem to be no control samples, i.e., those from cancer-free individuals. This aspect needs to be addressed in the manuscript.
 - Histological groups (serous, endometrioid, clear cell, mucinous) should be briefly characterized, especially with regard to their comparative malignancy.

- Control tissue staining for all galectins should be presented in Fig. 1 and in Table 5.
 - The result that “Gal-1 stromal staining serves as an independent prognostic factor for overall survival” has already been obtained in a previous study (Kim et al. High galectin-1 expression correlates with poor prognosis and is involved in epithelial ovarian cancer proliferation and invasion. *Eur J Cancer*. 2012 Aug;48(12):1914-21). This fact should be indicated, and the previous study must be appropriately cited.
 - The statement that “it is apparently nuclear and not cytoplasmic Gal-3 expression that has a major influence on patients’ outcomes” in lines 260-261 of the Discussion is premature and does not correspond to the facts. Other studies (17, 27) obtained the opposite results both in terms of Gal-3 localization and cancer prognosis, as they showed that cytoplasmic Gal-3 had a negative correlation with cancer prognosis. This statement should be deleted, as you do not present enough evidence for total dismissal of the previous findings. Instead, the reason for this discrepancy between the present and earlier studies should be discussed.
 - Correlations between expression patterns of Gal-1, -3, and -7 should be interpreted in view of your own findings regarding the distinct influence of these galectins on survival of ovarian cancer patients, i.e., the fact that nuclear Gal-3 indicates good prognosis and cytoplasmic Gal-1 and -7 indicate poor prognosis, whereas their expression showed positive correlation. Given these data, the statement that “This observation ... suggests that galectins might also share common functions in ovarian cancer molecular biology” (lines 286-288 in the Discussion) is not supported by the results, as high expression of Gal-3 inhibits cancer progression, while that of Gal-1 and -7 promotes it, indicating that their functions are far from common in ovarian cancer according to your data.
 - The Discussion should serve to emphasize the contribution of the study to the understanding of the prognostic potential of galectins in ovarian cancer. This is currently not the case; you should add a few lines to highlight this.
- **Minor issues – Likely to cause delays in journal acceptance.**
 - The Introduction does not provide a sufficient background for the problem studied. Mechanisms underlying the oncogenic effects of galectins should be outlined in view of their localization. Distinct functional activities of galectins in intracellular compartments should also be presented and appropriate references must be cited.
 - The information not relevant to the study, such as Gal oligomerization or the number of CRD domains (lines 40-45 in the Introduction), should be removed.
 - The Results section is not appropriately organized, and the presentation of data does not correspond to the data structure in the illustrations. As a rule, the data shown in a single illustration should be described in the same paragraph. However, the Results are structured according to individual galectins, and certain illustrations present the data related to all studied galectins (Fig. 1 – IF results; Fig. 2 – Survival; Table 2 - Multivariate analysis of prognostic factors), which complicates comparative analysis of the data and decreases the coherence and readability of the text.
 - **Does the paper present novel ideas/a novel direction with regard to the field of research?**
 - In clearly stating the gaps in the existing literature on the topic of your study and presenting the rationale for your study, you have established the novelty of the study in the Introduction section. However, the novelty of the study should also be discussed in the abstract. It should also be highlighted in the Discussion by stating how the study furthered understanding of the prognostic value of the investigated galectins in ovarian cancer.

- **Is the research rationale sound? (Is the reason for conducting the research explained clearly in the paper?)**
 - The study rationale is discussed to some extent. The rationale for studying the effect of galectins on survival in ovarian cancer depending on their cellular localization should be elaborated. Moreover, the second aim stated for the study is to investigate whether the expression levels of different galectins are correlated in ovarian cancer because “there is a critical need for a comprehensive study of various galectins in a representative ovarian cancer panel.” The purpose of this analysis is unclear, as you have not evaluated the correlation between combinations of different galectins and the outcome (survival).

- **Does the paper present novel ideas or build on the research published in the target journal? (Has the target journal previously published similar papers?)**
 - The paper presents novel ideas regarding the importance of galectin-1, -3, and -7 expression patterns in predicting overall survival in ovarian cancer.

Assessment by Paper Section

TITLE AND ABSTRACT

Are the Title and Abstract representative of the study? How can they be made more compelling?

Overall, the title and abstract give the readers a good idea of the paper. The novelty of the study should also be discussed in the abstract.

Can a wide readership understand the Title and Abstract independent of the main text? How can they be made more accessible to readers across disciplines?

The title and abstract are easy to follow for the readership of *Scientific Reports*. Once the abstract further highlights the novelty for the study, the content will be more accessible to a wider audience.

INTRODUCTION

Which other papers can the author cite to strengthen the review of literature?

The literature review is not complete. The biological functions of galectins related to tumorigenesis, including malignant transformation, invasion, and metastasis, are not described, and it is unclear how galectins are involved in all these processes. As the study specifically focused on the correlation of galectin expression in different cellular compartments (extracellular, cytoplasmic, and nuclear) with ovarian cancer, you should include an outline of localization-dependent functional activity of galectins. Thus, it should be indicated that extracellular galectins mediate cell–cell and cell–ECM contacts via binding to mucins, including cancer antigen 125, which promotes tumor cell adhesion, migration, and invasion. Through interaction with glycosylated cell surface receptors, galectins induce the expression of oncogenes, thereby promoting cell proliferation. In contrast, intracellular galectins regulate signaling pathways and gene transcription by interacting with cytoplasmic and nuclear proteins. Consider citing the following papers along with presenting this information:

- Funasaka et al. Nuclear transport of galectin-3 and its therapeutic implications. *Semin Cancer Biol.* 2014 Aug; 0: 30–38.
- Bhat et al. Nuclear repartitioning of galectin-1 by an extracellular glycan switch regulates mammary morphogenesis. *Proc Natl Acad Sci U S A.* 2016 Aug 16; 113(33): E4820–E4827.
- Patterson et al. Understanding the biochemical activities of galectin-1 and galectin-3 in the nucleus. *Glycoconj J.* 2002; 19(7-9): 499–506.

Are the study objectives clearly stated and do they align with the methods and results?

The study objective is stated and aligns with the methods and results.

METHODS

Is the research design appropriate? What are the gaps, and what should be done to fill the gaps?

Overall, the study design is appropriate. There are two major potential issues with the design as currently reported. First, the number of patients does not seem to be justified by a power analysis, and it is unclear

whether the sample size was sufficient to achieve statistical significance. A power analysis should be performed, and whether it confirmed that the results of the study were statistically conclusive should be indicated. Second, there were no control samples, i.e., samples from cancer-free individuals. This also needs to be addressed in the manuscript. Finally, the correlation between different combinations of galectins and survival should be analyzed.

Is the research methodology sound and relevant to the field? Are the methods detailed enough to be reproduced by a skilled researcher?

Overall, the methodology applied in the study is adequate to answer the research question. The two points mentioned above should be addressed to ensure that the reporting of the methodology is without errors. The histological groups (serous, endometrioid, clear cell, mucinous) should also be briefly characterized, especially with regard to their comparative malignancy.

Has the manuscript been prepared in accordance with the EQUATOR Network's research reporting guidelines? What are the gaps, and what should be done to fill the gaps?

The manuscript, in its current form, does not adhere to the EQUATOR Network's research reporting guidelines. The gaps have been highlighted in response to previous questions. Addressing these gaps will ensure adherence to the reporting guidelines.

RESULTS AND DISCUSSION

Does the data appear accurate, and has it been interpreted appropriately? Flag cases of insufficient or insignificant data with the author.

The purpose of performing the analysis presented in Table 5 is unclear, as correlations between galectin expression patterns and their significance in ovarian cancer are not interpreted. Nuclear Gal-3 correlated with cytoplasmic Gal-1 and -7; however, nuclear Gal-3 indicated good prognosis, whereas cytoplasmic Gal-1 and -7 indicated poor prognosis. Please explain this contradiction. Did any of the galectin combinations presented in Table 5 correlate with patient survival? What were such combinations in normal control samples? These issues must be addressed.

Should the author get their data verified by a statistician or submit analyzed datasets to the journal?

Further data verification and submission are not required. However, the journal does require a Data Availability Statement to be included in the Methods section of submitted manuscripts, and this should be added.

Are the research implications clearly mentioned? If they are mentioned, are they sound? If they are not mentioned, what tips should the author follow?

The implications of the results in the context of ovarian cancer are appropriately described in the Discussion section. However, as Scientific Reports caters to a broad audience, I recommend including any additional implications of your findings for other fields of research. For example, it appears that galectins have been implicated in a broad range of pathological conditions such as inflammation and fibrosis. A

brief mention of these in the Discussion or Conclusions sections would, therefore, improve the multidisciplinary appeal of your manuscript.

Are the concluding statements clear, and do they mention the contributions, limitations, and next steps for other researchers in the field?

A clear Conclusion section is provided. However, it can better emphasize the contribution of this study to the prognostic potential of galectins in ovarian cancer. Moreover, the limitations of the study need to be listed at the end of the Discussion before the scope for further research in the field is discussed.

SENIOR SCIENCE EDITOR'S AND MANAGING EDITOR'S COMMENTS ON LANGUAGE, PAPER STRUCTURE, AND FORMATTING

How was the paper's overall language quality prior to editing?

The language of the manuscript needed several improvements to make it submission-ready. Changes were made to correct grammatical errors related to article use, to improve word choice and sentence construction, and to ensure the use of formal language.

What were the top 3 recurring grammar and language issues found and edited for native tone?

1. Article use: Definite and indefinite articles were added wherever missing in the file. Editorial changes made in this regard are presented in boldface in the following examples.
 - According to our data, Gal-1 staining in **the** cytoplasm and stroma... (Body parts are one-of-a-kind entities and their names are preceded by the definite article)
 - Recently, **the** carbohydrate stem cell marker TF1 has been proposed as **a** negative prognostic marker in ovarian cancer displaying wild-type p53... (Specific, countable nouns take the definite article and non-specific, countable nouns take the indefinite article)
2. Word choice: "also" at the start of a sentence was replaced with the more formal alternative "In addition." In a few instances, terms were replaced with more appropriate alternatives based on the context. For example, "reduced outcomes" was changed to "poorer outcomes."
3. Sentence construction: The intended meaning did not come through very clearly in some sentences. For example, "However, it requires further investigations to explain cases without Gal-1 expression in cancer cells but in the stroma or vice versa" was changed to "However, further investigations are required to explain cases of Gal-1 expression in the stroma but not in cancer cells, and vice versa."

Does the edited paper adhere to the target journal's language preference?

The manuscript has been edited according to the target journal's language preference. The journal also asks authors to avoid the use of technical jargon without it being explained. The manuscript meets this requirement.

What types of changes were made for improvements to paper flow and how has the paper's readability improved because of these?

- Abstract: In the abstract, the conclusion, which was originally missing, was presented.
- Introduction: The context for the study was set, and the research objective and significance of the study were stated.
- Methods: The number of patients was not justified by power analysis, and it is unclear whether the sample size was sufficient to achieve statistical significance. There appear to be no control samples, i.e., those from cancer-free individuals. You need to address these two points prior to submission.
- Results: The results section requires greater re-organization, because the presentation of the data does not correspond to their placement in your illustrations. I have elaborated on this in my assessment of your manuscript.

- Discussion: The discussion section should better emphasize the contribution of this study to the prognostic potential of galectins in ovarian cancer. This is something that is lacking in the text. A good Discussion section usually begins with a brief summary of the aims or overall results of the study. I have added a sentence describing this.
- Conclusion: This section could be further enhanced by the addition of a discussion of any limitations of the study, as well as the broader implications of your study. As Scientific Reports caters to a broad audience, are there other fields of research that may be impacted by your study?

What details or documents are missing in the paper submission package based on the target journal's formatting and submission requirements?

The journal requires a separate cover letter to be provided with the submission, and one has been created for you. In the manuscript itself, please remember to add the author information on the title page and the reference list. A Data Availability Statement must be included at the end of the main text, before the References.

List out the journal's author preferences and formatting instructions (including the right file formats) that could not be followed and why.

All author preferences and instructions have been followed.

Does the target journal have a word count limit, and does the paper adhere to this limit after editing?

The title is within the 20-word limit. The target journal requires the abstract to be within 200 words; the edited abstract adheres to this limit. Finally, the main text is required to be no more than 4,500 words (not including Abstract, Methods, References and figure legends). This limit has also been met.

Does the paper need to be split for submission?

Typically, the journal requires individual figure files. However, for first submissions (i.e. not revised manuscripts), you may incorporate the manuscript text and figures into a single file up to 3 MB in size in either a Microsoft Word, LaTeX, or PDF format. Since your manuscript is a first submission, it does not need to be split.

Does the paper need to be blinded for review, and has it been blinded?

The paper does not need to be blinded for review.

Are all the references present and in the right format?

The references have not been provided for editing. Please ensure that a reference list is provided and formatted per the requirements of the target journal. These are the following formats that you should be aware of for different types of references:

Published papers

Printed journals

- Schott, D. H., Collins, R. N. & Bretscher, A. Secretory vesicle transport velocity in living cells depends on the myosin V lever arm length. *J. Cell Biol.* **156**, 35-39 (2002).

Online only

- Bellin, D. L. et al. Electrochemical camera chip for simultaneous imaging of multiple metabolites in biofilms. *Nat. Commun.* **7**, 10535; 10.1038/ncomms10535 (2016).

For papers with more than five authors include only the first author's name followed by 'et al.'

Books

- Smith, J. Syntax of referencing in *How to reference books* (ed. Smith, S.) 180-181 (Macmillan, 2013).

Online material

- Manaster, J. Sloth squeak. *Scientific American Blog Network*
<http://blogs.scientificamerican.com/psi-vid/2014/04/09/sloth-squeak> (2014).
- Hao, Z., AghaKouchak, A., Nakhjiri, N. & Farahmand, A. Global integrated drought monitoring and prediction system (GIDMaPS) data sets. *figshare*
<http://dx.doi.org/10.6084/m9.figshare.853801> (2014).

JOURNAL EDITORIAL DESK CHECKS

The submission readiness of your manuscript has been evaluated by performing checks that are typically done on manuscripts by the journal's Editorial Desk. We have included comments to explain the rationale behind the rating for each parameter and included suggestions for revisions to address the identified focus areas. We hope you find this feedback useful!

Article Checks

PARAMETER	DESCRIPTION	RATING
Scope Match	Does the scope of the research presented in the manuscript match the scope of the target journal?	EXCELLENT
<p><i>Notes: Scientific Reports caters to a broad scientific audience and welcomes research from all areas across the natural and clinical sciences. Your paper will definitely fit this broad scope.</i></p>		
Article Type	Does the article type selected align with the structure presented in the manuscript?	EXCELLENT
<p><i>Notes: Scientific Reports publishes original research in the following format: Article. Your manuscript has been correctly structured in accordance with an Article.</i></p>		
Data & Methods	Has data collection been described in the Methods section (according to the article type) and presented appropriately via tables and figures in the Results section?	GOOD
<p><i>Notes: Data collection has been described clearly for the most part. The following major changes are required to the figures and tables.</i></p> <ul style="list-style-type: none"> ○ <i>In Fig. 1, nuclear and cytoplasmic staining for Gal-3 and -7 is not clearly visible and should be indicated by arrows or asterisks.</i> ○ <i>In Table 1, the last line (≤ 60) should be changed to > 60.</i> <p><i>It is unclear what statistical significance (p-value) is related to in Histology (Tables 1, 3, and 4). There are four histological tumor types in these tables and three levels of expression (negative, low, and high) in Table 4, but only one p-value is shown, and it is unclear which groups were compared. It should be clearly indicated in the column (p versus ...) or in a footnote to each table.</i></p>		
References	Is the cited literature relevant, recent, and sufficient?	Choose an item.
<p><i>Notes: No reference list was included in the edit.</i></p>		

Desk Checks

PARAMETER	DESCRIPTION	RATING
Plagiarism Check	Is the Similarity Index within acceptable limits per standard journal requirements (<15%)?	EXCELLENT
<p><i>Notes: Please refer to the attached iThenticate report for details on overlapping text.</i> EXCELLENT: SI<15% AND single-source overlap<5%</p>		

PARAMETER	DESCRIPTION	RATING
	<p>➤ <i>SI within acceptable limits for standard journals</i></p> <p>Next steps and tips to reduce the SI</p> <p>➤ <i>As the manuscript's SI is within acceptable limits for standard journals, no major revisions are needed. However, here are some tips you may find useful to reduce the overlap in portions with relatively large matches.</i></p> <p>➤ <i>The findings of previous studies in the Discussion section should be paraphrased.</i></p>	
Ethical Compliance	Have all necessary consents and approvals have been obtained from authors to publish their work (including IRB approval and informed consent, as needed)	EXCELLENT
<i>Notes: All necessary ethics statements have been provided.</i>		
Data Availability Statement	Does the Data Availability Statement accurately describe the data and its presentation in the manuscript?	POOR
<i>Notes: A data availability statement has not been provided. As per journal guidelines, you must include a Data Availability Statement in all submitted manuscripts (at the end of the main text, before the References section). Data availability statements should include, where applicable, accession codes, other unique identifiers and associated web links for publicly available datasets, and any conditions for access of non-publicly available datasets. Where figure source data are provided, statements confirming this should be included in data availability statements. Please refer to https://www.nature.com/srep/journal-policies/editorial-policies#availability for examples of such statements.</i>		
Funding Information	Has funding information been provided, when needed, or a statement been made about it not being needed?	EXCELLENT
<i>Notes: Financial declarations have been provided.</i>		

Submission Checks

	PARAMETER	GAPS ANALYSIS	EXAMPLE/ISSUE
1.	List of Contributing Authors	Absent	Please provide the full list of contributing authors in the title page. Guidance on authorship here
2.	Author Contributions Statement	Present	A section on author contributions has been provided
3.	Corresponding Author Email	Absent	Please provide the corresponding author information in the title page. Guidance on the role of the corresponding author here
4.	Conflict of Interest Statement	Present	A conflict of interest statement has been provided
5.	Figure and Table Citation	Present	The citation of figures and tables is adequate

	PARAMETER	GAPS ANALYSIS	EXAMPLE/ISSUE
6.	List of Keywords	NA	The journal guidelines do not ask for keywords
7.	Data Access Statement This describes where the data associated with the paper is available, and under what conditions the data can be accessed.	Absent	Please provide a data access statement at the end of the manuscript Guidance on how to write a Data Access Statement here
8.	Figure Legends	Included and appear consecutively	Included and adequate.
9.	Table Legends	Included and appear consecutively	Included and adequate.

Journal Editorial Desk Checks: Assessment and Recommendations

Our assessment is based on the manuscript submission requirements for Scientific Reports.

ASSESSMENT

This manuscript requires several changes before it is ready to submit.

RECOMMENDATIONS

Major issues likely to be raised by the journal's Editorial Desk and lead to rejection include

1. The corresponding author has not been identified.
2. The Data Availability Statement is missing.
3. The list of contributing authors is missing.

Acknowledging Editage's support

Several authors choose to acknowledge Editage's support in their paper. According to prominent publication guidelines such as the ICMJE guidelines on authorship, editing or writing support should be acknowledged in the paper.

If you would like to acknowledge our support for this paper, you can do so by including the following sentence in the Acknowledgments section of your paper:

We would like to acknowledge Editage's (<http://www.editage.cn>) support in manuscript preparation.

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