June 23rd, 2017

Dear Drs. Garcia-Olmo, Strom, and Tarnawski,

We would like to submit the revisions for our manuscript entitled, "High Yield Reproducible Rat Model Recapitulating Human Barrett's Carcinogenesis," for publication consideration by *World Journal of Gastroenterology*.

We would like to kindly thank the reviewers for their thoughtful assessment of our submitted manuscript. In addition to the reviewers' comments, we have fully addressed all of the tracked comments and suggestions embedded within the manuscript file and subsequently updated our revised version accordingly. Additionally, all authors have approved the resulting changes.

Thank you for your kind consideration.

Sincerely,

Blair A. Jobe, MD, FACS
Director, Esophageal and Lung Institute
Allegheny Health Network

Responses to Comments to Authors:

Reviewer #1:
Reviewer's code: 03529755

COMMENTS TO AUTHORS
Thanks for giving opportunity to me to review this article. I would like to congratulate all the authors for this study. I would like to accept this properly written article as it is.

RESPONSE TO REVIEWER
We would like to thank the reviewer for their consideration and kind comments regarding our manuscript.

Reviewer #2:
Reviewer's code: 03699916

COMMENTS TO AUTHORS
Ms: ESPS Manuscript NO: 34613 Authors: Daisuke Matsui, Ashten N Omstead, Juliann E Kosovec, Yoshihiro Komatsu, Emily J. Lloyd, Hailey Raphael, Ronan J Kelly, Ali H Zaidi, Blair A Jobe Title: High Yield Reproducible Rat Model Recapitulating Human Barrett's Carcinogenesis

GENERAL COMMENTS: This is a well-designed basic study. Authors in this study generated modified End-to-side esophagojejunostomy (EJ) rat model. Using this model, authors determine respective rates of carcinogenic development and gene expression levels of MUC2, CK19, and CK20. In order to better understand the underlying biology and prevent and treat esophageal adenocarcinoma (EAC), the modified EJ model generated in the present study and the data obtained are important for understand disease progression spectrum from Barrett's esophagus to metastatic EAC. Therefore, the manuscript is good for the readership of WJG.

SPECIFIC COMMENTS:
Title and sub-title: The title and sub-title accurately reflect the major topic and contents of the study.
Abstract: The description of aim, material and methods, result and conclusion sections is fine. However, the number of animals in each time point should be indicated in the method section.
Introduction: It is well written.

Material and methods: Generally, this section is well written. Again, the number of animals in each time point should be indicated. I notice from figure 1 that the number of animals is different among different groups, why? Furthermore, the extent of each disease type in 200uM of tissue may differ with the marked H&E slide. How the authors can be sure that the enough lesion exists in the 200uM of tissue. Inhomogeneous lesions may affect the results of gene expression analysis

Results: This section generally reflects the results obtained from the study clearly. Three comments are as following:

1, About the effective numbers of rats examined for study endpoints, is it true about non-operated (n=0)?

2, About the EAC, did you find any evidence grossly about EAC at any time points post-surgery?

3, In two animals with macro-metastases, did authors also find the macro-lesion of EAC in the surface of esophageal mucosa? It will be interesting to show the photos for such macro-metastases in the paper.

Discussion: The discussion is well written. However, it should be good to provide some information about the overall incidence of EAC from other similar studies.

References: The references are appropriate, relevant, and updated.

Tables and figures: Figures are appropriately presented.

RESPONSE TO REVIEWER

Thank you to the reviewer for their thorough consideration of our submitted manuscript. The following modifications have been made in accordance with your suggestions:

Abstract

1. *The number of animals at each time point has been included in the methods section of the abstract.*

Materials and Methods

1. *The number of animals at each time point has been included in the materials and methods section of the manuscript. The number of animals differed among different groups due to health considerations that resulted in some animals being euthanized before their assigned time points. This clarification has been added to the methodology as "The higher effective n at 17 and 24 weeks is reflective of animals that were prematurely sacrificed from the original designated time point due to health considerations."*
2. *The reviewer raises a valid limitation regarding the homogeneity of lesions obtained through macrodissection, as described in the highlighted discussion text. Special care was taken to mark the specific areas of the highest disease level to maximize homogeneity, and*

this has been clarified in the methodology section as "and special care was taken to ensure all collections were highly representative of the disease states."

Results

1. *The number of non-operated animals was modified to correctly reflect the true size of the cohort (n=6).*
2. *We were able to observe the gross tumors on necropsy before histological confirmation. Images of the visible lesions are available in our previously published works, including the following:*
    a. *Kosovec JE, Zaidi AH, Komatsu Y, Kasi PM, Cothron K, Thompson DV, Lynch E, Jobe BA. Establishing magnetic resonance imaging as an accurate and reliable tool to diagnose and monitor esophageal cancer in a rat model. PLoS One. 2014;9(4):e93694. [PMID: 24705451 DOI: 10.1371/journal.pone.0093694]*
3. *In the animals with macro-metastases, we were able to identify the gross macro-lesion of EAC in the esophagus. We appreciate the authors suggestion to include such figures, and we have added a new Figure 4, accordingly. Additionally, we have previously validated the true metastatic nature of such lesions in our previous work.*

Discussion

1. *Additional information about EAC incidence in similar studies has been added in response to the reviewer's suggestion as, "The overall incidence of EAC reported in this study was higher compared to other previously reported rates of only 17.4% at 30 weeks and 74% at 40 weeks for the rat surgical reflux model."*

Reviewer #3
Reviewer's code: 00036825

COMMENTS TO AUTHORS
the surgical model do not meet the human pathology. In the model described the acid reflux is missing.The content of reflux remains unknown, the analysis is missing.Evaluation of pathophysiological processes provoking EAC are also missing. The terminology of dysplasia is confusing: " dysplastic squamous cell epithalium " is applied, while in BE the field of dysplasia is the glandular/intestinal type metaplasia.

RESPONSE TO REVIEWER
Thank you to the reviewer for their comments. As presented in the Introduction of the submitted manuscript, the modified Levrat model of esophagojejunostomy in a rat model is a well-established and validated replicative translational model of human pathology leading to

esophageal adenocarcinoma through cross-species conserved mechanisms. Analysis of the reflux contents were beyond the novelty and scope of this study, as this validation has been previously well-documented in the following studies referenced in this manuscript:

*13. **Fein M**, Peters JH, Chandrasoma P, Ireland AP, Oberg S, Ritter MP, Bremner CG, Hagen JA, DeMeester TR. Duodenoesophageal reflux induces esophageal adenocarcinoma without exogenous carcinogen. J Gastrointest Surg. 1998;2(3):260-8. [PMID: 9841983 DOI]*

*14. **Chen X**, Yang G, Ding WY, Bondoc F, Curtis SK, Yang CS. An esophagogastroduodenal anastomosis model for esophageal adenocarcinogenesis in rats and enhancement by iron overload. Carcinogenesis. 1999;20(9):1801-8. [PMID: 10469627 DOI]*

*15. **Pera M**, Trastek VF, Carpenter HA, Fernandez PL, Cardesa A, Mohr U, Pairolero PC. Influence of pancreatic and biliary reflux on the development of esophageal carcinoma. Ann Thorac Surg. 1993;55(6):1386-92; discussion 92-3. [PMID: 8512386 DOI]*

*16. **Clark GW**, Smyrk TC, Mirvish SS, Anselmino M, Yamashita Y, Hinder RA, DeMeester TR, Birt DF. Effect of gastroduodenal juice and dietary fat on the development of Barrett's esophagus and esophageal neoplasia: an experimental rat model. Annals of surgical oncology. 1994;1(3):252-61. [PMID: 7842295 DOI]*

*17. **Li H**, Walsh TN, O'Dowd G, Gillen P, Byrne PJ, Hennessy TP. Mechanisms of columnar metaplasia and squamous regeneration in experimental Barrett's esophagus. Surgery. 1994;115(2):176-81. [PMID: 8310406 DOI]*

*18. **Miyashita T**, Tajima H, Munemoto M, Shah FA, Harmon JW, Watanabe T, Shoji M, Okamoto K, Nakanuma S, Sakai S, Kinoshita J, Makino I, Nakamura K, Hayashi H, Oyama K, Inokuchi M, Nakagawara H, Takamura H, Ninomiya I, Kitagawa H, Fushida S, Mukaisho K, Fujimura T, Ohta T. Impact of histone deacetylase 1 and metastasis-associated gene 1 expression in esophageal carcinogenesis. Oncol Lett. 2014;8(2):758-64. [PMID: 25009653 DOI: 10.3892/ol.2014.2176]*

*19. **Cheng P**, Gong J, Wang T, Chen J, Liu GS, Zhang R. Gene expression in rats with Barrett's esophagus and esophageal adenocarcinoma induced by gastroduodenoesophageal reflux. World J Gastroenterol. 2005;11(33):5117-22. [PMID: 16127739 DOI]*

The novelty of this particular manuscript was to further refine and increase the efficiency of the previously-validated surgical model through improved surgical technique, and to further authenticate the model through evaluation of newly identified conserved EAC disease progression markers, such as mucin and cytokeratins. Finally, the histological evaluation was performed by multiple pathological experts according to standard criteria for the esophageal lesions as previously described in the following referenced studies:

*9. **Su Y**, Chen X, Klein M, Fang M, Wang S, Yang CS, Goyal RK. Phenotype of columnar-lined esophagus in rats with esophagogastroduodenal anastomosis: similarity to human Barrett's esophagus. Lab Invest. 2004;84(6):753-65. [PMID: 15094711 DOI: 10.1038/labinvest.3700079]*

*17. **Li H**, Walsh TN, O'Dowd G, Gillen P, Byrne PJ, Hennessy TP. Mechanisms of columnar metaplasia and squamous regeneration in experimental Barrett's esophagus. Surgery. 1994;115(2):176-81. [PMID: 8310406 DOI]*

*25. **Goldstein SR**, Yang GY, Curtis SK, Reuhl KR, Liu BC, Mirvish SS, Newmark HL, Yang CS. Development of esophageal metaplasia and adenocarcinoma in a rat surgical model without the use of a carcinogen. Carcinogenesis. 1997;18(11):2265-70. [PMID: 9395230 DOI]*

*27. **Buskens CJ**, Hulscher JB, van Gulik TM, Ten Kate FJ, van Lanschot JJ. Histopathologic evaluation of an animal model for Barrett's esophagus and adenocarcinoma of the distal esophagus. J Surg Res. 2006;135(2):337-44. [PMID: 16926029 DOI: 10.1016/j.jss.2006.04.023]*