



Life Science Alliance

# Snail augments fatty acid oxidation by suppression of mitochondrial ACC2 during cancer progression

Jong Yook, Ji Hye Yang, Nam Kim, Jun Seop Yun, Eunae Cho, Yong Cha, Sue Bean Cho, Seon-Hyeong Lee, So Cha, Soo-Youl Kim, Jiwon Choi, Tin-Tin Manh Nguyen, Sunghyok Park, and Hyun Kim

**DOI:** <https://doi.org/10.26508/lisa.202000683>

*Corresponding author(s): Jong Yook, Yonsei University College of Dentistry and Hyun Kim, College of Dentistry Yonsei University*

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## Review Timeline:

Submission Date:	2020-02-19
Editorial Decision:	2020-04-28
Revision Received:	2020-05-07
Editorial Decision:	2020-05-18
Revision Received:	2020-05-20
Accepted:	2020-05-22

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## Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

April 28, 2020

Re: Life Science Alliance manuscript #LSA-2020-00683-T

Prof. Jong In Yook  
Yonsei University College of Dentistry  
Department of Oral Pathology  
50-1 Yonsei-ro, Seodaemoon-gu  
Seoul 120-752  
Korea, Republic of

Dear Dr. Yook,

Thank you for submitting your manuscript entitled "Snail augments fatty acid oxidation by suppression of mitochondrial ACC2 during cancer progression" to Life Science Alliance. Please excuse the delay in getting back to you. It was very difficult to secure reviewers on your work and we also had to give them more time. In the end, only one reviewer submitted a report, which is mostly positive, while another reviewer refused to provide a full report given the unbalanced way you put your work into the context of the existing literature. You will find the reviewer report below and I also copy the constructive comments of the other reviewer at the end of this message (advisor comments).

Based on the input received, we decided that we can invite you to submit a revised version of your work to us. We would expect that you fully address the reviewer's comments. Furthermore, we'd expect that you include a much more complete and balanced discussion of the role of Snail in metabolism - including citing and discussing key papers that show that Snail increases glycolysis, and inclusion of either experimental data addressing the controversial findings or clearly discussing the discrepancies and the need to resolve them.

To upload the revised version of your manuscript, please log in to your account:

<https://lsa.msubmit.net/cgi-bin/main.plex>

You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

We would be happy to discuss the individual revision points further with you should this be helpful.

While you are revising your manuscript, please also attend to the below editorial points to help expedite the publication of your manuscript. Please direct any editorial questions to the journal office.

The typical timeframe for revisions is three months. We are aware that many laboratories cannot function fully during the current COVID-19/SARS-CoV-2 pandemic and therefore encourage you to take the time necessary to revise the manuscript to the extent requested above. We will extend our 'scooping protection policy' to the full revision period required. Please note that papers are generally considered through only one revision cycle, so strong support from the referee on the revised version is needed for acceptance.

When submitting the revision, please include a letter addressing the reviewers' comments point by point.

We hope that the comments below will prove constructive as your work progresses.

Thank you for this interesting contribution to Life Science Alliance. We are looking forward to receiving your revised manuscript.

Sincerely,

Andrea Leibfried, PhD  
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#### A. THESE ITEMS ARE REQUIRED FOR REVISIONS

-- A letter addressing the reviewers' comments point by point.

-- An editable version of the final text (.DOC or .DOCX) is needed for copyediting (no PDFs).

-- High-resolution figure, supplementary figure and video files uploaded as individual files: See our detailed guidelines for preparing your production-ready images, <http://www.life-science-alliance.org/authors>

-- Summary blurb (enter in submission system): A short text summarizing in a single sentence the study (max. 200 characters including spaces). This text is used in conjunction with the titles of papers, hence should be informative and complementary to the title and running title. It should describe the context and significance of the findings for a general readership; it should be written in the present tense and refer to the work in the third person. Author names should not be mentioned.

#### B. MANUSCRIPT ORGANIZATION AND FORMATTING:

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We encourage our authors to provide original source data, particularly uncropped/-processed electrophoretic blots and spreadsheets for the main figures of the manuscript. If you would like to add source data, we would welcome one PDF/Excel-file per figure for this information. These files will be linked online as supplementary "Source Data" files.

**\*\*\*IMPORTANT:** It is Life Science Alliance policy that if requested, original data images must be made available. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original microscopy and blot data images

before submitting your revision.\*\*\*

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Reviewer #2 (Comments to the Authors (Required)):

### Summary

This paper provides mechanistic evidence that the transcription factor Snail, previously implicated in the epithelial to mesenchymal transition, enhances fatty acid oxidation (FAO) to promote cancer cell survival in glucose-starved conditions. Further studies suggest Snail expression leads to enhanced fatty acid transporter activity. This builds on the team's recent paper, Kim et al., 2017 Nat Comm, which focuses on Snail's ability to re-programme glucose metabolism by suppressing PKFP to facilitate cancer cell survival.

### Main points and supporting data

The paper has a logical and thoughtful flow. Its main points seem well supported by a combination of pharmacological and genetic knockdown studies in vitro, analysis with xenograft models in vivo and some limited cancer patient data. The study focusses on breast cancer, except for some patient data that do not directly link to Snail; this should be made clearer in the Abstract, Discussion, etc. Overall, mechanistic insights are supported by rescue experiments, eg use of the CPT1 inhibitor ETX blocks palmitate-induced ATP recovery and rescue of Snail knockdown-mediated reprogramming by inducible ACC2 knockdown. I do not have expertise in fatty acid oxidation metabolism, but the general conclusions appear novel and interesting. There is a potential mechanism proposed for Snail's effect on the ACC2 promoter, but the authors do not flag this in the Abstract, which might have been useful.

It would have been preferable to see the western data performed in triplicate, so that statistical differences could be shown, but this may be difficult in the current difficult circumstances.

### Additional issues that should be addressed

For the most part, the paper is clearly written. I have, however, identified a few places where the text needs changing and there are others, so the text should be carefully proof-read and edited prior to resubmission.

When first mentioned (page 7) the authors should explain what palmitate does.

Page 3. Highlight, and elsewhere: 'combinatorial' instead of 'combinational'.

Page 4, line 2 from top: replace 'had' with 'has'

Page 5, line 6 from bottom: replace 'cancer stemness' with 'cancer cell stemness'

Page 6, bottom line: specify what pharmacological strategy is for

Page 7, line 10 from top; Page 9, line 11 from top: rephrase 'Snail repressor'

Page 7, line 6 from bottom: replace 'treated' with 'administered'

Page 11, line 8 from top: I think 'leading to cancer pro-survival' could be phrased better

Page 16, line 10 from top: replace 'level' with 'levels'

Page 16, line 14 from top: rephrase 'constitute fatty acid metabolism'

Figure 1A legend (Page 33) mentions an immunoblot analysis, which is missing from the figure

Figure 3D legend (Page 36) 'knockdowned' should be replaced by 'knocked down'

### Advisor comments:

- It is difficult to reconcile the data with previous knowledge on Snail and metabolism. The authors have already published that Snail inhibits glycolysis by repressing phosphofructokinase (PFK1), but several others have reported the opposite. Lu et al 2015 show that Snail increases glycolysis attenuating gluconeogenesis and Dong et al 2013 show that Snail represses fructose 1,6

biphosphatase (FBP1), therefore increasing glycolysis as well. - In this paper, the authors start by mentioning their previous data on glycolysis inhibition mediated by Snail and then in the discussion, they use Dong et al (inhibition of FBP1) to say that it is of special interest. They see that Snail enhances Fatty acid biosynthesis (FAO) they now propose that maybe this compensates for FBP1 Loss. Snail inhibiting PFK and FBP is very difficult to understand and they use one paper or another. - The authors use references in many instances all throughout the paper that do not fit with what is stated in the text.

**Response to Reviewers (LSA-2020-00683-T)**

**We appreciate the reviewers' constructive criticism and helpful comments on our manuscript. Our point-by-point responses to the reviewers are provided in bold below.**

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**Reviewer #2 (Comments to the Authors) :**

Summary

This paper provides mechanistic evidence that the transcription factor Snail, previously implicated in the epithelial to mesenchymal transition, enhances fatty acid oxidation (FAO) to promote cancer cell survival in glucose-starved conditions. Further studies suggest Snail expression leads to enhanced fatty acid transporter activity. This builds on the team's recent paper, Kim et al., 2017 Nat Comm, which focuses on Snail's ability to re-programme glucose metabolism by suppressing PKFP to facilitate cancer cell survival.

Main points and supporting data

The paper has a logical and thoughtful flow. Its main points seem well supported by a combination of pharmacological and genetic knockdown studies in vitro, analysis with xenograft models in vivo and some limited cancer patient data. The study focusses on breast cancer, except for some patient data that do not directly link to Snail; this should be made clearer in the Abstract, Discussion, etc. Overall, mechanistic insights are supported by rescue experiments, eg use of the CPT1 inhibitor ETX blocks palmitate-induced ATP recovery and rescue of Snail knockdown-mediated reprogramming by inducible ACC2 knockdown. I do not have expertise in fatty acid oxidation metabolism, but the general conclusions appear novel and interesting. There is a potential mechanism proposed for Snail's effect on the ACC2 promoter, but the authors do not flag this in the Abstract, which might have been useful.

It would have been preferable to see the western data preformed in triplicate, so that statistical differences could be shown, but this may be difficult in the current difficult circumstances.

**Authors' Response) We appreciate the reviewer's helpful comment on our manuscript. We have added the comments on breast cancer in the Abstract and Discussion, and on transcriptional repression in Abstract.**

Additional issues that should be addressed

For the most part, the paper is clearly written. I have, however, identified a few places where the text needs changing and there are others, so the text should be carefully proof-read and edited prior to resubmission.

When first mentioned (page 7) the authors should explain what palmitate does.

Page 3. Highlight, and elsewhere: 'combinatorial' instead of 'combinational'.

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Figure 1A legend (Page 33) mentions an immunoblot analysis, which is missing from the figure

Figure 3D legend (Page 36) 'knockdowned' should be replaced by 'knocked down'

**Authors' Response) We appreciate the reviewer's careful comments on our manuscript. We have corrected the mistakes in the revised manuscript following the reviewer's suggestions.**

**Advisor comments:**

It is difficult to reconcile the data with previous knowledge on Snail and metabolism. The authors have already published that Snail inhibits glycolysis by repressing phosphofruktokinase (PFK1), but several others have reported the opposite. Lu et al 2015 show that Snail increases glycolysis attenuating gluconeogenesis and Dong et al 2013 show that Snail represses fructose 1,6 biphosphatase (FBP1), therefore increasing glycolysis as well. In this paper, the authors start by mentioning their previous data on glycolysis inhibition mediated by Snail and then in the discussion, they use Dong et al (inhibition of FBP1) to say that it is of special interest. They see that Snail enhances Fatty acid biosynthesis (FAO) they now propose that maybe this compensates for FBP1 Loss. Snail inhibiting PFK and FBP is very difficult to understand and they use one paper or another.

**Authors' Response) We understand the reviewer's concern regarding Snail's role in glycolysis and gluconeogenesis. In this study, we focused on Snail's role in metabolic reprogramming, especially for the efficient supply of essential catabolic metabolites (ATP and NADPH) in a glucose-limited environment. Previously, we reported that inhibition of PFK-1 by Snail increased metabolic flux into the pentose phosphate pathway (PPP) for NADPH generation. Interestingly, suppression of FBP-1 followed by decreased endoergonic gluconeogenesis can contribute to activating nonoxidative PPP by bi-directional transketolase and transaldolase (Berg JM, Tymoczko JL, & Stryer L. Biochemistry, 7<sup>th</sup> ed. 2010, p606-p609). We have recently summarized those catabolic circuits, as referenced in this manuscript (Cho ES et al, Biomol Ther, 2018, 26, 29-38). The suppression of PFK-1 and FBP-1 may utilize Mode3 and Mode4 circuits, respectively (please see Fig. 2 in Cho ES et al). These suggest the possibility of several catabolic circuits to provide ATP and NADPH according to cancer subtypes or oncogenic activation. To avoid confusing readers, we have added detailed comments on metabolic outcomes by inhibition of PFK-1 or FBP-1 in the Discussion section.**

- The authors use references in many instances all throughout the paper that do not fit with what is stated in the text.

**Authors' Response) We have thoroughly and carefully reviewed the manuscript again. Please specify the reference if further correction is required.**

- END -

May 18, 2020

RE: Life Science Alliance Manuscript #LSA-2020-00683-TR

Prof. Jong In Yook  
Yonsei University College of Dentistry  
Department of Oral Pathology  
50-1 Yonsei-ro, Seodaemun-gu  
Seoul 03722  
Korea (South), Republic of

Dear Dr. Yook,

Thank you for submitting your revised manuscript entitled "Snail augments fatty acid oxidation by suppression of mitochondrial ACC2 during cancer progression". We have re-evaluated your work within our editorial team. While we think that the findings are still difficult to reconcile with the previous literature, we appreciate your response to this concern and would be happy to publish your paper in Life Science Alliance pending final revisions necessary to meet our formatting guidelines:

- LC-MS raw data should get included or deposited in a repository (eg. MetaboLights).
- Since data on the Broad GDAC firehose changes with time, it is important to include the raw data used in your analyses to allow others to reproduce your findings. Please do so by either providing the raw files as "data set" files or by depositing them in a repository and provide an accession code in the manuscript text.
- Please list in each figure legend the statistical test used and p-values (description is lacking in many places). It seems that those currently already described, are not necessarily adequate ones - please revise.
- All figures need to get uploaded as individual files, including the supplementary files; the legends (including supplementary figure legends) should remain in the main manuscript docx file; please use the same style for the legends in both main and suppl figure legends
- We can only publish figures that adhere to our guidelines, please revise accordingly (figure 1 spans two pages at the moment, figure quality not sufficient (blurry) in some instances)
- Please add a scale bar to Fig S1H and increase visibility of the scale bar in Fig 6E

If you are planning a press release on your work, please inform us immediately to allow informing our production team and scheduling a release date.

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You will be guided to complete the submission of your revised manuscript and to fill in all necessary information. Please get in touch in case you do not know or remember your login name.

To avoid unnecessary delays in the acceptance and publication of your paper, please read the following information carefully.

A. FINAL FILES:

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**\*\*Submission of a paper that does not conform to Life Science Alliance guidelines will delay the acceptance of your manuscript.\*\***

**\*\*It is Life Science Alliance policy that if requested, original data images must be made available to the editors. Failure to provide original images upon request will result in unavoidable delays in publication. Please ensure that you have access to all original data images prior to final submission.\*\***

**\*\*The license to publish form must be signed before your manuscript can be sent to production. A link to the electronic license to publish form will be sent to the corresponding author only. Please take a moment to check your funder requirements.\*\***

**\*\*Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.\*\***

Thank you for your attention to these final processing requirements. Please revise and format the manuscript and upload materials within 7 days.

Thank you for this interesting contribution, we look forward to publishing your paper in Life Science Alliance.

Sincerely,

Andrea Leibfried, PhD  
Executive Editor  
Life Science Alliance  
Meyerhofstr. 1  
69117 Heidelberg, Germany  
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[www.life-science-alliance.org](http://www.life-science-alliance.org)

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May 22, 2020

RE: Life Science Alliance Manuscript #LSA-2020-00683-TRR

Prof. Jong In Yook  
Yonsei University College of Dentistry  
Department of Oral Pathology  
50-1 Yonsei-ro, Seodaemun-gu  
Seoul 03722  
Korea (South), Republic of

Dear Dr. Yook,

Thank you for submitting your Research Article entitled "Snail augments fatty acid oxidation by suppression of mitochondrial ACC2 during cancer progression". I appreciate the introduced changes and it is a pleasure to let you know that your manuscript is now accepted for publication in Life Science Alliance. Congratulations on this interesting work.

The final published version of your manuscript will be deposited by us to PubMed Central upon online publication.

Your manuscript will now progress through copyediting and proofing. It is journal policy that authors provide original data upon request.

Reviews, decision letters, and point-by-point responses associated with peer-review at Life Science Alliance will be published online, alongside the manuscript. If you do want to opt out of having the reviewer reports and your point-by-point responses displayed, please let us know immediately.

**\*\*\*IMPORTANT:** If you will be unreachable at any time, please provide us with the email address of an alternate author. Failure to respond to routine queries may lead to unavoidable delays in publication.\*\*\*

Scheduling details will be available from our production department. You will receive proofs shortly before the publication date. Only essential corrections can be made at the proof stage so if there are any minor final changes you wish to make to the manuscript, please let the journal office know now.

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Authors are required to distribute freely any materials used in experiments published in Life Science Alliance. Authors are encouraged to deposit materials used in their studies to the appropriate repositories for distribution to researchers.

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Again, congratulations on a very nice paper. I hope you found the review process to be constructive and are pleased with how the manuscript was handled editorially. We look forward to future exciting submissions from your lab.

Sincerely,

Reilly Lorenz  
Editorial Office Life Science Alliance  
Meyerhofstr. 1  
69117 Heidelberg, Germany  
t +49 6221 8891 414  
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[www.life-science-alliance.org](http://www.life-science-alliance.org)