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Glutamate dehydrogenase1 supports HIF-1α stability to promote colorectal tumorigenesis under hypoxia

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(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. Depending on transfer agreements, referee reports obtained elsewhere may or may not be included in this compilation. Referee reports are anonymous unless the Referee chooses to sign their reports.)

1st Editorial Decision **21st Oct 2022**

Dear Dr Wang,

Thank you for submitting your manuscript for consideration by the EMBO Journal. It has now been seen by three referees whose comments are shown below.

Should you be able to address these criticisms in full (i.p. ref#2's pts4,5), we could consider a revised manuscript. I should remind you that it is EMBO Journal policy to allow a single round of revision only and that, therefore, acceptance or rejection of the manuscript will depend on the completeness of your responses in this revised version. I do realize that addressing all the referees' criticisms will require a lot of additional time and effort and be technically challenging. I would therefore understand if you wish to publish the manuscript rapidly and without any significant changes elsewhere, in which case please let us know so we can withdraw it from our system.

If you decide to thoroughly revise the manuscript for the EMBO Journal, please include a detailed point-by-point response to the referees' comments. Please bear in mind that this will form part of the Review Process File, and will therefore be available online to the community. For more details on our Transparent Editorial Process, please visit our website: https://www.embo.org/embo-press

We generally allow three months as standard revision time. As a matter of policy, competing manuscripts published during this period will not negatively impact on our assessment of the conceptual advance presented by your study. However, we request that you contact the editor as soon as possible upon publication of any related work, to discuss how to proceed. Should you foresee a problem in meeting this three-month deadline, please let us know in advance and we may be able to grant an extension.

Thank you for the opportunity to consider your work for publication. I look forward to your revision.

Yours sincerely,

Daniel Klimmeck

Daniel Klimmeck, PhD
Senior Editor
The EMBO Journal

Instruction for the preparation of your revised manuscript:

1) a .docx formatted version of the manuscript text (including legends for main figures, EV figures and tables). Please make sure that the changes are highlighted to be clearly visible.

2) individual production quality figure files as .eps, .tif, .jpg (one file per figure).

3) a .docx formatted letter INCLUDING the reviewers' reports and your detailed point-by-point response to their comments. As part of the EMBO Press transparent editorial process, the point-by-point response is part of the Review Process File (RPF), which will be published alongside your paper.

4) a complete author checklist, which you can download from our author guidelines (https://wol-prod-cdn.literatumonline.com/pb-assets/embo-site/Author Checklist%20-%20EMBO%20J-1561436015657.xlsx). Please insert information in the checklist that is also reflected in the manuscript. The completed author checklist will also be part of the RPF.

5) Please note that all corresponding authors are required to supply an ORCID ID for their name upon submission of a revised manuscript.

6) It is mandatory to include a 'Data Availability' section after the Materials and Methods. Before submitting your revision, primary datasets produced in this study need to be deposited in an appropriate public database, and the accession numbers and database listed under 'Data Availability'. Please remember to provide a reviewer password if the datasets are not yet public (see https://www.embopress.org/page/journal/14602075/authorguide#datadeposition).
In case you have no data that requires deposition in a public database, please state so in this section. Note that the Data Availability Section is restricted to new primary data that are part of this study.
*** Note - All links should resolve to a page where the data can be accessed. ***

7) Our journal encourages inclusion of *data citations in the reference list* to directly cite datasets that were re-used and

obtained from public databases. Data citations in the article text are distinct from normal bibliographical citations and should directly link to the database records from which the data can be accessed. In the main text, data citations are formatted as follows: "Data ref: Smith et al, 2001" or "Data ref: NCBI Sequence Read Archive PRJNA342805, 2017". In the Reference list, data citations must be labeled with "[DATASET]". A data reference must provide the database name, accession number/identifiers and a resolvable link to the landing page from which the data can be accessed at the end of the reference. Further instructions are available at .

8) At EMBO Press we ask authors to provide source data for the main and EV figures. Our source data coordinator will contact you to discuss which figure panels we would need source data for and will also provide you with helpful tips on how to upload and organize the files.

Numerical data can be provided as individual .xls or .csv files (including a tab describing the data). For 'blots' or microscopy, uncropped images should be submitted (using a zip archive or a single pdf per main figure if multiple images need to be supplied for one panel). Additional information on source data and instruction on how to label the files are available at .

9) We replaced Supplementary Information with Expanded View (EV) Figures and Tables that are collapsible/expandable online (see examples in https://www.embopress.org/doi/10.15252/embj.201695874). A maximum of 5 EV Figures can be typeset. EV Figures should be cited as 'Figure EV1, Figure EV2" etc. in the text and their respective legends should be included in the main text after the legends of regular figures.

- For the figures that you do NOT wish to display as Expanded View figures, they should be bundled together with their legends in a single PDF file called *Appendix*, which should start with a short Table of Content. Appendix figures should be referred to in the main text as: "Appendix Figure S1, Appendix Figure S2" etc. See detailed instructions regarding expanded view here: .

- Additional Tables/Datasets should be labelled and referred to as Table EV1, Dataset EV1, etc. Legends have to be provided in a separate tab in case of .xls files. Alternatively, the legend can be supplied as a separate text file (README) and zipped together with the Table/Dataset file.

10) When assembling figures, please refer to our figure preparation guideline in order to ensure proper formatting and readability in print as well as on screen:
http://bit.ly/EMBOPressFigurePreparationGuideline

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

11) For data quantification: please specify the name of the statistical test used to generate error bars and P values, the number (n) of independent experiments (specify technical or biological replicates) underlying each data point and the test used to calculate p-values in each figure legend. The figure legends should contain a basic description of n, P and the test applied. Graphs must include a description of the bars and the error bars (s.d., s.e.m.).

Please remember: Digital image enhancement is acceptable practice, as long as it accurately represents the original data and conforms to community standards. If a figure has been subjected to significant electronic manipulation, this must be noted in the figure legend or in the 'Materials and Methods' section. The editors reserve the right to request original versions of figures and the original images that were used to assemble the figure.

Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

The revision must be submitted online within 90 days;

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Referee #1:

This is a very extensive and detailed study identifying regulation of GDH1 acetylation and how this controls HIF-1a levels via action on PHD2 affinity. The data presented is extensive and the figures incredibly busy, so to facilitate reading it would be best to reduce the number of panels and split these into multiple figures. Major issue is the lack of detail of the methods. Not sure how the MS was run, how the antibodies were raised etc. The data focus on HIF-1, but given that PHD2 equally regulates HIF-2, is this also altered?

Another question from the data provide arises regarding the localisation of the acetylation. p300 is mostly if not exclusively nuclear, while the data provided suggests that GDH1 is mostly cytoplasmic. Where does this acetylation occur?

Minor issue is related to the sentence construction and some strange information. i.e. page 3, lines 48-52. PHD function is very sensitive to oxygen. This has been widely demonstrated.
Page 8 line 222, "has no regularity" no idea what this is meant to say.

This plus the density of the figures with data make the research not clear in message.

Referee #2:

The concept behind this manuscript is sound - enzymes that produce or catabolise aKG are likely to have an effect on the availability of aKG for other reactions.
Major
1. The language throughout is not conventional scientific prose - statements are not always correct due to English use (e.g. 'For response to hypoxia, HIF1a is reported to be overexpressed in various cancer cells' - HIF1a has not been substantially reported at overexpressed in any cancers I'm aware of - stabilised by hypoxia or other pathogenic stimuli, yes, but not overexpressed). This is observed throughout the manuscript and requires a full re-write to be of sufficient standard to be considered for publication
2. It isn't possible to perform any statistical analysis on n=2 (Figure 1b, S1b, S1c, S1f, S5g, maybe some others I've not noticed), and there is no mention of any of the blots being representative rather than a single experiment. There are therefore concerns over the presentation of data from n=2 with significance values next to it, and whether the knockdown of gene expression presented was always the case. Other aspects of the statistical analysis are not consistent: why is a one-way ANOVA used in 1e, while an unpaired t-test in 1f when the same type of data with the same number of groups being analysed?
3. While the high oxygen and low oxygen conditions were presented in Figure 1b, in Figure S1c no high oxygen data are presented. This should be included as it is possible that the methylated derivative of aKG has an effect in normoxia.
4. Given that HIF1a was not stabilised in hypoxia in the shGDH cells (Figure 1d), this could suggest increased aKG rather than decreased. It is therefore unclear why the authors use aKG supplementation to rescue the shGDH1 effect. It isn't particularly clear why the authors also supplemented 2HG, given that the Schofield lab showed that it can activate EGLN1 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959194/). The authors measure aKG levels in these different conditions (Figure 1e) and show an increase from ~90-100 uM in hypoxia after shGDH1. It would be surprising if this increase of around 10% in aKG levels resulted in such a significant decrease in HIF1a without other accompanying metabolic changes (succinate and fumarate), or changes in respiration accompanying this. The authors will need to show more detail about the outcomes of shGDH1 in order for an appropriate interpretation of these data.

5. The result of the above problems, particularly point #4 mean that while there is clearly an issue with tumourigenesis after deletion of GDH1, which is obvious in the model, the mechanistic part is not supported, so the conclusions of the paper are not. The key experiments are the lack of substantial increase in aKG after shGDH, and the lack of a rescue with esterified aKG. The authors don't perform the other important measurements - was aKG increased after esterified aKG addition? What happens to glutamate after shGDH1? Was HIF1a decreased in hypoxia after me-aKG treatment? Does transamination of aKG to glutmate not support aKG:glutamate levels after knockdown of GDH? Is ammonia involved, which could be inferred from the Spinelli Nature paper a few years ago

Referee #3:

This manuscript demonstrates that glutamate dehydrogenase 1 (GDH1) promote colorectal cancer (CRC) by stabilizing HIF1a. The authors performed elegant biological and biophysical studies to reveal that two lysine residues in GDH1 promote HIF1a level under hypoxic conditions. Mechanistically, the authors demonstrate that Ac-K503 reduces a-KG level by promoting a-KG glutamate reaction, while Ac-K527 in GDH1 promotes EGLN1 interaction and activity. These factors together work to promote HIF1 stability. The clinical correlation between GDH1 acetylation, HIF1a level, and clinical outcome is validated using CRC patient tumor specimens. Overall, this research revealed a new role of acetylated GDH1 in hypoxic CRC. The mechanistic studies are well conducted and will improve the understanding of GDH1 role in CRC. A few minor issues as listed below should be addressed to strengthen the finding.

1. Figure 1a offers a schematic view of the RNAi screen and the result. The scheme shows two distinct siRNAs were used for

each gene under both normoxia and hypoxia conditions. But the results show three GDH1 siRNAs and how these siRNAs induced cell death further under hypoxia than under normoxia. The screening strategy including the origin of siRNA library is not described in the method section. The authors need to describe how the screening was performed and what are the 163 dehydrogenases investigated by the screening.

2. Based on the data, the authors show that acetylation at K503 decreases a-KG level by promoting the reverse reaction and the decreased a-KG level modulates EGLN1 and consequently HIF1a stability. However, in Figure 6k, the authors demonstrate that HIF1A knockdown attenuates GDH1 binding to its acetyltransferase p300 mitigating GDH1 acetylation. How are these factors in p300-GDH1-EGLN1-HIF1a axis connected with each other? Are these factors in a feedback loop system? The authors need to at least discuss.

3. In Figure 1d, the authors confirmed that knockdown of GDH1 abolishes HIF1a induction under hypoxic conditions in CRC. Does overexpression of GDH1 enhances HIF1a level? Or can rescue expression of GDH1 replenish the HIF1a induction in GDH1 knockdown cells? The data shows a single GDH1 shRNA clone's effect. To show that the effect of GDH1 loss on HIF1a expression decrease is indeed due to GDH1 target downregulation, the authors either need to use multiple shRNA clones or perform the GDH1 rescue experiment.

4. Clinical annotations of the CRC patients should be provided for the human tumor specimens used in Figure 7. Do any of the correlations provided in Figures 7e-7l depend on clinical treatment the patients received?

According to the comments and suggestions, we improved this study. All changes made in the manuscript are marked as Green. Of note, we formatted the manuscript and Figures as EMBO Journal required, hence, Expanded View (EV) Figures mean Supplemental Figures, Expanded View (EV) Tables mean Supplemental Tables. **Below are detailed point-by-point responses to reviewers' comments:**

Referee #1:

This is a very extensive and detailed study identifying regulation of GDH1 acetylation and how this controls HIF-1a levels via action on PHD2 affinity. The data presented is extensive and the figures incredibly busy, so to facilitate reading it would be best to reduce the number of panels and split these into multiple figures. Major issue is the lack of detail of the methods. Not sure how the MS was run, how the antibodies were raised etc. The data focus on HIF-1, but given that PHD2 equally regulates HIF-2, is this also altered?

Response: Thanks for your mention. We had added the methods of MS, antibodies preparation, measurement of reversible GDH1 activity and GDH1 enzyme kinetics, measurement of αKG, measurement of EGLN1-bound αKG, isothermal titration calorimetry and other methods in the methods section, which were previously described in the supplemental information.

Also, we supplemented the detection of HIF2a protein which was slightly affected by GDH1(Figure 1D).

Honestly, we tried to split the complicated experiments into multiple figures, however, limited to the total Figure number of EMBO Journal, we have no way to alleviate the panel congestion by augmenting the Figure number.

Another question from the data provide arises regarding the localization of the acetylation. p300 is mostly if not exclusively nuclear, while the data provided suggests that GDH1 is mostly cytoplasmic. Where does this acetylation occur?

Response: Thanks for your mention. In fact, we have some experiments to preliminarily indicate that p300 mediated GDH1 acetylation mainly occurred in the cytoplasm. The evidences were described as follow. Firstly, Appendix Figure S1B-C showed that p300 was not only localized in the nucleus but also in the cytoplasm and interacts with cytoplasmic proteins. Secondly, the result in Figure 6C demonstrated that acetyl-GDH1 was present in the both cytoplasm and the nucleus. Most importantly, Figure 6G clearly showed that the interaction of p300 with GDH1 was mainly in the cytoplasm. Taken together, we speculated that GDH1 acetylation at K503/527 occurred in the cytoplasm. The nuclear GDH1 acetylation at K503/527, it may be due to the interaction of acetyl-GDH1 with HIF1α which carried part of acetyl-GDH1 into the nucleus.

Minor issue is related to the sentence construction and some strange information. i.e. page 3, lines 48-52. PHD function is very sensitive to oxygen. This has been widely demonstrated.
Response: Thanks for your mention. According to your suggestion, we deleted the overstated description.

Page 8 line 222, "has no regularity" no idea what this is meant to say.
Response: We improved the description as this "HIF1α protein has not been regulated by global change of αKG level." Thanks, your mention prompted us to precisely use the language.

This plus the density of the figures with data make the research not clear in message.
Response: For clearly describing the idea in this study, we greatly improved the language and simplified the description by improving it with ourselves and handing over the manuscript to English native speakers.

Referee #2:

The concept behind this manuscript is sound - enzymes that produce or catabolize αKG are likely to have an effect on the availability of αKG for other reactions.
Major
1. The language throughout is not conventional scientific prose - statements are not always correct due to English use (e.g. 'For response to hypoxia, HIF1α is reported to be overexpressed in various cancer cells' - HIF1α has not been substantially reported at overexpressed in any cancers I'm aware of - stabilized by hypoxia or other pathogenic stimuli, yes, but not overexpressed). This is observed throughout the manuscript and requires a full re-write to be of sufficient standard to be considered for publication
Response: Thanks a lot for the reviewer's mention. Sorry for the incorrect enough expression: what we indeed mean is that the increased stabilization of HIF1α resulted in the higher HIF1α protein expression level. Accordingly, we rewrote the introduction, results and discussion of this manuscript to highlight the reversed GDH1 enzyme activity upon hypoxia stimulation. Meanwhile, we improved the accuracy of language by improving it by ourselves and handing over the manuscript to English native speakers.

2. It isn't possible to perform any statistical analysis on n=2 (Figure 1b, S1b, S1c, S1f, S5g, maybe some others I've not noticed), and there is no mention of any of the blots being representative rather than a single experiment. There are therefore concerns over the presentation of data from n=2 with significance values next to it, and whether the knockdown of gene expression presented was always the case. Other aspects of the statistical analysis are not consistent: why is a one-way ANOVA used in 1e, while an unpaired t-test in 1f when the same type of data with the same number of groups

being analyzed?

Response: Thanks for the reviewer's mention. Firstly, we apologized for the not clear or correct enough data presentation in the reviewer mentioned places. We have checked the whole paper for figure panels with n=2, and increased the biologically independent sample numbers to n=3 in the related experiments (Figure 1B, EV1B, EV1C, EV1F, 5M, EV5G, EV5M, 7A, Appendix Figure S2A, Appendix Figure S2D). The figure panels had been updated and the statistical p-values had been re-calculated in the revised edition using three biologically independent samples, which did not affect our original conclusions. Additionally, all the experiments were repeated three times and the representative data were shown.

Secondly, as for the statistical analysis in Figure 1E, we compared the αKG levels of hypoxia vs normoxia in shNT vs shGDH1 cells for indicating that αKG increased under hypoxia compared with normoxia, and loss of GDH1 impaired the overall αKG level under normoxia but not hypoxia. This is a comparison among four groups of data, so we used one-way ANOVA followed by Tukey's HSD post hoc test for multiple comparisons. For example, the group of shNT under hypoxia was used to be compared with shGDH1 under hypoxia or shNT under normoxia for twice. In contrast, in Figure 1f, we only compared shNT vs shGDH1 under hypoxia or shNT vs shGDH1 under normoxia, in order to show that HIF1A gene transcription was slightly impaired after GDH1 depletion in both hypoxia and normoxia conditions. This is a comparison among just two groups of data respectively, so the unpaired two-tailed student's t test was used. For example, the group of shNT under hypoxia, compared with shGDH1 under hypoxia, only was used to be compared once, which would not increase the risk of the first type errors of statistics. Additionally, we have also modified the figure panel 1e by making the four columns evenly distributed to avoid the misleading.

3. While the high oxygen and low oxygen conditions were presented in Figure 1b, in Figure S1c no high oxygen data are presented. This should be included as it is possible that the methylated derivative of αKG has an effect in normoxia.

Response: Thanks a lot for the reviewer's suggestion. We supplemented this data in Figure EV1E by adding the condition of normoxia.

4. Given that HIF1α was not stabilized in hypoxia in the shGDH1 cells (Figure 1d), this could suggest increased αKG rather than decreased. It is therefore unclear why the authors use αKG supplementation to rescue the shGDH1 effect. It isn't particularly clear why the authors also supplemented 2HG, given that the Schofield lab showed that it can activate EGLN1 (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3959194/).

The authors measure αKG levels in these different conditions (Figure 1e) and show an increase from ~90-100 uM in hypoxia after shGDH1. It would be surprising if this increase of around 10% in αKG levels resulted in such a significant decrease in HIF1α without other accompanying metabolic changes (succinate and fumarate), or changes in respiration accompanying this. The authors will need to show more detail about the outcomes of shGDH1 in order for an appropriate interpretation of these data.

Response: Thanks a lot for the reviewer's mention. We interpreted as follows. We firstly performed the experiment of supplementing αKG to shGDH1 cells under hypoxia and normoxia for the reason that GDH1 generally functions as glutamate dehydrogenase, whose loss would decrease cellular αKG level. Though αKG did not decrease, but increased a little (10%) in the shGDH1 cells under hypoxia, it truly decreased greatly in shGDH1 cells under normoxia. After supplementing me-αKG, αKG level of shNT cells under hypoxia was supplemented to be nearly equal to the shGDH1 group, eliminating the difference of cellular αKG, which did not recuse the phenotype of shGDH1 under hypoxia (Figure EV1E), implying that the increase of around 10% in αKG levels of shGDH1 cells was not responsible for the function of GDH1 on survival under hypoxia, as well as the HIF1α stability regulation by GDH1 (Figure EV1C-E). Therefore, it came the question how GDH1 regulated HIF1α stability under hypoxia. That was also the reason why we further utilized LC-MS to discover GDH1 post-translational modifications, such as lysine acetylation (Figure 3) and GDH1 interacting proteins (Figure 5).

Hence, our logic is to find phenomena that seems to be contradicted with the established conclusions and then we explored the inner regulatory mechanisms behind this phenomenon. In the present study, eventually we found that once cells suffer hypoxia stress, GDH1 targeted the EGLN1/HIF1α complex after GDH1 acetylation by p300. In detail, AcK527 induced the formation of the GDH1/EGLN1/HIF1α axis, while GDH1 acetylation at K503 reversed GDH1 dehydrogenase activity and resulted in glutamate production using αKG as a substrate by alleviating the restriction of αKG binding to GDH1. As a consequence, combined GDH1 acetylation at K503 and K527 likely consumed αKG around the GDH1/EGLN1/HIF1α complex, which led to the inhibition of EGLN1 activity and in turn stabilized HIF1α.

Meanwhile, given previous report that 2HG activated EGLN1 from the Schofield lab, we supplemented octyl-2HG in GDH1 deficient HCT116 cells under hypoxia. Data were presented in Figure EV1H. This result was partially supported by a previous report showing that the IDH1-R132H mutant utilized αKG as a substrate and produced R-2HG, which did not stabilize HIF1α protein (Koivunen et al, 2012). Conversely, forced expression of an IDH1-R132H mutant reduced αKG but increases HIF1α protein (Zhao et al, 2009). However, the *Km* value of EGLN1 for αKG is 1.3 μM (Lorenzo et al, 2014), while in different cell contexts, the intracellular αKG concentration changed in the range of 10 μM-200 μM, which was insufficient to affect EGLN1 enzyme activity. The inconsistent results suggest that global changes in intracellular αKG or 2HG may not uniformly affect EGLN1 activity.

Thus, we should shift our perspective toward signal transduction-mediated local changes of αKG around EGLN1 to regulate its activity and consequent HIF1α stability under hypoxia, thus triggering a GDH1/P300/EGLN1/HIF1α axis for the response to hypoxia stress.

5. The result of the above problems, particularly point #4 mean that while there is clearly an issue with tumourigenesis after deletion of GDH1, which is obvious in the model, the mechanistic part is not supported, so the conclusions of the paper are not.

The key experiments are the lack of substantial increase in αKG after shGDH1, and the lack of a rescue with esterified αKG. The authors don't perform the other important measurements - was αKG increased after esterified αKG addition? What happens to glutamate after shGDH1? Was HIF1α decreased in hypoxia after me-αKG treatment? Does transamination of αKG to glutmate not support αKG: glutamate levels after knockdown of GDH? Is ammonia involved, which could be inferred from the Spinelli Nature paper a few years ago

Response: Thanks a lot for the reviewer's mention and insightful suggestions.

According to the suggestions, we supplemented the data of important measurements, such as αKG and glutamate determination after methyl-αKG supplementation(Figure EV1C); we also investigated HIF1α protein level in hypoxia after me-αKG treatment(Figure EV1D); The transamination of αKG to glutamate by GDH1 under hypoxia supported the increased glutamate while knockdown of GDH1 would impair the level of glutamate in the cells under hypoxia(Figure EV1F); After GDH1 depletion, ammonia in increased under normoxia but decreased under hypoxia, strongly suggested the reversed activity of GDH1 occurred from dehydrogenase under normoxia to transamination under hypoxia(Figure EV1G).

The supplementation of above data greatly improved our study. Thanks again.

Referee #3:

This manuscript demonstrates that glutamate dehydrogenase 1 (GDH1) promote colorectal cancer (CRC) by stabilizing HIF1α. The authors performed elegant biological and biophysical studies to reveal that two lysine residues in GDH1 promote HIF1α level under hypoxic conditions. Mechanistically, the authors demonstrate that Ac-K503 reduces a-KG level by promoting a-KG glutamate reaction, while Ac-K527 in GDH1 promotes EGLN1 interaction and activity. These factors together work to promote HIF1 stability. The clinical correlation between GDH1 acetylation, HIF1α level, and clinical outcome is validated using CRC patient tumor specimens. Overall, this research revealed a new role of acetylated GDH1 in hypoxic CRC. The mechanistic studies are well conducted and will improve the understanding of GDH1 role in CRC. A few minor issues as listed below should be addressed to strengthen the finding.

1. Figure 1a offers a schematic view of the RNAi screen and the result. The scheme shows two distinct siRNAs were used for each gene under both normoxia and hypoxia conditions. But the results show three GDH1 siRNAs and how these siRNAs induced cell death further under hypoxia than under normoxia. The screening strategy including the origin of siRNA library is not described in the method section. The authors need to describe how the screening was performed and what are the 163 dehydrogenases investigated by the screening.

Response: So sorry for this mistake. We corrected it in the scheme. Three single

siRNAs for each gene with two repeats were used for the RNAi screen. The schematic view had been updated in Figure 1A. For the 163 dehydrogenases investigated by the screening, we first downloaded the known metabolic enzymes from KEGG metabolic pathways. Then we obtained the rate limited dehydrogenases and other important dehydrogenases, a total of 163 dehydrogenases were used for this screening. siRNAs were specifically extracted from the above siRNA screening libraries.
As for the origin of siRNA library:
https://horizondiscovery.com/en/screening/screening-libraries/gene-modulation/sirna/products/human-on-targetplus-sirna-library-genome
We added the information about the process of screening for dehydrogenases required for hypoxia resistance in Figure 1A legend section.

2. Based on the data, the authors show that acetylation at K503 decreases a-KG level by promoting the reverse reaction and the decreased a-KG level modulates EGLN1 and consequently HIF1α stability. However, in Figure 6k, the authors demonstrate that HIF1A knockdown attenuates GDH1 binding to its acetyltransferase p300 mitigating GDH1 acetylation. How are these factors in p300-GDH1-EGLN1-HIF1α axis connected with each other? Are these factors in a feedback loop system? The authors need to at least discuss.
Response: Thanks a lot for your suggestion. We discussed it as follows: in this study, we observed that GDH1 regulated cell viability upon hypoxia. Upon hypoxia stress, HIF1α, but not HIF2a, linked p300 and GDH1 together, driving GDH1 acetylation at K503 and K527 by p300, which in turn decreased a-KG level by the reverse reaction to modulate EGLN1 activity and HIF1α stability. Consequently, these factors formed the p300-GDH1-EGLN1-HIF1α axis.

Thanks so much for your attention and suggestion in this feedback axis, we agreed that it's a positive feedback loop initiated by a basal level of HIF1α, which triggers p300 acetylating GDH1 and then acetylated GDH1 further promotes HIF1α stability possibly for the rapid response to hypoxia stress, eventually resulting in cancer cells survival and tumor progression. We added this explanation in the discussion section.

3. In Figure 1d, the authors confirmed that knockdown of GDH1 abolishes HIF1α induction under hypoxic conditions in CRC. Does overexpression of GDH1 enhances HIF1α level? Or can rescue expression of GDH1 replenish the HIF1α induction in GDH1 knockdown cells? The data shows a single GDH1 shRNA clone's effect. To show that the effect of GDH1 loss on HIF1α expression decrease is indeed due to GDH1 target downregulation, the authors either need to use multiple shRNA clones or perform the GDH1 rescue experiment.
Response: Thanks a lot for your suggestion and mentions. We performed the experiment and showed that overexpression of GDH1 enhances HIF1α level in both HCT116 and SW480 cells under hypoxia (Figure EV1I). We also performed the rescued experiments for shGDH1 in Figure 5H, J-P, and Figure EV5G-M, for testing rescued expression of WT- or mutant GDH1 could affect HIF1α stability under

hypoxia and normoxia. Also, our previous studies have confirmed that the shRNA targeting GDH1 does not direct the off target by two distinct shRNA sequence (PMID: 31447391,34269483). Taken together, above data and our previous reports excluded the possibility of GDH1 knockdown with non-specificity.

4. Clinical annotations of the CRC patients should be provided for the human tumor specimens used in Figure 7. Do any of the correlations provided in Figures 7e-7l depend on clinical treatment the patients received?

Response: Thanks a lot for your mention and suggestion. We had added the related clinical annotations into the method part about clinical specimens, as well as the notification that detailed clinicopathological information was supplied in the Table EV1-4. As stated in the clinical annotations, we collected the tumor samples from the patients who had not received any chemotherapies or other treatments yet before surgery treatment or diagnosis at the hospital, including the patients with high or low AJCC stage level. Thus, we could conclude that the correlations in Figure 7 did not depend on the clinical treatments of the patients.

Dear Dr Wang,

Thank you for submitting your revised manuscript (EMBOJ-2022-112675R) to The EMBO Journal, as well as for your patience with our response. Your amended study was sent back to the three referees for their re-evaluation, and we have received comments from two of them, which I enclose below. The third referee was unfortunately at this point not able to provide additional feedback, however we have considered your response to this expert editorially and found the raised issues to be addressed satisfactorily. As you will see, the other experts stated that the work has been substantially improved by the revisions and they are now in favour of publication, pending minor revision.

Thus, we are pleased to inform you that your manuscript has been accepted in principle for publication in The EMBO Journal.

Please consider the remaining comment of expert #3 carefully and address them by complementing the data annotation.

We also need you to take care of a number of minor issues related to formatting and data annotation as detailed below, which should be addressed at re-submission.

Please contact me at any time if you have additional questions related to below points.

As you might have noted on our web page, every paper at the EMBO Journal now includes a 'Synopsis', displayed on the html and freely accessible to all readers. The synopsis includes a 'model' figure as well as 2-5 one-short-sentence bullet points that summarize the article. I would appreciate if you could provide this figure and the bullet points.

Thank you for giving us the chance to consider your manuscript for The EMBO Journal. I look forward to your final revision.

Again, please contact me at any time if you need any help or have further questions.

Kind regards,

Daniel Klimmeck

Daniel Klimmeck PhD
Senior Editor
The EMBO Journal

****

Formatting changes required for the revised version of the manuscript:

>> Introduce ORCID IDs for all corresponding authors (H.A.) via our online manuscript system. Please see below for additional information.

>> Author Contributions: Remove the author contributions information from the manuscript text. Note that CRediT has replaced the traditional author contributions section as of now because it offers a systematic machine-readable author contributions format that allows for more effective research assessment. and use the free text boxes beneath each contributing author's name to add specific details on the author's contribution.

>> Figures: EV figures need to be uploaded separately and the legends added to the manuscript after the main figure legends.

>> Funding information: 'Operation Cost of Guangdong Provincial Key Laboratory of Guangdong Provincial Science and Technology Plan Project (2020B1212060019)' needs to be added in our online system.

>> Appendix: the appendix file needs a ToC on its first page. Figure resolution should be improved for Appendix Figures S1 and S2.

>> Tables EV1-4 need to be uploaded as individual files.

>>Data availability section: please substitute current statement by 'This study contains no data amenable to large scale repository deposition.' .

>> Consider additional changes and comments from our production team as indicated by the .doc file enclosed and leave changes in track mode.

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Please note that as of January 2016, our new EMBO Press policy asks for corresponding authors to link to their ORCID iDs. You can read about the change under "Authorship Guidelines" in the Guide to Authors here: http://emboj.embopress.org/authorguide

In order to link your ORCID iD to your account in our manuscript tracking system, please do the following:

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We regret that we cannot do this linking on your behalf for security reasons. We also cannot add your ORCID iD number manually to our system because there is no way for us to authenticate this iD number with ORCID.

Thank you very much in advance.

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Further information is available in our Guide For Authors: https://www.embopress.org/page/journal/14602075/authorguide

We realize that it is difficult to revise to a specific deadline. In the interest of protecting the conceptual advance provided by the work, we recommend a revision within 3 months (10th Jun 2023). Please discuss the revision progress ahead of this time with the editor if you require more time to complete the revisions.

Referee #1:

Although this manuscript is still enormous, the authors had addressed my concerns. They have provided detailed methods, make the figures easier to follow.

Referee #3:

The authors addressed most of the concerns raised during the initial review. However, the information on 163 dehydrogenases used for RNAi screen is still missing as stated in comment #1.

The authors have addressed all minor editorial requests.

Dear Dr Wang,

Thank you for submitting the revised version of your manuscript. I have now evaluated your amended manuscript and concluded that the remaining minor concerns have been sufficiently addressed.

Thus, I am pleased to inform you that your manuscript has been accepted for publication in the EMBO Journal.

Please note that it is EMBO Journal policy for the transcript of the editorial process (containing referee reports and your response letter) to be published as an online supplement to each paper.

Also, in case you might NOT want the transparent process file published at all, you will also need to inform us via email immediately. More information is available here:
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On a different note, I would like to alert you that EMBO Press is currently developing a new format for a video-synopsis of work published with us, which essentially is a short, author-generated film explaining the core findings in hand drawings, and, as we believe, can be very useful to increase visibility of the work. This has proven to offer a nice opportunity for exposure i.p. for the first author(s) of the study. Please see the following link for representative examples and their integration into the article web page:
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Please let me know, should you be interested to engage in commissioning a similar video synopsis for your work. According operation instructions are available and intuitive.

If you have any questions, please do not hesitate to call or email the Editorial Office.

Thank you again for this contribution to The EMBO Journal and congratulations on a successful publication! Please consider us again in the future for your most exciting work.

Kind regards,

Daniel Klimmeck

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EMBO Reports - Author Guidelines
Molecular Systems Biology - Author Guidelines
EMBO Molecular Medicine - Author Guidelines

Reporting Checklist for Life Science Articles (updated January

This checklist is adapted from Materials Design Analysis Reporting (MDAR) Checklist for Authors. MDAR establishes a minimum set of requirements in transparent reporting in the life sciences (see Statement of Task: 10.31222/osf.io/9sm4x). Please follow the journal's guidelines in preparing your

Please note that a copy of this checklist will be published alongside your article.

Abridged guidelines for figures

1. Data

The data shown in figures should satisfy the following conditions:

- the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
- ideally, figure panels should include only measurements that are directly comparable to each other and obtained with the same assay.
- plots include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical
- if n<5, the individual data points from each experiment should be plotted. Any statistical test employed should be justified.
- Source Data should be included to report the data underlying figures according to the guidelines set out in the authorship guidelines on Data

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

- a specification of the experimental system investigated (eg cell line, species name).
- the assay(s) and method(s) used to carry out the reported observations and measurements.
- an explicit mention of the biological and chemical entity(ies) that are being measured.
- an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.
- the exact sample size (n) for each experimental group/condition, given as a number, not a range;
- a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
- a statement of how many times the experiment shown was independently replicated in the laboratory.
- definitions of statistical methods and measures:

  - common tests, such as t-test (please specify whether paired vs. unpaired), simple χ2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  - are tests one-sided or two-sided?
  - are there adjustments for multiple comparisons?
  - exact statistical test results, e.g., P values = x but not P values < x;
  - definition of 'center values' as median or average;
  - definition of error bars as s.d. or s.e.m.

Please complete ALL of the questions below.
Select "Not Applicable" only when the requested information is not relevant for your study.

Materials

Newly Created Materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
New materials and reagents need to be available; do any restrictions apply?	Not Applicable	

Antibodies	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
For **antibodies** provide the following information: - Commercial antibodies: RRID (if possible) or supplier name, catalogue number and or/clone number - Non-commercial: RRID or citation	Yes	We provided related information of commercial antiboies in the section of Materials and Methods.

DNA and RNA sequences	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Short novel DNA or RNA including primers, probes: provide the sequences.	Yes	We provided siRNA seqences and its RT-PCR primers in Supplemetary Table 5, and plamid construct primers and the shRNA sequence were stated in the section of Materials and Methods, listed in our previous report.

Cell materials	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Cell lines: Provide species information, strain. Provide accession number in repository **OR** supplier name, catalog number, clone number, and/**OR** RRID.	Yes	We provided related information in the section of Materials and Methods.
Primary cultures: Provide species, strain, sex of origin, genetic modification status.	Not Applicable	
Report if the cell lines were recently **authenticated** (e.g., by STR profiling) and tested for mycoplasma contamination.	Yes	We reported it in the section of Materials and Methods.

Experimental animals	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Laboratory animals or Model organisms: Provide species, strain, sex, age, genetic modification status. Provide accession number in repository **OR** supplier name, catalog number, clone number, **OR** RRID.	Yes	We provided related information in the section of Materials and Methods.
Animal observed in or captured from the field: Provide species, sex, and age where possible**.**	Yes	We provided related information in the section of Materials and Methods.
Please detail **housing and husbandry conditions**.	Yes	We provided related information in the section of Materials and Methods.

Plants and microbes	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Plants: provide species and strain, ecotype and cultivar where relevant, unique accession number if available, and source (including location for collected wild specimens).	Not Applicable	
Microbes: provide species and strain, unique accession number if available, and source.	Not Applicable	

Human research participants	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If collected and within the bounds of privacy constraints report on age, sex and gender or ethnicity for all study participants.	Yes	We provided related information in the section of Materials and Methods, and Supplemetary Tables.

Core facilities	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
If your work benefited from core facilities, was their service mentioned in the acknowledgments section?	Yes	We thanked our core facilities in the section of acknowledgment.

Design

Study protocol	Information included in the manuscript?	In which section is the information available? (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)

If study protocol has been **pre-registered, provide DOI in the manuscript**. For clinical trials, provide the trial registration number **OR** cite DOI.	Not Applicable	
Report the **clinical trial registration number** (at ClinicalTrials.gov or equivalent), where applicable.	Not Applicable	

Laboratory protocol	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Provide DOI OR other citation details if **external detailed step-by-step protocols** are available.	Not Applicable	

Experimental study design and statistics	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Include a statement about **sample size** estimate even if no statistical methods were used.	**Yes**	We provided the statement in the section of Materials and Methods. No statistical methods were applied to pre-evaluate sample size. The sample size was determined based on our experiences and similar with reported
Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. **randomization procedure**)? If yes, have they been described?	Not Applicable	No methods were used to minimize the effects of subjective bias. For in vivo and in vitro assays, groups were randomly allocated in this study.
Include a statement about **blinding** even if no blinding was done.	**Yes**	We provided the statement in the section of Materials and Methods. Except the IHC scoring, the investigators were not blinded to allocation.
Describe **inclusion/exclusion criteria** if samples or animals were excluded from the analysis. Were the criteria pre-established? If sample or data points were omitted from analysis, report if this was due to attrition or intentional exclusion and provide justification.	Not Applicable	No data were excluded from the analyses.
For every figure, are **statistical tests** justified as appropriate? Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it. Is there an estimate of variation within each group of data? Is the variance similar between the groups that are being statistically compared?	**Yes**	We provided related information in the section of Materials and Methods, and Figures.

Sample definition and in-laboratory replication	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
In the figure legends: state number of times the experiment was **replicated** in laboratory.	**Yes**	We provided related information in the section of Materials and Methods, and Figures.
In the figure legends: define whether data describe **technical or biological replicates**.	**Yes**	We provided related information in Materials and Methods, and Figure legends.All experiments were performed with at least three biologically independent samples or three biologically independent experiments.

Ethics

Ethics	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Studies involving **human participants**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval.	**Yes**	We provided related information and the approval number in the section of Materials and Methods.
Studies involving **human participants**: Include a statement confirming that **informed consent** was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	**Yes**	We provided the statement in the section of Materials and Methods.
Studies involving **human participants:** For publication of **patient photos**, include a statement confirming that consent to publish was obtained.	Not Applicable	We did not publish patient photos.
Studies involving experimental **animals**: State details of **authority granting ethics approval** (IRB or equivalent committee(s), provide reference number for approval. Include a statement of compliance with ethical regulations.	**Yes**	We provided related information and the approval number in the section of Materials and Methods.
Studies involving **specimen and field samples:** State if relevant **permits** obtained, provide details of authority approving study; if none were required, explain why.	**Yes**	We provided related information in the section of Materials and Methods.

Dual Use Research of Concern (DURC)	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Could your study fall under dual use research restrictions? Please check biosecurity documents and list of **select agents and toxins** (CDC): https://www.selectagents.gov/sat/list.htm	Not Applicable	
If you used a select agent, is the security level of the lab appropriate and reported in the manuscript?	Not Applicable	
If a study is subject to dual use research of concern regulations, is the name of the **authority granting approval and reference number** for the regulatory approval provided in the manuscript?	Not Applicable	

Reporting

The MDAR framework recommends adoption of discipline-specific guidelines, established and endorsed through community initiatives. Journals have their own policy about requiring specific guidelines and recommendations to complement MDAR.

Adherence to community standards	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
State if relevant guidelines or checklists (e.g., **ICMJE, MIBBI, ARRIVE, PRISMA**) have been followed or provided.	Not Applicable	
For **tumor marker prognostic studies,** we recommend that you follow the **REMARK** reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	Not Applicable	
For **phase II and III randomized controlled trials**, please refer to the **CONSORT** flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	Not Applicable	

Data Availability

Data availability	**Information included in the manuscript?**	**In which section is the information available?** (Reagents and Tools Table, Materials and Methods, Figures, Data Availability Section)
Have **primary datasets** been deposited according to the journal's guidelines (see 'Data Deposition' section) and the respective accession numbers provided in the Data Availability Section?	Not Applicable	
Were **human clinical and genomic datasets** deposited in a public access-controlled repository in accordance to ethical obligations to the patients and to the applicable consent agreement?	Not Applicable	
Are **computational models** that are central and integral to a study available without restrictions in a machine-readable form? Were the relevant accession numbers or links provided?	Not Applicable	
If publicly available data were reused, provide the respective **data citations in the reference list**.	Not Applicable	