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Reviewer A

Major revisions

Comment A1: I have question about the lack of correlation of the immunohistochemical scores between KMT2C and HRR factors after dividing the group to H-FLACs and common adenocarcinomas, while the coefficients were noticeable when all cases were taken into account? What could be the reason?

Reply A1: Firstly, we must inform you about our mistake in Figure 3. We demonstrated good positive correlation of KMT2C mRNA expression with that of HRR genes in H-FLACs in Figure 3C and poorer correlation in common adenocarcinomas in Figure 3D. However, correctly, Figure 3C, showing good correlation, was for common adenocarcinoma and Figure 3D for H-FLACs. We have corrected this mistake in Figure 3 and revised corresponding descriptions in Abstract, Results, and Discussion. Lack of correlation in H-FLAC cases could be partly due to low expression levels of *KMT2C* and HRR genes examined in addition to the small numbers of cases analysed.

The main reason for the lack of significant correlation is thought to be that the number of cases decreased due to the division into the two groups. In addition, immunohistochemical evaluation based on semiquantitative scores is considered to be a factor preventing significant correlation. A significant correlation might have been obtained if the number of cases could have been increased. Considering the correction of our coefficient analysis on RNA sequencing data for H-FLAC, generally low expression levels of the genes and encoded proteins in H-FLAC in comparison with common adenocarcinoma could be an additional cause for lacking correlation especially in analysis for small numbers of cases. However, due to the rarity of H-FLACs and the cost of analysis, it was difficult to increase the number of cases.

Changes in the text: We have added the following sentences (see Page 19, line 354–357): If more cases could have been analysed, a significant correlation between expressions of KMT2C and HRR factors might have been obtained after dividing the group into H-FLACs and common adenocarcinomas. However, due to the rarity of H-FLACs and the cost of analysis, it was difficult to increase the number of cases.

Comment A2: I would slightly change the conclusion (lines 394-395), as this analysis did

not evaluate the usefulness of PARP inhibitors. The better conclusion is presented in lines 382-383.
Reply A2: Following your suggestion, we have changed the conclusion (see Page 23, line 439–441).
Changes in the text: We speculated that PARP inhibitors could be an effective drug for treatment of H-FLACs with KMT2C mutation/low expression.

Minor revisions
Comment A3: I would suggest moving the statistical analysis to one paragraph, as it is partially present in section 2.3 (lines 188-199) and later in section 2.5, where only protein analysis is explained.
Reply A3: Following your suggestion, we have moved the statistical analysis to one paragraph (see Page 13, line 225–232).
Changes in the text: The threshold value to identify the DEGs was a greater than 2-fold change in expression, with a *P*-value < 0.05 and an adjusted *P*-value < 0.05. In IPA analysis, we evaluated the probability of the identified pathways by calculating the *P*-value using the right-tailed Fisher's exact test, and pathways with a *P*-value < 0.05 were considered significant. Additionally, z-scores were automatically calculated by the IPA, and positive and negative z-scores indicated activation and inactivation of pathways, respectively. Z-scores for pathways involving DEGs without enough information regarding their biological significance in the IPA dataset were ineligible for assignment and were designated as NaN.

Comment A4: Figure 1 - I suggest moving the A and B letters above the pictures, as they are not readable enough right now.
Reply A4: Following your suggestion, we have moved the letters above the pictures.
Changes in the text: None. Changes were made to Figure 1.

Comment A5: In figure 3A, what represents the box, line and whiskers? I can’t seem to find any legend.
Reply A5: We have provided information of the boxplot in the Figure legend.
Changes in the text: Boxes indicate the first and the third quartile counts, lines in boxes indicate median values, and ends of whiskers indicate minimum and maximum values, respectively. Dots demonstrate outliers.

Comment A6: In Figure 3A, I suggest changing the gray background to white, as it would

be more visible.
Reply A6: The grey background was changed to white.
Changes in the text: Figure 3A was changed.

Reviewer B

Firstly, we must inform you about our mistake in Figure 3. We demonstrated good positive correlation of KMT2C mRNA expression with that of HRR genes in H-FLACs in Figure 3C, and poorer correlation in common adenocarcinomas in Figure 3D. However, correctly, Figure 3C, showing good correlation, was for common adenocarcinoma and Figure 3D for H-FLACs. We have corrected this mistake in Figure 3 and revised corresponding descriptions in Abstract, Results, and Discussion. Lack of correlation in H-FLAC cases could be partly due to low expression levels of *KMT2C* and HRR genes examined in addition to the small numbers of cases analysed.

Major revisions
Comment B1: It would be valuable to compare KMT2C mutants with KMT2C WT within H-FLAC as well as comparing H-FALC with common primary LUAD. Was the cohort used in this paper the same as the one used in your previous study (Suzuki M. et al., 2021)? If so, could you repeat figures 2 and 3a by comparing KMT2C mutants with KMT2C WT within the H-FLAC population?
Reply B1: The cohort used in the present study was the same as the one used in our previous report. Therefore, we first compared the mRNA expression of *KMT2C* and HRR genes *ATM*, *ATR*, *BRCA1*, and *BRCA2* between six *KMT2C* mutated H-FLAC with the rest nine *KMT2C* wild type ones. No genes showed significantly different expression between the two. We further identified 19 differentially expressed genes (DEGs) between the *KMT2C* mutated and the wild type H-FLACs using the same DEG criteria (greater than 2-fold change in expression, with a *P*-value < 0.05 and an adjusted *P*-value < 0.05) for the analysis between H-FLAC and the corresponding common adenocarcinoma. IPA analysis for the 19 DEGs identified only one pathway, nNOS signalling in skeletal muscle cell without no activity pattern. These nonspecific results may partly be due to the small numbers of cases examined and may raise a possibility that the inactivation of KMT2C is common in H-FLAC but with different mechanisms of genetics and epigenetics.
Changes in the text: We added new paragraphs describing the results at the end of "3.2. Transcriptome analysis using PCA, DESeq2, and IPA" and at the end of "3.3. *KMT2C* expression and HRR genes in H-FLAC" in Results, and the data was provided as

Supplementary Fig. 3. We further discussed this result in “4.2 Strengths and limitations” as the second limitation of the present study.

Comment B2: Unless you use the previously reported mutations responsible for the impairment of KMT2C function in their study, I suggest you rephrase your claims KMT2C dysfunction" to "KMT2C expression".
Reply B2: We have rephrased “KMT2C dysfunction” to “low KMT2C expression”, “KMT2C aberration”, or “KMT2C mutation/low expression”.
Changes in the text: As stated above.

Comment B3: To enhance the impact of your study, you could explore the association between KMT2C mutation (or low expression) and RAD51 foci, a surrogate marker of HRR functionality. RAD51 foci have been linked to resistance to PARP inhibitors in breast cancer (Cruz et al., 2018). Can you look at the presence of RAD51 foci by IHC in KMT2C mutants and WT, H-FLAC versus common adenocarcinoma (and or KMT2C high versus low)?
Reply B3: Following your suggestion, we performed immunohistochemical analysis of RAD51. Low nuclear expression (score 0 or 1+) of RAD51 was observed in 12/16 cases of H-FLAC and 12/16 cases of common adenocarcinoma, and RAD51 expression showed no significant correlation with KMT2C expression and mutation.
Changes in the text: We have added the following sentences:
Low nuclear expression (score 0 or 1+) of RAD51 was observed in 12/16 cases of H-FLACs and 12/16 cases of common adenocarcinomas (Figures 4 and 5F). Only one case (F7) showed cytoplasmic RAD51 expression. (Page 17, line 315–317)
RAD51 expression showed no significant correlation with KMT2C expression. (Page 18, line 326–327)

Comment B4: Could you please present boxplots in Figure 4 to illustrate the statements "Low KMT2C expression with a score of 1+ was identified in more H-FLAC components than in common adenocarcinomas" and "H-FLACs showed significantly lower KMT2C expression than in common adenocarcinomas"? Also, please specify the p-value on the graph.
Reply B4: We have presented boxplots of expressions of KMT2C and HRR factors in Supplementary Fig. 4 and added a legend for Supplementary Fig. 4.
Changes in the text: We have added the following sentences to the legend of Supplementary Fig. 4:

Boxplots of immunohistochemical expressions of KMT2C and HRR factors. Boxes indicate the first and the third quartile counts, lines in boxes indicate median values, and ends of whiskers indicate minimum and maximum values respectively. Dots demonstrate outliers.

Minor revisions

Comment B5: Could you please specify the RIN cut-off you used to include RNA samples in the study (Line 164)? This would be useful information for readers.

Reply B5: The description was not accurate, and line 164 has been corrected.

Changes in the text:

The RNA integrity was assessed with RNA integrity numbers (RIN) and DV200 using the RNA Nano 6000 Assay Kit and Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA, USA). Library synthesis was performed on samples with DV200 greater than 20%. (Pages 10–11, line 171–174)

Comment B6: In Figure 4, please state what F and C mean in the legend and explain when you use a line in the cells in the legend (already specified in the text).

Reply B6: Following your suggestion, we have added the following to the legend of Figure 4:

Changes in the text: F1-16 are H-FLAC cases and C1-16 are common adenocarcinoma cases. F1, F3, F7, and F9 were almost entirely comprised of H-FLAC components and could not be evaluated in non-fetal-type components (shown in a slant line).

Comment B7: Regarding Figure 5, it is unclear whether the images showing H-FLAC and common ad are matched (coming from the same patients) or not (since the H-FLAC cases can show non-H-FLAC areas).

Reply B7: H-FLAC and common Ad are separate cases, and we have corrected the description as follows in the legend of Figure 5.

Changes in the text: Expression of KMT2C (**A**), ATM (**B**), ATR (**C**), BRCA1 (**D**), and BRCA2 (**E**) tended to be lower in the fetal components of the H-FLAC cases than in cancer tissues of the common adenocarcinoma cases.

Comment B8: I suggest clarifying in the text and in the legends why you present H-FLAC and non-H-FLAC values for the H-FLAC samples in Figure 4 but only one image per patient in Figure 5.

Reply B8: The difference of expression between fetal components and non-fetal components of H-FLAC cases was unclear. Therefore, only images of fetal components of the H-FLAC cases and cancer tissues of the common adenocarcinomas are shown in Figure 5 to make the difference easier to understand. We have added the following in the text (Page 17, line 317–320).
Changes in the text: As the difference between the expression of fetal and non-fetal components of H-FLAC cases was unclear, only images of fetal components of the H-FLAC cases and cancer tissues of the common adenocarcinomas are shown in Figure 5.

Reviewer C

Major revisions
Comment C1: evidence provided by the authors to establish a link between KMT2C downregulation and decreased expression of HRR genes is purely correlative. This link is fortunately supported by KMT2C depletion experiments in the Rampias et al. study, although this one was done in bladder cancer. One may still argue that in lung cancer cells KMT2C does not regulate HRR genes, but is only co-regulated with HRR genes. It must be acknowledged that the authors clearly state this possibility in the article, and present a well-balanced discussion on this subject.
Reply C1: As you point out, we did not directly clarify the KMT2C regulatory effect on the expression of the HRR factors in lung cancers with an H-FLAC component. We think that further studies are required to determine this function of KMT2C in H-FLAC.
Changes in the text: None.

Comment C2: Another questionable point is the claim that the correlation between KMT2C and HRR gene expression exists only in H-FLACs and not in common LUAD. It is true that the correlation is not significant in LUAD alone, but I notice that the correlation is better in H-FLAC + LUAD, than in H-FLAC alone. I performed a quick analysis of expression data in LUAD samples from TCGA (n=510) and found a very significant positive correlation between KMT2C and HRR gene expression. The same is true for the LUAD lines in the CCLE (n=74). The correlation therefore seems to exist in all forms of lung adenocarcinoma. The lack of correlation observed by Suzuki et al. is probably related to the fact that KMT2C is less often downregulated in common Adenocarcinoma, and therefore that more of such samples are needed to see a significant correlation. I would suggest that the authors include TCGA datasets in their analyses.

Reply C2: Firstly, we must inform you about our mistake in Figure 3. We demonstrated good positive correlation of KMT2C mRNA expression with that of HRR genes in H-FLACs in Figure 3C and poorer correlation in common adenocarcinomas in Figure 3D. However, correctly, Figure 3C, showing good correlation, was for common adenocarcinoma and Figure 3D for H-FLACs. We have corrected this mistake in Figure 3 and revised corresponding descriptions in Abstract, Results, and Discussion. Lack of correlation in H-FLAC cases could be partly due to low expression levels of *KMT2C* and HRR genes examined in addition to the small numbers of cases analysed. As the reviewer suggested the correlation seems to exist in all forms of lung adenocarcinoma. We added the analysis of TCGA LUAD in the Figure 3E. Although *BRCA2* expression did not show correlation to that of *KMT2C*, other genes did.
Changes in the text: We have added the analysis of TCGA LUAD as Figure 3E. Accordingly, we provided the method for the TCGA analysis in Method. The result was shown in Results, lines 322–324, and we added a discussion "The correlated expression of *KMT2C* and HRR genes examined seemed to be a characteristic of adenocarcinoma of the lung including ones with H-FLAC components" in Discussion, 4.1 Key Findings, Lines 343–344.

Comment C3: it would be useful to link the expression data of the present study with the mutation analyses previously carried out (both studies were performed on the same H-FLAC samples). What effect do mutations in KMT2C have on HRR gene expression?
Reply C3: We first compared the mRNA expression of *KMT2C* and HRR genes *ATM*, *ATR*, *BRCA1*, and *BRCA2* between six *KMT2C* mutated H-FLAC with the rest nine *KMT2C* wild type ones. No genes showed significantly different expression between the two. We further identified 19 differentially expressed genes (DEGs) between the *KMT2C* mutated and the wild type H-FLACs using the same DEG criteria (1 or higher log2 fold change of expression with 0.05 or smaller *padj*) for the analysis between all H-FLAC and the corresponding common adenocarcinoma. IPA analysis for the 19 DEGs identified only one pathway, nNOS signalling in skeletal muscle cell without no activity pattern. These nonspecific results may partly be due to the small numbers of cases examined and may raise a possibility that the inactivation of KMT2C is common in H-FLAC but with different mechanisms of genetic and epigenetic.
Changes in the text: We added new paragraphs describing the results at the end of "3.2. Transcriptome analysis using PCA, DESeq2, and IPA", and at the end of "3.3. *KMT2C* expression and HRR genes in H-FLAC" in Results, and the data was provided as Supplementary Fig. 4. We further discussed this result in "4.2 Strengths and limitations"

as the second limitation of the present study.

Minor revisions

Comment C4: - Line 95: “high frequency of KMT2C mutations”. Please specify here what frequency.

Reply C4: We have added a specific frequency of KMT2C mutations (Page 7, line 98–99).

Changes in the text: Our recent study showed that lung cancers with H-FLAC components rarely harboured druggable driver gene mutations but exhibited a high frequency of *KMT2C* mutations (6/16 cases, 38%).

Comment C5: - Fig. 2: I don’t understand the difference between “z-score=0“ (white) and “no activity pattern available“ (grey). Also, color intensities seem to reflect quantitative differences, but this is not explained in the legend.

Reply C5: I have changed the description of Figure 2.

Changes in the text:

The significant pathways identified by IPA analysis for the DEGs in H-FLAC and the common adenocarcinoma counterpart are depicted. The y-axis displays the -log of the P-value calculated by the right-tailed Fisher's Exact Test, and the height of each bar indicates the probability of the association. Only the pathways with -log (P-value) greater than 2 (corresponding to a P-value less than 0.01) were depicted in this figure. Blue bars indicate the pathways inactivated in H-FLAC and orange ones indicate pathways activated in H-FLAC. White bars represent unpredicted pathway activation/inhibition. Gray bars indicate those that are ineligible for activity prediction analysis. The title of the pathway is shown on the x-axis.

Comment C6: - Fig. 3: Expression values are unreadable (too small), yet important.

Reply C6: I have changed the scale size text on the figure to be larger.

Changes in the text: None. Changes have been made to Figure 3.

Comment C7: - Fig. 5: There is no indication of the tissue sample order. Samples should be identified on the figure, or in the legend. My advice would be to order H-FLAC samples (upper line) and Common Ad samples (lower line) according to the level of KMT2C expression (left to right), and to keep these orders for the other proteins. But maybe this is already how they were ordered.

Reply C7: We have edited the description of the legend because it was unclear and added

case numbers in Figure 5. To make it easier to see the tendency of lower expression of KMT2C and HRR factors in H-FLAC, immunohistochemical images for each marker are arranged from left to right in ascending order of expression. Additionally, we have added case numbers in Figure 5.
Changes in the text: Immunohistochemical images for each marker are arranged from left to right in ascending order of expression.

Comment C8: - Table 2: Only p value are given, but with no information on the direction of the difference: lower or higher? Please include fold change in table.
Reply C8: Immunostaining of each marker was evaluated as an ordinal variable with scores from 0 to 3+, so comparative analysis was based on the Mann-Whitney U test. We have added boxplots to show if each expression is high or low.
Changes in the text: None.