Title: A new method for diagnosing adipocytic tumors by real-time polymerase chain reaction analysis of MDM2 and CDK4 expression using total RNA from core-needle biopsies.

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Author's response to reviews: see over
Dear Dr. Obiajulu Iwenofu

Thank you very much for your letter with regard to our manuscript together with the comments from the three reviewers. We have revised the manuscript “A new method for diagnosing adipocytic tumors by real-time polymerase chain reaction analysis of MDM2 and CDK4 expression using total RNA from core-needle biopsies” on the basis of the Referee’s comments.

Our responses to the reviewers’ reports are as follows:

Reviewer Michael Arnold

Major compulsory revisions
1. p.11, line 27-32. Sentences of explanation have been added.
   There are not so many data of MDM2 and CDK4 expression level by real-time PCR that it was difficult to establish a cut off value to favor the diagnosing lipoma or ALT/WDL.
    The data of each individual sample of MDM2 and CDK4 expression have been added in Figure 1 and 3.

2. p.8, line 17-20. Sentences have been added.

Minor essential revisions
1. The table numbers have been changed consecutively.

2. p.2, line 17. “of same the tumor” has been inserted and p.5, line 2-3. A sentence of explanation has been added.

3. p.6, line 29-30. A sentence has been added.

Discretionary Revisions
1. There are not so many samples which have been analyzed by immunohistochemistry that it was difficult to compare MDM2 and CDK4 expression level by real-time PCR with immunohistochemistry.
Reviewer Benjamin Swanson

Major compulsory revisions
1. Figure 3. A footnote “Amplification of target genes by real-time PCR in core-needle biopsy sections” has been added. p.8, line 28-30 and p.11, line 14-16. Sentences have been added.
2. p.11, line 27-32. Sentences of explanation have been added.
3. p.6, line 22-30. Sentences of explanation have been added.

Reviewer Adrian Mariño-Enriquez

Major compulsory revisions
1. In the discussion, end of second paragraph, I had missed that I had not described the references. p.9, line 26. “Bartuma et al reported that” has been inserted. I have added the summary table, Supplemental table S1 “The summary of each performed methods”.
2. The explanation for the ALT/WDL case with HMGA2-LPP fusion gene was added, p.10, line 13-19 and Figure 5 “There are variation in adipocytic size and extensive collagenous stroma”. And two lipomas with MDM2 and CDK4 amplification have been described, Figure 6 “L27 showed an infiltrative pattern with mature adipocytes” and Figure 7 “L30 was a superficial intramuscular lipoma composed of mature adipocytes” have been added and p.11, line 1-6. Sentences have been added.
3. Our two pathologists have reviewed the samples that have a discrepancy between histological diagnosis and cytogenetic analysis. It is possible that the sample L27 and L30 might be ALT/WDL. p.11, line 6-10. Sentences have been added.
4. Two experienced pathologists independently reviewed the cases in which it was difficult to distinguish between lipoma and ALT/WDL. So, co-author who is Ohashi Riuko have been added. p.1, line 6. “Riuko Ohashi” has been added.
5. We have obtained some samples by core-needle biopsy just after resection in the operating room as an experimental procedure.

Minor essential revisions
6. Figure 5, 6, 7 have been added.
7. The abbreviations were changed as suggested.
8. “HMGIC” has been changed into “HMGA2”.

9. Table 1 and 2. A footnote “Abbreviations: U, upper extremity; L, lower extremity; T, trunk; H, head and neck.” has been added.

10. Tables “Primer sequences of the fusion genes” and “Primer used to amplify target genes” are changed as Supplemental table.

11. p.11, line 6. The sentence has been changed as suggested, and we have got English proofreading.

12. Some references to other neoplasms have been deleted.

13. Repetition in the discussion has been deleted.

14. The title has been changed as “Real-time polymerase chain reaction analysis of MDM2 and CDK4 expression using total RNA from core-needle biopsies is useful for diagnosing adipocytic tumors”

Discretionary Revisions

15. Supplemental table S1 “The summary of the performed methods” was added.

I believe the manuscript has been improved satisfactorily and hope it will be accepted for publication in BMC cancer.

Best regards,

Sincerely yours,

Taro Sasaki

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