Reviewer’s report

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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Reviewer: Adrian Mariño-Enriquez

Reviewer’s report:

In this manuscript, the authors analyze the expression of MDM2 and CDK4 by qRT-PCR in a large series of lipomas and atypical lipomatous tumors/well-differentiated liposarcoma; an interesting angle is provided by the inclusion of some core biopsies in the study. The authors also present cytogenetic information for a subset of cases, which is correlated with the gene expression analysis. A subset of samples are studied by RT-PCR for a number of fusion genes involving HMGA2, characteristic of lipoma. The resulting manuscript is essentially a descriptive report, collecting a reasonable amount of data regarding evaluation of MDM2 and CDK4 expression at the RNA level.

While the data substantiating this report is of potential interest, the manuscript in its present form has some limitations, both in its structure/presentation and in the interpretation of the results. Several particular points should be addressed, in my opinion, as follows:

Major Compulsory Revisions

1. The number of cases included in the study, and the techniques performed on each is essentially impossible to understand. In the methods, section specimens, the authors mention 168 cases (159 primary and 9 recurrent; 96 men, 72 women). But the results are presented separately for 104 cases (cytogenetics), 128 cases (RT-PCR), 149 cases (qRT-PCR) and 38 cases (core needle biopsies). In the discussion, end of second paragraph, the authors refer to “272 cases… in the present series” and “5/272 local recurrences” (?). This is confusing, and it’s impossible to correlate the results from one section to the next. Perhaps the authors could include a summary table, explaining the subgroups and the techniques performed on each of them.

2. The molecular findings for several cases are most surprising. According to the authors, there are lipomas with MDM2 and CDK4 amplification (i.e. cases LP27, LP24, and LP8, figure 3). Conversely, there are ALT without MDM2 and CDK4 amplification (at least cases 2, 3 and 5 in the same figure 3), and one case with a HMGA2-LPP fusion. This is all rather unlikely and, if true, the authors should explain those cases in detail (perhaps including histologic pictures). Initially, these are most likely misdiagnoses. Did those lipomas show atypia? Were they deep-seated? Is there follow-up information, to confirm benign course? The
authors mention in the discussion that some cases of “lipoma” did recur, in relationship with the presence of ring chromosomes (although it is unclear if they are referring to their series, or to the literature); this is very unusual for conventional, adipocytic-type, benign lipoma.

3. Similarly to the previous point, it is unconceivable that the upper limit of the range of expression of MDM2 and CDK4 is so high in lipomas (range 0.2-54.1 and 0.1-19.9, respectively) compared to atypical lipomatous tumor/well-differentiated liposarcoma (0.2-52.5 and 0.4-22.4). This is also obvious in figures 1 and 2. The underlying problem, again, is most likely misdiagnosis of some cases (or, if that was not the case, extraordinary cases that deserve to be presented in detail).

4. Given the apparent difficulties in diagnosis, a central histopathological review, ideally by two or more experienced pathologists, would be advisable. The diagnostic criteria, if specific problems are encountered, could be listed in the methods section beyond the vague reference to the WHO classification.

5. It should be clarified how the core needle biopsies were obtained. The authors state that “Core-needle biopsy sections were sampled prior to or after surgical resection using a 16G Tru-Cut trocar with at least two passes or until an adequate sample was obtained.” Does this mean that some core biopsies were obtained after surgical resection, as an experimental procedure in the grossing room during macroscopic examination?

Minor Essential Revisions

6. Histologic pictures of critical cases (for sure any case with unexpected molecular findings) would be very informative.

7. LP is not a standard abbreviation for lipoma. Also, the authors use some inconsistent abbreviations that should be corrected.

8. HMGIC should be consistently designated by its official name HMGA2.

9. In tables 4 and 5, it is impossible to understand the location of the tumors (U, L, T, H?)

10. Tables 1 and 2 can be published as supplemental materials.

11. The text needs a very thorough revision, not only regarding language issues, but also precision and content (i.e. “…were reported to show MDM2 or CDK4 protein amplification [24].” When it should say “gene amplification).

12. The paper should be focused on lipoma and ALT/WDL. References to other adipocytic neoplasms (myxoid liposarcoma, which is mentioned in a couple of sections, and others like hibernoma or lipoblastoma, are basically an unnecessary distraction).

13. Large sections of the discussion are already mentioned in the introduction. Repetition should be avoided; the manuscript length can be substantially reduced.
and would be much easier to read.

14. The title is a bit confusing. This is hardly a new method for diagnosis, rather a nice application of a well establish method. I suggest something more realistic and concrete.

Discretionary Revisions

15. It may be helpful to include an initial subsection in the results section, describing all the cases and the different categories be would help (adding maybe a supplementary table 1, comprehensively listing all the cases, with all the tests performed, including the gene expression values). This would take care of points #1, and to some extent simplify #2 and #3 of this review.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

I declare that I have no competing interests.