Reviewer’s report

Title: MIA is a potential biomarker for tumor load in neurofibromatosis type 1

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Reviewer: Rudi Beschorner

Reviewer’s report:

In the present study the authors investigated if Melanoma Inhibitory Activity (MIA) might be a suitable biomarker for tumor load in patients with neurofibromatosis type 1 (NF1). The authors determined MIA expression in the cartilage of an NF1 mouse model (Nf1flox; ISH, qRT-PCR,) as well as in tumor samples (immunohistology, in situ) from NF1 patients and MIA levels in blood from NF1 patients and healthy controls (ELISA). Tumor load in NF1 patients was determined by clinical examination (cutaneous tumors) and MR imaging (internal tumors). 43 NF1 patients and 22 healthy controls were investigated. The NF1 cases included cases with plexiform neurofibromas (n=17) and MPNST (n=7). The authors found significantly increased serum levels of MIA1 in NF1 patients compared to controls. Furthermore, among NF1 cases, significantly higher MIA1 levels were found in patients with plexiform NF and in patients with high numbers of tumors (>100 internal or >1000 cutaneous tumors) and with total tumor burden. The authors conclude that MIA1 is a potential biomarker of tumor load in NF1 patients.

The scientific background for the hypothesis that MIA1 might be a biomarker in NF1 is reasonable. The experimental setting and the study design are excellent. The data encourage to investigating MIA1 serum levels in larger series of NF1 patients to elucidate its value as a biomarker for tumor load (and prognosis?).

In summary, this study presents new data that are highly relevant to the clinical management of NF1 patients. However some points that should be clarified prior to publication are listed below.

Major compulsory revisions:
none

Minor essential revisions:
1. The numbers of cases/samples investigated in each group/subgroup is not always clear. Some times it seems that the numbers that are the given in the text are not congruent with in numbers of cases/samples illustrated in the figures. Examples for such "obscurities" are the following:
   - Fig. 3B: there are approx. 26 data points on patients with +pNF (n=17 in the text) and approx. 15 data points on -pNF patients (43 - 17 pNF -3 7 MPNST =19 ?).
- p10: In the text the authors mention 10 cases with more than 100 subcutaneous neurofibromas (n=10) but in figure 3C only 9 data points are visible in this group(s).

- Overall, in figures 3C (subcutaneous NF) and 3D (cutaneous NF) there are 39 and approximately 79 (!?) data points, respectively.

- Figure 3E, right shows only 28 data points.
These data should be checked and it should be more clearly stated (material and methods, results) how many cases (or how many blood samples from how many cases) were investigated each.

2. The internal tumor load was determined on the basis of semi-automated volumetric measuring using MRIs. Patients were then divided in groups with no, low, moderate or high internal tumor load. The authors should briefly add information how these groups were separated (cut offs).

3. Immunohistochemistry (p9-10, Fig. 2): The authors state on page 10 that the proportion of MIA1 positive cells was about 1:1 in neurofibromas and up to 10:1 in MPNST and that "In general, MPNSTs exhibited higher cellular density and thus showed higher proportion of immunopositive tumor cells (Figure 2)." However, a higher cellular density does not necessarily result in a higher proportion of positive cells. The figures show a higher cell density and a higher number of MIA1-positive cells in MPNST when compared to plexiform and non-plexiform neurofibromas. However, the (absolute) number of immunonegative cells is also higher in MPNST and the estimated proportion of immunostained cells seems to be approx. 50% in non-plexiform NF, approx. 70% in plexiform NF and approx. 50% in MPNST. Thus, if the figures already show representative results the statement in the section results needs to be corrected. Otherwise, the figures should be replaced and show roughly representative results.

4. Figure 1B is not cited in the text (pleas add on page 10).

5. Figure 3B: in the text a significant differences between cases with (+pNF) and without (-pNF) plexiform neurofibromas is stated (p=0.032). This should appropriately be illustrated in the figure.

Discretionary Revisions:
none

**Quality of written English:** Acceptable

**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.