Reviewer’s report

Title: Macrophage migration inhibitory factor engages PI3K/Akt signalling and is a prognostic factor in metastatic melanoma

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Reviewer: Sebastian Kobold

Reviewer’s report:

The manuscript by Oliveira et al. reports on the role of MIF in proliferation and signaling in melanoma cell lines. Using publicly available expression data bases, the authors show that MIF expression is associated with disease progression in metastatic but not in primary melanoma. The paper is well written and the data is sound supporting most of the conclusions made. The data on the inhibition of cell growth is mostly confirmatory but is a sound addition to current literature in human melanoma cells. PI3K is one of the known downstream signaling pathways of MIF but signaling of MIF through PI3K in melanoma cell lines was so far undemonstrated. MIF expression in primary and metastatic melanoma samples has been published previously but the authors of the present manuscript show for the first time an association with disease progression in the metastatic setting. Overall, the manuscript will be helpful to the field, once the points limiting its present quality have been addressed.

Major compulsory revisions:

1) In the introduction and later in the discussion, the authors put emphasis on the problem of endotoxin contamination in recombinant MIF samples. While the importance or rather the problem of endotoxin contamination is an issue when studying recombinant cytokines, the problem is certainly not limited to MIF but extends to any cytokine used. Given that the authors neither solve nor address the issue experimentally, the point should be taken out of manuscript or at least confined to the discussion on the methods used. Also the references used in this respect only partially support the statements made: for example reference 4: a review of MIF on innate immunity by Calandra and Roger not addressing this issue of endotoxin contamination.

2) In several passages (for example line 97), the authors write about “clinically relevant melanoma samples”. Since it is hard to conceive that any tumor sample may be clinically irrelevant, this statement should be corrected in a way that reflects the true nature of the samples.

3) In the discussion the authors state that so far only two studies have dealt with MIF in melanoma (one reference on a human, the other one on a murine cell line). Given that a pubmed search reveals at least several more references (mostly in murine B16 melanoma, but also in human uveal melanoma). This should be corrected and the references included in the discussion.

4) The authors should extend their discussion on additional sources on MIF in
the melanoma environment besides tumor cells.

5) In the conclusion and in several parts of the paper, the authors state that “MIF expression levels in metastatic melanoma is prognostic”. Given that the authors are studying retrospective cohorts of patient, no statement on prognosis can be made. It should say “association” throughout the text which reflects the data more accurately.

6) The cell lines used should all be named in the method section, a cross reference to previous publication is not appropriate. The authors should also specify how cell line identity has been assured.

7) Concerning the siRNA-sequences: the method and/or the discussion should contain a statement on whether or not similar or identical sequences have been used by other authors to silence MIF. Given the large number of available siRNA, this would be an important information to the reader.

8) In figure 5E, the authors use a student’s t test to compare MIF levels in patient samples which is confusing, since they used a Mann-Whitney test in the same figure, which is more appropriate for samples likely not normally distributed.

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

**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