Reviewer's report

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

Version: 3
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Reviewer: Christophe M Queva

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Major Compulsory Revisions:
- Given the potential toxicities and lack of specificity of siRNA (see for example Kassner, Combinatorial chemistry and High Throughput screening, 2008, 11, 175-184), the authors should present the data acquired from the 2 independent siRNA, a single one is currently shown. I assume it wouldn’t be complicated adding to fig 1 the extent of MIF knockdown, the relative cell number and viability observed with the second targeting siRNA.

- siRNA targeting MIF have a clear effect inhibiting the viability of Me1007 and to a lesser extend of MelCV. This effect cannot be explained by the decreased proportion of cells in S-phase on the cell cycle and need to be further explored as it may be much more important than the cell cycle inhibitory effect. Does MIF inhibition trigger apoptosis? Is Akt through BAD phosphorylation important in this process? The effect on viability should be discussed.

- Present the cell cycle data as a % of cells in the different phases of the cell cycle and repeat the experiment synchronizing cells. Is the effect of MIF inhibition on progression through G1 (as CDK4 and p27 would suggest) or are cells blocked and accumulating in S-phase?

- The link between MIF mRNA (assessed from publicly available dataset) is weak (less than 2 fold changes, small number of patients). The presumed link to metastasis is not represented by the biological data. The effect of decreasing MIF expression with siRNA on behavior expected to influence metastatic behavior (migration, invasion etc.) has not been studied.

- Acute inhibition of MIF pathway or stimulation by exogenous addition of recombinant MIF may allow deciphering whether changes in P-Akt, p27 and CDK4 are mere consequences of cell cycle inhibition or the direct outcome of MIF signaling. The current time point (3 days after transfection) is too late to provide this understanding.

Discretionary Revisions:

- MIF inhibition can be achieved by neutralizing mAb to MIF or to CD74 or using small molecule DDT inhibitors. These reagents are available and would be very useful providing an independent confirmation of MIF inhibition in melanoma.

- Receptors for MIF have been characterized. Are CD74/CD44, CXCR2 and/or CXCR4 expressed in melanoma cell lines? Which one of these receptors
mediate activation of the PI3K pathway upon MIF addition in melanoma cell lines?

Minor essential revisions:
- The viability assay is not described in the material and methods
- Fig 2E, please explain how a 25% reduction in S phase in MelFH can be statistically significant when a 30% reduction for MM200 with a tighter error bar is not.
- Akt is phosphorylated on multiple residues. Phosphorylation on T308 and S473 are important for full Akt kinase activation. The authors should specify which P-Akt entities they are looking at.

Level of interest: An article of limited interest

Quality of written English: Acceptable

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests:
I declare that I have no competing interests'