Reviewer's report

Title: Evidence for a positive feedback loop in the HGF/c-met/Stat3 signaling pathway during tumor cell invasion

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Reviewer: Yitzhak Zimmer

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Major Compulsory Revisions

(1) It is not appropriate/accurate to use the word "evidence" in the title as well as in the text itself, since the observations made here are done, as also the authors admit, using one single cell line and are not robust enough to be considered evidence and should be presented rather as indications;

(2) In the Background section it's not very clearly stated what is the clinical relevance/rationale of the study; if there are Stat3 inhibitory approaches in the development or use, it would be worthwhile to mention what types and this way to bring the presented data into some kind of logical background: also, it would be useful to explain better the system the authors are using – which features of normal Stat3 activity are still functional (Tyr phosphorylation?) in the DN cells and which are not (DNA binding?) and why and what can be observed by the partial suppression as compared to total suppression of Stat3 activity;

(3) total c-met levels: in vitro – page 13 – "levels of total c-met expression were slightly reduced in the DN cell lines"; in vivo – page 16 – "Unexpectedly, we found that unlike in S3DN cells grown in culture, total c-met levels were diminished in DN2 tumors compared to WT". Is it indeed "unlike"?; thus, also the interpretation of these observations ("This effect may be the result of...", page 16) is very speculative and actually not based on real data;

Minor Essential Revisions

(1) How was the number of cells lacking the cell-cell contact evaluated? How were those cells counted?

(2) How much of total protein was taken for the immunoprecipitations? Was this normalized for all the samples? (this information could be added to Materials and Methods section)

(3) There is possibly a confusion in the marking of the figures – on page 13, the text is calling figure 2C, however, most probably figures 2C-2H are meant (as denoted in the figure itself)

(4) - p. 15, 3rd line – more precisely, the text probably refers to Fig. 4A (not Fig. 4)

Discretionary Revisions

(1) One needs to be careful to consider the findings concerning c-met/Stat3
regulations and functions as well as Stat3 role to cell motility as very novel, since there are already studies linking these events (e.g., Cheng, N., Mol Cancer Res, 2008)

(2) Concerning the positive feedback loop itself: if Stat3 regulates the expression of HGF gene (among others), isn't that natural that there is a positive regulation of c-met activity?

(3) The authors are stating (page 11) the following: "It is unlikely that these effects are due to differences in cell survival or proliferation due to...". However, for the interpretation of other data presented in the study, it would be interesting to know, if there are indeed differences in these hallmarks.

(4) - p. 14 – paragraph "We note that differing...single tumors in mice." Perhaps it's not necessary to mention this?

(5) - in the Results section “Expression of S3DN alters the localization of active Stat3 in the cell", it would be maybe nice to confirm the subcellular localization of p-Stat3 also by looking at cytoplasmic and nuclear fractions of cell lysates (by Western blotting); this assay would distinguish more clearly between cytoplasmic and nuclear proteins as compared to microscopy, where (apart of the protein of interest) no indicative co-staining of nucleus or cytoplasm has been done (Fig. 4A);

(6) - in the discussion, the authors are commenting on association between c-met and FAK; maybe it would be worthwhile anyway to add these data to this study;

(7) - why in some figures/results, the NEO4 and DN5 cell lines are omitted while in the others they are present?

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.