Reviewer's report

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

Version: 1 Date: 20 July 2010

Reviewer: Faye Johnson

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In this manuscript, the authors express a dominant negative form of STAT3 in a single SCC skin cell line. The cells that express the dominant negative form of STAT3 were less invasive in vitro; were markedly less able to migrate in response to HGF; had decreased basal c-Met activation; had decreased c-Met activation after HGF stimulation; had decreased total c-Met as compared to wt cells; had markedly less STAT3-Met binding; were less invasive in vivo; and produced more / larger colonies in soft agar. HGF did not affect pSTAT3 levels in any of the cell lines. MMP2 and MMP9 levels were reduced in tumors that express the dominant negative form of STAT3 as compared to wt tumors.

The topic is very relevant as STAT3 is clearly important for the progression of multiple tumor types, including skin cancer. The paper is well-written and easy to understand. The data are clean and presented well.

Major Compulsory Revisions:

1. The entire study was conducted in a single cell line.

2. I think that readers will be confused about the effect of the dominant negative form of STAT3 on STAT3 activity because cells expressing the dominant negative form do not have decreased pSTAT3 and do have higher nuclear pSTAT3 (Fig. 4A), which is usually associated with increased STAT3 activity. The authors should indicate the extent of the decrease of STAT3 DNA-binding activity of the clones expressing the dominant negative form of STAT3 (showing DNA-binding data would also be helpful).

3. Please provide statistics for Figure 3B. The wide variability between wt and neo suggests that the changes observed may be due to chance alone.

4. Usually the observation of larger and more numerous colonies in soft agar is interpreted as the cancer cells being more tumorogenic, having higher rates of proliferation, and/or have less apoptosis. Here, the authors have interpreted this result as being due to increased homotypic adhesion. How is this novel interpretation of the soft agar results supported by the data?

5. The presented data do not support a feedback loop. The most straight forward interpretation of the signaling data presented in this manuscript is that both HGF and activated STAT3 can activate c-Met independently. MMP2 and MMP9 are downstream of STAT3. MMP2 and MMP9 are needed for invasion and motility. There is no direct evidence that the activation of c-Met is needed for the effects
of STAT3 on invasion.

Minor Essential revisions

1. Given that STAT3 can affect proliferation and survival, it is possible that the observed decrease in invasive cells was due to apoptosis. The authors state that the 10 hour assay time is too short for any differences in proliferation and survival to affect results but provide no data to support this claim.

2. Please explain why the total c-Met levels were increased in wt cells incubated with HGF (Fig. 2A).

3. Add the pSTAT3 data to Figure 2A.

Please note that I had technical difficulty opening the supplemental files and I did not personally review these data.

Level of interest: An article of importance in its field

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.