Reviewer's report

Title: A synergistic interaction between transcription factors nuclear factor-kappaB and signal transducers and activators of transcription 3 promotes gastric cancer cell migration and invasion

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Reviewer: brendan jenkins

Reviewers report:

The upregulation of transcription factors, NF-κB and STAT3, has been implicated in many cancers, including gastric cancer. This manuscript by Yoon et al aims to bridge the gap between the crosstalk of these signaling molecules in gastric cancer. More specifically, they showed that the inhibition of NF-κB reduced STAT3 activation in a gastric cancer cell line (SNU-638), and inhibition of these two signaling molecules significantly suppressed the metastatic potential of SNU-638 cells. Overall, the manuscript is clearly written, however there are several major concerns with the manuscript as presented, some of which include unconvincing data and the lack thereof. With respect to this, this manuscript requires major revisions and would not warrant publication unless these concerns are addressed.

Specific comments:

Minor essential revisions
1) On page 10, the title of the first paragraph needs to be more informative to the reader.
2) Figure 1, scale bars are missing.

Major compulsory revisions
3) While Figure 2 shows that SNU-638 cells over-expressing IκBαM displayed reduced levels of STAT3 protein expression and activation, the mechanism by which this is occurring has not been addressed. For instance, is NF-κB directly regulating the transcription of STAT3 and are there any NF-κB binding sites in the STAT3 promoter? To address this, STAT3 luciferase reporter assays should be conducted. In addition, given that a study has shown that STAT3 and p65 can heterodimerize to transcriptionally regulate NF-κB-dependent genes (Yang et al, Genes and Development, 21, 1396-1408), it is therefore important to investigate this by performing co-immunoprecipitation experiments on these cell lines. Furthermore, the 2nd paragraph on page 13, the authors state that their findings are consistent with Wani et al (2011), however they have not shown that IL-6 is reduced in the SNU-638 cells over-expressing IκBαM, which may account for the reduced STAT3 levels.
4) Figure 3B and 4B show that IκBαM and siSTAT3 over-expressing SNU-638 cells are significantly less invasive. Given that their immunoblot assays showed
that I#B#M and siSTAT3 reduced pRelA and pSTAT3 by approximately 50%, respectively, the marginal decrease in the percentage of invasion does not look convincing. Therefore, I would recommend showing these experiments in a second gastric epithelial cell line.

5) While Figure 3C and 4C shows that EMT markers are altered in I#B#M and siSTAT3 over-expressing SNU-638 cells, this was not looked at in SNU-638 cells that over-express both I#B#M and siSTAT3. Since EMT is linked to cell migration and invasion, and Figure 5B and 5C suggest that a reduction of pRelA and pSTAT3 synergistically contributed to cell migration and invasion, it is important to show that these EMT markers are also altered in SNU-638 cells that over-express both I#B#M and siSTAT3.

Level of interest: An article of limited interest

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' below.