Author’s response to reviews

Title: Unphosphorylated STAT3 in heterochromatin formation and tumor suppression in lung cancer

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Version: 1 Date: 15 Jan 2020

Author’s response to reviews:

We thank the editor and reviewers for their constructive suggestions. We have revised the manuscript accordingly as detailed below.

Editor Comments:

We have discussed your manuscript in-house and provide you with an editorial decision based on the Reviewer's reports and our own assessment. Please be informed we might seek an additional opinion for your revision.

1) Please provide the raw data of presented Western Blots images for our assessment attached in your revised manuscript.
Reviewer reports:

We provided these as Figure S1

Sung-Hoon Kim (Reviewer 1): Authors suggested that unphosphorylated STAT3 (uSTAT3) in the cytoplasm acts as a tumor suppressor due to heterochromatin-promoting activity in A549 cells in vitro and in vivo. Despite interesting data, it has some concerns as follows:

1. Show STAT3Y705F and STAT3S727 and STAT3Y705 in cytoplasm and nucleus

As shown in Figure 2B, STAT3Y705F is localized in both the nucleus and cytoplasm, but more in the former, whereas STAT3V462A (with HP1-binding motif disrupted) is mainly localized in the cytoplasm. STAT3S727 is not investigated in the study.


Hu et al show that uSTAT5A associates with HP1 and plays a role in tumor suppression. Our uSTAT3 results are consistent with the finding. A comprehensive analysis of transcriptome profile, including the oncogenes mentioned above, is indeed informative but is beyond the scope of this paper. We are planning to do RNA-seq experiment with different transfections.
3. It is well documented that nuclear translocation of STAT3 enhances tumor growth. But what's the significance of uSTAT3 in nucleus? Dual roles of stat3? What ratio between uSTAT3 and p STAT3 in nucleus? How can the final action of STAT3 be decided after completion between uSTAT3 and p STAT3 in nucleus, since unphosphorylated STAT3 (uSTAT3) can have significant transcriptional control over the expression of genes such as RANTES, IL6, IL8, MET and MRAS, which do not respond directly to phosphorylated STAT3 (pSTAT3). Unphosphorylated STAT3 accumulates in response to IL-6 and activates transcription by binding to NFκB) and the unphosphorylated form of STAT3 binds to regulatory regions of proapoptotic genes and prevents their expression in tumor cells but not normal cells (STAT3 suppresses transcription of proapoptotic genes in cancer cells with the involvement of its N-terminal domain)

As we stated in Background, uSTATs (including uSTAT3) can translocate into and prominently exist in the nucleus (9-16), yet their functions are unclear, although transcription activity was also reported for uSTAT3, as the reviewer described above. Our manuscript describes an underappreciated epigenetic function of uSTAT3 in promoting heterochromatin formation.

4. Show the novelty of your MS compared to previous evidences in Introduction

We stated in the Introduction (Background), non-canonical function of uSTATs was shown for STAT5, we found that STAT3 and HP1α partially colocalize in the nucleus and might physically interact, and that uSTAT3 promotes heterochromatin formation and suppresses lung cancer cell proliferation in vitro and in vivo. These results suggest that the non-canonical functions of STAT3 operate in lung cancer cells, in which uSTAT3 plays a role in suppressing cancer growth.

5. Add LMO approval for shSTAT3 cell lines using lentiviral vector

We added “Cell culture and transfection procedure was approved by UCSD BUA R1347” to Methods (Page 8, line 11).
Zhipei Zhang (Reviewer 2): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Tao Ren (Reviewer 3): JAK/STAT pathway plays an important role in many types of human cancers. Usually this pathway exert its role by phosphorylating STAT, but the role of unphosphorylated STAT is still unknown. In this study the author found that uSTAT3 may has a function in promoting heterochromatin formation in lung cancer. However, more experiments are necessary to make the results more convince. Although the authors verified STAT3 interacts with HP1a and regulates heterochromatin formation, the mechanism is not of that clear.

We have shown that uSTAT3 is found in the nucleus and partially interacts with HP1, uSTAT3 promotes heterochromatin formation, and uSTAT3 suppresses lung cancer growth. We agree that the mechanism is still not clear but we are continuing to investigate. At this stage, we think it is worth publishing what we have to stimulate research in this area.