Reviewer's report

Title: Stat3 is a positive regulator of gap junctional intercellular communication in cultured, human lung carcinoma cells

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Reviewer: Morten Schak Nielsen

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Major Compulsory Revisions

1. The rationale of the study is a bit unclear. The authors have previously shown that expressing activated Src in liver epithelial cells down regulates GJIC independent of Stat3 (PMID 19456249). Is there reason to believe that lung cancer cells with activated Src should be different? The aim should be clarified and the introduction updated to include what is known about stat3 and GJIC.

2. The authors claim that the reduction in coupling upon Stat3 inhibition is unexpected despite the fact that they already demonstrated this in liver epithelial cells (PMID 19456249). This is the major finding according to the conclusion, but I think that some mechanistic investigations should be performed. Does Stat3 induce Cx43 transcription? This is the case when activating the JAK/Stat pathway in astrocytes (PMID 15342787) and during differentiation of stem cell to cardiomyocytes (PMID 17823373). Does Stat3 preserve Cx43 indirectly by preventing apoptosis? This was suggested from the work in rat liver epithelial cells (PMID 19456249), but if so what is the mechanism?

3. What is the rationale of measuring Src and Stat3 at 50 % confluence given the fact that Stat3 and GJIC change with cell density (see also under minor essential revisions)? I think this compromises the conclusion that CPA7 and sh-Stat3 treatment was efficient in the communication-competent cell lines. At best the experiments should be redone at the same densities as the GJIC measurements, or at least the problem should be stated clearly as a limitation.

4. Statistical analysis is completely lacking and in some places the number of experiments is unclear (ie for Cx43 quantification in E10 cells). This should be fixed.

5. The data of table I is repeated in table II. They should be merged to one. Are data presented as average +/- SEM? And why is the error lacking for GJIC in some cell types? Please clarify.

6. Methods are appropriate, but their description is unbalanced. The electroporation technique is described in great detail including two figures and a description of the slide manufacturing. In contrast, western blotting is hardly described, lacking clear identification of the antibodies used (cat. numbers and dilutions) which could be important for reproducing the data.

Minor Essential Revisions
1. On page 8: “….had very low Cx43 at all densities tested”; add “(data not shown)”

2. On page 10: “As shown…….were assessed at a confluence of 50%...”; to me it makes no sense to compare GJIC data with Src and Stat3 data at a different density (see above). Please revise.

3. On page 10: “…cannot be solely responsible for the lack of junctional communication…”; data shows that Stat3 is not responsible at all.

4. On page 14: “….so that the net effect of Src expression is gap junction closure.”; since Cx43 is not detectable, Src can hardly inhibit it directly. Please revise.

**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