Author's response to reviews

Title: SSeCKS/Gravin/AKAP12 attenuates expression of proliferative and angiogenic genes during suppression of v-Src-induced oncogenesis

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Author’s response to reviews: see over
To Whom It May Concern:

I am resubmitting MS-8569035909157100v.3 for publication in BMC Cancer. Below are the point-by-point responses to the reviewers’ comments. In brief, in this second resubmission we address several points that remain from the two reviewers.

Responses to Reviewers

Reviewer- FOSTER

1) It was pointed out that the major weakness of the report – in the opinion of this reviewer - was that the data are only correlative and the supposition that “SSeCKS reprograms proliferative and angiogenic gene expression” over-interprets the impact of the work. This point was not addressed by the authors. I believe that the title overstates the impact of the work. Also, as pointed out below, the authors have not adequately addressed the minor, but important, points made.

Response: I agree that the title may be overstated based on just the correlative microarray data. Given our background data on the tumor- and metastasis-suppressive effects of SSeCKS, would the reviewer be more comfortable with: “SSeCKS/Gravin/AKAP12 attenuates expression of proliferative and angiogenic genes during suppression of v-Src-induced oncogenesis”?

2) Figure 1b – Src protein is up in HT29 cells relative to HCT116 – the activity – as measured by Y416 phosphorylation is not elevated if one normalizes for total Src protein. They claim to have put in a new figure that clarifies. I still see that there is as big an increase in S src protein as there is in the level of Y416 phosphorylation. A densitometer tracing would clarify if my eyes are deceiving me. It looks like there is no increase – or even a decrease.

Response: Given that human cancers rarely encode mutated Src, but rather overexpress c-Src (as is the case with HT29 relative to HCT116), the relevant issue is the total level of Src activity in a cell rather than the specific activity (total activity divided by the protein level). Thus, the work of Neal Rosen, Rich Jove and Bart Sefton in the 1990’s underlines the notion that high levels of total Src activity (due to increased transcript levels or gene amplification) contribute directly to the oncogenic phenotype of human cancer cells. This contrasts with the example of mutated Src alleles (v-Src or c-Src527F) that are sufficient to transform cells due to their increased Src specific activities as well as to constitutive activation levels. I apologize if this did not come across clearly. However, the data in Fig. 1B are correct (and more clearly presented than in the first submission), and they agree fully with published data on the Src protein and activity levels in the two CoCa lines.

3) Figure 2b does not reveal the “significant” increases in expression of the genes shown with...
the exception of HMGA2. The increases are very marginal. The authors point out claim that there is a big increase in HMGA2. I too saw the increase. It was the other genes that appear to be marginal in there change in expression. I don’t believe that there is a problem with the copy of the figure I received because I do see the increase in HMGA2.

Response: We have performed a densitometric analysis (GeneTools software, Syngene; measure pixel density per set area) of the data from Fig. 2B, and most importantly, they show similar trends to the gene expression changes induced by Src in 3T3 cells (Table 4, column B). For example, Src induces FOSL1 and PSCDBP roughly 3-fold in Table 4B and in Fig. 2B. Although CDC20A was not identified as directly induced by Src in Table 4, it was downregulated ~2-fold by SSeCKS re-expression in the presence of v-Src in Table 5; this correlates with its ~2-fold upregulation in Fig. 2B. Thus, the main point here is that activated Src levels in the HT29 seems to recapitulate several of the gene expression trends we attributed to Src control in the 3T3 cells. I agree that these changes are not “substantial”, however, we specifically used the word “significant” because they were statistically significant and reproducible.

Reviewer- Dittmer

1) Table 1: The sequences of the primer pairs used for the detection of the additional genes as listed in Table 6 should be added.

Response: Because of the 3 week deadline to resubmit the previous version of MS, we opted to purchase pre-validated Q-RT-PCR kits from Qiagen (QuantiTect) for each of the genes requested by the reviewer (11 sets in total). These kits produced the correct sized products and in duplicate assays, showed similar v-Src and/or SSeCKS-induced effects to their mRNA levels as detected in the microarrays. Unfortunately, the company will not release the primer sequences due to propriety (see attached letter from Dr. Janina Lehmann, Global Assoc. Business Director, Qiagen), and thus, the best that can be done is to cite each gene’s catalogue number in Table 6. This will still allow others in the field to get the exact same reagent we used. I am sorry for this inconvenience, but I have argued my point with the company to no avail.

I am confident that the revised MS addresses all the comments made by the reviewers. Please contact me if there is anything else I can do to help in the publication of this data.
Dear Dr. Gelman,

it was nice talking to you and thank you for sharing your opinion on the QuantiTect Primer Assays. As explained QIAGEN would like to keep the QuantiTect Primer Assay sequence information confidential to protect QIAGEN's competitive advantage and to avoid risking R&D investments. However, we would like to support you and would like to make an exception. By signing a CDA we are open to disclose all sequence information of QuantiTect Primer Assays you used for your research. The same applies for the BMC Cancer reviewer who requested this information. Please notice that the sequence information will be for your and the reviewer information only not for publishing.

To clarify on BMC Cancer policy please find attached a link of a 2006 BMC Cancer publication which describes the use of 49 Applied Biosystems TaqMan Assays for Microarray validation. The publication has been accepted without giving any sequence information

http://www.biomedcentral.com/1471-2407/6/54

Cross-platform expression profiling demonstrates that SV40 small tumor antigen activates Notch, Hedgehog, and Wnt signaling in human cells

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If you have any further questions please don't hesitate to contact me.

Best regards,

Janina Lehmann