Reviewer's report

Title: SSeCKS/Gravin/AKAP12 Reprograms Proliferative/Angiogenic Gene Expression During Suppression of v-Src-Induced Oncogenesis

Version: 2 Date: 13 February 2006

Reviewer: Jürgen Dittmer

Reviewer's report:

General

The manuscript by Liu and Gelman describes microarray experiments performed to identify genes regulated by v-src and Src suppressed C kinase substrate (SSeCKS) in murine fibroblasts. Three sets of microarrays were carried out. In the first one, the effect of SSeCKS on gene expression was determined by using S24 cells, NIH3T3 derivatives harboring tet-regulated SSeCKS cDNA. In a second set of microarrays, v-Src-dependent changes in gene expression were monitored in S24/ts72v-Src containing a temperature-sensitive form of v-src. Finally, SSeCKS alterations of gene expression were measured in the presence of activated v-src by keeping S24/ts72v-Src cells at permissive temperature. Twelve genes were found whose RNA levels were changed in response to SSeCKS expression irrespective of whether active v-src was present or not. Of these, four genes (Hmgb3, Gfra1, Il1r1 and Afp) were inversely regulated by v-src suggesting that these genes are important targets for SSeCKS to suppress v-src-induced oncogenesis.

Overall, the data are interesting and allow new insights into the mechanism of v-src and SSeCKS actions. However, some issues have to be addressed before publication of the data can be recommended.

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Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Major points:

Fig. 1A: The authors claim that the SSeCKS protein in tet-treated S24/ts72v-Src cells as detected by Western blot analysis is derived from endogenous expression and that the reduced SSeCKS level relative to that in tet-treated S24 cells is due to the presence of activated v-Src. How can the author exclude the possibility that the SSeCKS levels resulted from leakage of the tet system rather than being the consequence of endogenous expression and how can the authors be sure that the reduced SSeCKS level in S24/ts72v-Src cells vs S24 cells is not simply due to different degree of leakage as S24 and S24/ts72v-Src are two different cell lines? The authors need to compare the SSeCKS level in temperature-permissive vs. non-permissive S24/ts72v-Src cells in order to substantiate their claim.

Fig. 1B: To me, the src activity seems quite similar in HCT116 and HT29 cells. Hence, I don’t agree with the authors claiming that the lower SSeCKS expression in HT29 cells is the result of higher src activity. Rather, these data suggest an inverse relationship between src and SSeCKS expression in human colon cancer cells which may be the consequence of a coordinated regulation of these genes by a common factor.

Fig. 2: It is important to verify the microarray data by RT-PCR for all 12 genes commonly found to be regulated by SSeCKS in the presence and absence of active v-src. At least the expression pattern of those four genes that are inversely regulated by v-src and SSeCKS need to be verified and their
expression analyzed in HCT116 and HT29 cells. Generally, quantitative PCR should be preferred over semi-quantitative PCR to quantify gene expression.

Table 3-5: The authors mentioned in Materials and Methods that at least three independent microarray analyses have been performed for each condition. To get an idea of how strongly the data vary within the same condition, the individual data of each of the three experiments should also be shown in addition to the average values.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Minor point: “NC” should also be explained in legend of Table 4.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article of importance in its field

Quality of written English: Acceptable

Statistical review: No

Declaration of competing interests:

I declare that I have no competing interests