Reviewer's report

Title: TSPY potentiates cell proliferation and tumorigenesis by promoting cell cycle progression in HeLa and NIH3T3 cells

Version: 1 Date: 7 April 2006

Reviewer: Joachim Arnemann

Reviewer's report:

General

--------------------------------------------------------------------------------

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

TSPY has been linked to a variety of human tumor tissues, supporting the hypothesis of a human Y-chromosomal gene with oncogenic potential. Due to a SET/NAP domain a potential function in cell cycle regulation and replication has been postulated.

The authors tried to approach TSPY's cellular function by classical cell transfection experiments and controlled overexpression of the TSPY gene using the Tet-off system.

Comments:

a) The methods are not always appropriate. Not sufficient details are provided for the microarray analysis and the evaluation of the data. The semi-quantitative RT-PCR analysis lacks proper controls.

b) From my point of view they failed to answer this "generalized" question of TSPY's cellular function. The finding that over-expression of TSPY in Hela cells accelerates the cell cycle in culture, especially G2/M transition, is of some interest, as there is the observation that NIH3T3 Tet-off cells overexpressing TSPY are capable of inducing tumors in nude mice.

However, as long as the basic molecular mechanisms are unknown, it cannot be excluded that the accelerated cell cycle is a secondary effect. What is the proven molecular link to cyclin B1?

c) The presented microarray analysis cannot be accepted for the following reasons:

- It is unclear how many biological and/or technical replicas have been carried out. I have to assume only one set of experiments/data. According to Affymetrix's customer informations there is already a deviation of approx. 5% in technical replicas. From this aspect it is advisable to present the expression data as decimals only with the tenths and to drop the hundredths.

- It has not been mentioned that the expression values in table 2 most likely will represent fold-changes. It is unusual to present the down-regulated genes in this format. It is more common to use negative values for the downregulated fold changes, however, I have to admit that it will be possible. For example, IGFBP has an expression value of 0.49 which corresponds to -2.04 according to the alternative scale.

- The p-values have not been included. From our experiences and from the informations given by Affymetrix the significance of expression data is reduced in a lower range between 2.0 and -2.0 fold changes.

- What about the false-discovery rate?

- In case the expression values of all 54.000 genes of the Affymetrix U133 Plus2 array had been used for the biostatistical analysis a so-called multiple test problem will come up. Or has a filter been used to select for certain probe sets?
d) The presented semi-quantitative RT-PCR data (Fig.5) are unacceptable. The method of choice would be the use of a real-time PCR equipment, which is usually available at places where microarray data have to be validated. However, in the case semi-quantitative RT-PCR has to be used, a control gene, like GAPDH or BIP, should be included in the PCR assay to monitor any differences between the samples.

e) The result/conclusion that “numerous genes involved in the cell cycle and apoptosis are affected by TSPY expression in HeL cells” is not supported by the presented data!

f) In a recent paper, Krick et al. (2006) reported on similar experiments, where they expressed TSPY as an EGFP fusionprotein in HEK 293 cells, but did not find this scattered locations along the periphery and within the nuclei, as shown in Fig 1F,H. This fact has to be discussed.

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

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No

**Declaration of competing interests:**

I declare that I have no competing interests.