Reviewer's report

Title: Musashi1 modulates cell proliferation genes in the medulloblastoma cell line Daoy

Version: 2 Date: 25 June 2008

Reviewer: Joel Yisraeli

Reviewer's report:

Major compulsory revisions:

1. At the beginning of the results section, the authors conclude from the fact that Msi1 is upregulated 2-fold in neurospheres as compared with monolayer cultures that “Msi1 may contribute to cancer cell progression.” If anything, neurospheres may be some sort of measure of “stemness,” which the authors themselves suggest in the introduction. Given that the neurospheres are grown in the presence of several different growth factors that may have different effects on a wide range of genes, it seems somewhat gratuitous to conclude anything about the connection between Msi1 and cancer progression from this experiment.

2. On page 9, the authors demonstrate that the ability of Daoy cells to form colonies in soft agar is significantly reduced when Msi1 has been knocked down. This effect on anchorage-independent growth, however, does not automatically mean that Msi1 promotes cell proliferation, as the authors conclude.

3. The analysis of neurosphere formation following Msi1 knockdown requires additional clarification. In this case, the number of neurospheres larger than 50 um in diameter is observed to drop 2-fold as compared to cells with no knockdown. Is this due to an effect on cell proliferation, sustaining cancer cells, sustaining stem cells, rate of cell growth, or apoptosis – all equally possible, but different, explanations? The authors first suggest that this assay evaluates cell proliferative potential, and then later in the same paragraph conclude that this indicates a role for Msi1 in sustaining cancer cells. No reasoning is given for choosing one explanation over another. In addition, although the text indicates that both the number and overall size of the spheres was affected, no data is presented showing the effect on overall size.

4. As mentioned in comment #1, the authors suggest that upregulation of Msi1 in neurospheres reflects its role in cancer progression. If one wants to learn what target genes are being activated by Msi1 in this cancer environment, then why not compare the effects on gene expression of Msi1 knockdown in neurospheres, rather than in monolayers? The shRNA used for this analysis should be mentioned in the paper. Did all three of the shRNAs give similar gene expression profiles?

5. I find the description/interpretation of figure 5 unclear. First of all, the neurospheres that grew from the KD cells in 20uM cyclopamine look larger than
those that grew in 5uM cyclopamine. Second, I do not see a difference between
the control and KD neurospheres that grew in tomatidine, a negative control,
even though figure 3 argues that there should be a reduction in both number and
size of the KD spheres. Third, given that 5uM cyclopamine has a clear effect on
the KD spheres, one can argue that Hedgehog signaling is important for
formation and/or growth and/or maintenance of neurospheres, at least in the
Msi1 knockdown environment. Is this the source of the defect in the Msi1 KD
spheres? In this experiment, it is very hard to know, because the KD spheres
grown in tomatidine look like the control spheres. In short, I am not sure what we
learn from this experiment.

6. The fact that there is less bcl2 mRNA and protein in the Msi1 KD does not
prove that there is more apoptosis. To check apoptosis, one should check
TUNEL or caspase activity.

**Level of interest:** An article of limited interest

**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