Reviewer's report

Title: Methylthioadenosine (MTA) inhibits melanoma cell proliferation and in vivo tumor growth.

Version: 2 Date: 23 March 2010

Reviewer: Stefan Wiemann

Reviewer's report:

In manuscript 2038747753348999 Andreu-Perez and co-workers describe methylthioadenosine (MTA) as a potent inhibitor of melanoma cell proliferation as well as tumor growth, using in vitro and in vivo models. MTA is a naturally occurring nucleoside that is metabolized by the enzyme 5'-methylthioadenosine phosphorylase to produce adenine and methylthioribose-1-phosphate. These products are further metabolized to AMP and Methionine, respectively. Some tumors have been identified that lack the 5'-methylthioadenosine phosphorylase activity and that in consequence cannot metabolize MTA. Accumulation of MTA in some tumor cells has been described to induce tumor suppressive effects. Since MTA has been proven to be well tolerated in vivo, also in humans, this molecule could be an interesting candidate for therapeutic intervention. Here, Andreu-Perez tested the effects of MTA using several melanoma cell lines as well as employing xenograft mouse models. Their most important finding was that MTA was indeed able to inhibit proliferation of melanoma tumor cells in vitro as well as in vivo. The authors conclude that MTA is a novel therapeutic candidate for the treatment of melanoma.

Major Compulsory Revisions

The authors tested the cell line models for their respective mutational status in the NRAS and BRAF genes. Why have they not looked into the gene encoding 5'-methylthioadenosine phosphorylase? The relevance of that protein in the effects MTA exerted on different tumor cell lines was recently described (Lubin and Lubin, 2009 PLoS One, 4(5):e5736). It should be essential to extend the analysis of mutation status to include at least also the MTAP gene. The small number of cell lines that was analyzed in the present study, however, is not really able to support the claim stating that the observed effects would indeed originate from mutations in NRAS or BRAF genes. 1. Have the data that are shown in Figures 1 and 2 been statistically analyzed, and are the differences significant? There appear to be some differences in the effect sizes for all these cell lines and it should not be so easy to really pinpoint any effect to exactly the NRAS or BRAF mutations. 2. All these cell lines have different genetic backgrounds. More recent evidence from other tumors suggests that every tumor carries genetic alterations in a number of genes rather than in only just a few. These differences may be SNPs, i.e., classical mutations, copy number variations or aberrations, and epigenetic changes. All these contribute to the different phenotypes of these tumors and to cell lines that have been derived thereof. It should be over
simplistic to attribute effects to just one mutation, without knowing the genetic background of the systems as well as not having tested this hypothesis experimentally (e.g., with help of knock-in mutants).

On page 8 (MTA reduces melanoma cells survival), the authors found that “interestingly, 1 micromolar concentration of MTA promoted an increase in the number of colonies in” some cell lines. This is a rather disturbing observation. In the light that MTA is quickly metabolized in the blood by MTAP, the concentration of MTA will quickly lower after administration of a dose. Hence, the concentration at the effective dose will not last for long, while the concentration having adverse effects might not be able to be controlled. There will be a need for further research on this phenomenon before it can really be claimed that MTA could become an effective drug. Again, the genetic background of the cell systems could provide answers as to which patients could benefit from MTA treatment and which would not. For example, recently the mutation status in the BRAF gene was found to be correlated with the rather opposite effects that were induced by a BRAF inhibitor (Nature 2010 Mar 18;464(7287):431-5).

On page 9 (MTA promotes cytostatic effects rather than pro-apoptotic responses), the authors write that in their system the killing of melanoma cells is via cytostatic effects of MTA rather than via induction of apoptosis. On the one hand this is a little in contrast to the data shown in Figure 4B, where a significant increase in apoptotic cells is shown after treatment with MTA. On the other hand it should be interesting to investigate why some cell systems appear to preferentially undergo apoptosis while treatment with MTA rather induces cytostatic effects in others.

On page 11, the authors state that their “findings are in agreement with previous publications showing the inhibitory effects of MTA on proliferation and invasion in different types of tumor cell lines”. While I do see a significant effect MTA has on proliferation, the authors appear to not having tested cell invasion phenotypes experimentally. A few sentences later, it is said that the “BRAF mutant melanoma cell lines showed the highest sensitivity to MTA treatment, where concentrations of 1 micromolar of MTA blocked proliferation and viability”. This statement is not really supported by the data shown in Figure 1, where I could not see any differences in the cell numbers between control samples and cells treated with 1 micromolar MTA. In fact, a statistical analysis of the effects MTA should have on cell numbers is lacking and thus it is difficult to infer any effects just based on visual inspection of the graphs.

Figure 1: All cell lines display effects upon treatment with MTA. It would have been advisable to include some control cell line that was not affected by, for example, 600 micromolar MTA. Are the differences in the growth characteristics between the cell lines statistically significant?

Figure 2: An analysis of this experiment appears to be not trivial, as the ability of cell lines to form colonies is very different already under conditions of the controls (without MTA). While cell line 37-31E appears to very effectively grow in soft agar (?)/polyHEMA (?), SKMel147 appears to do not. Hence, a potential reduction in
the number of clones in response to MTA treatment should be hard to detect with the latter cell line. Some statistical analysis should help to demonstrate any significance of the results. Why are there no colonies at all in the dish showing Colo820 cells treated with 1 micromolar MTA? The data from Figure 1 would suggest that MTA should be growth promoting at that concentration. This apparent discrepancy should be discussed.

Discretionary Revisions

Page 6, (Colony formation assays): “At least two experiments in triplicates per cell line were performed”. I should suggest to change this to “At least two biological replicates with three technical replicates each were performed for every cell line.”

Page 7, qRT-PCR: 18S is said to having been used as an internal control. I would guess that this is the 18S RNA. Am I right that random priming of cDNA was carried out also to reverse transcribe the mRNAs having been quantified? The order numbers of Taqman probes for the tested genes and control should be provided.


Page 11, last sentence of first paragraph: “..., we observed a mild increase of apoptosis in our tumor samples.” What exactly is a ‘mild’ increase?

Figure 3: What kind of tissues is shown in the right panel of Figure 3A? This should be mentioned in the legend for that figure. Did the mice develop one tumor each after the injection of cells.

Table 1: The insignificant differences in DMSO and MTA treated cells with respect to ALT and AST could be stated as such in the text. The table is not necessary.

The authors say that MTA would be a novel drug potentially useful for melanoma treatment (abstract). In the light of literature, e.g., reference 29, where in 2004 a therapeutic potential for MTA was put in context with cancer, I would not really speak of a ‘novel’ drug.

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests