Reviewer's report

Title: Systematic validation of predicted microRNAs for cyclin D1

Version: 1 Date: 22 January 2009

Reviewer: Richard Pestell

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Comments for paper review:

This work used a luciferase reporter system to validate the target interaction of miRnada database predicted miRNAs on cyclin D1 3'UTR reporter activity. These studies represent an extension of substantial prior published work and thus should be formally described as such in the introduction. 45 miRNAs were successfully cloned and overexpressed in 293T cells. 7 miRNA repressed the luciferase activity carrying the 3'UTR of cyclin D1 in vitro. miR-503 decreased cyclin D1 abundance in vivo and inhibited cell proliferation. This work provides more information on the miRNA regulating cyclin D1. The key concerns with this study are the lack of detailed information within the text to understand the authors conclusion and the lack of integration with the previously published work on this exact topic (miRNA regulating of the cyclin D1 3'UTR).

Several concerns arise that should be addressed by the authors.

1. In the abstract, result and discussion, the authors state that miR-503 functions as a tumor suppressor. It is too early to conclude this based on one MTT assay.
2. In the materials and methods, the name of the PCR cloning vector should be indicated. Also the exact base pair positions of the cyclin D1 3'UTR-1, 3'UTR-2 and deletion clones should be indicated.
3. In page 11, authors have to be very careful to say that “different programs often predict different microRNAs for a given coding gene.” Different programs may not predict the same results, but most of the miRNAs predicted for one given gene should be the same.
4. In page 12, the exact length of the cyclin D1 UTR should be indicated.
5. Fig. 1 and table 2 provide the same information. The authors should remove redundant information.
6. In this paper, reporter assays and miRNA overexpression data were from 293T cells, while the western blot, FACS and MTT assays were performed on UMSCC10B cells. The authors should show evidence that the functional analysis and miRNA expression are linked in the same cell system. The authors should show the basal and overexpressed level of miR-503 in UMSCC10B cells. Was the cyclin D1 protein level suppressed by miR-503 in 293T cells? The authors should show reporter assays from UMSCC10B cells.
7. In the MTT assay, authors chose day 1 and day 5 as two time points. Usually
cells will be overgrown after 5 days of incubation. What was the confluence of this cell type at day 5?

Comments 6 and 7 are major compulsory revisions. Comments 1 through 5 are minor essential 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, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.