Title: siRNA Inhibition of Telomerase Enhances the Anti-Cancer Effect of Doxorubicin in Breast Cancer Cells

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Reviewer: Lynne Elmore

Reviewer's report:

Major Compulsory Revisions:
· The authors nicely demonstrated that their hTERT si-RNA knock-down strategy efficiently reduces hTERT at the mRNA and protein levels. However, they do not measure levels of telomerase activity and they do not assess telomere lengths. Instead they reference a previously published paper that states hTERT levels correlate with activity. While this is true, it is important to determine whether their level of knock-down is sufficient to cause a marked reduction in telomerase activity. If not, it is unlikely that this is a telomere length-mediated cell death.
· The authors fail to cite and discuss an important publication: Cerone, et al. Molecular Cancer Therapy 2006; 5(7): 1669-1675 (“Telomerase inhibition enhances the response to anticancer drug treatment in human breast cancer cells”). In many ways this publication undermines the novelty of the paper under review. Other papers that should at least be cited include Tauchi et al, Methods Mol Bio 2007, 405:181-189 and Ludwig et al, Cancer Res 61, 3053-3061, 2001. Both of these papers relate to telomerase inhibition and sensitization of breast cancer cells to topoisomerase inhibitors. Since multiple papers have been already been published on telomerase inhibition enhancing anti-cancer effects of doxorubicin in breast cancer cells, Dong et al need to clearly articulate how their study advances the field.

Minor Essential Revisions:
· Several studies have reported that a similar dose of AdR causes senescence rather than apoptosis in MCF-7 cells. At a minimum, these studies should be briefly described and cited.
· There is an inconsistency in the number of cells inoculated into nude mice. Is it 2 million or 20 million? If it is 20 million, this is an unusually high number and needs to be justified. There is mention that MDA-MB453 cells are not tumorigenic in nude mice. I believe these cells are tumorigenic, but they are not invasive or metastatic.
· Numerous places in the text the authors conclude that there are “statistically significant” differences, yet no statistical test was performed. Inclusion of a statistical test, such as a student’s t-test, is appropriate with the p-values and statistical differences being noted on the figures and/or in the legends.
· The gel in Figure 1D needs to be replaced with a better quality image.
Discretionary Revisions:
· This study limited the AdR concentration to a single acute dose at 0.5uM. If a range of concentrations (i.e., 25-500nM) + hTERT inhibition was included one could determine whether knock-down by siRNA sensitizes breast cancer cells to AdR, which would be encouraging pre-clinical data since AdR is highly toxic to patients.
· This study would be greatly strengthened by inclusion of a cytogenetic analysis (i.e., GTG-banding showing a high frequency of end-to-end fusion, rings and radials, which would indicate telomere dysfunction);
· To complement the apoptosis data, inclusion of senescence (i.e., SA #-galactosidase activity) as an end-point would strengthen the manuscript.
· Rather than include gross images of the xenografts (Figure 3A), including a tumor section revealing apoptotic cell death would integrate nicely with the in vitro data. This could include a TUNEL assay as simply visualization of apoptotic bodies on an H&E stained tumor section.
· In Figure 1, it would be helpful to directly label the lanes rather than include 1, 2 and 3.

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

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

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”.