Reviewer’s report

Title: SIRT1 Activation Mediates Heat-Induced Survival of UVB Damaged Keratinocytes

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Reviewer: Heinrich Kovar

Reviewer's report:

In this study, Calapre et al. report on the combined effect of in vitro UVB and heat stress on keratinocytes and skin biopsies. They report modulation of a p53-driven apoptosis response due to heat induced activation of SIRT1 and consequent deacetylation of p53. In essence, this study recapitulates a previously published study of the same authors (Calapre et al., BMC Dermatol. 2016 May 26;16:6) with very little new information. The only new finding is that the SIRT1 inhibitor Ex-527 can phenotypically reverse the protective heat effect on keratinocytes. However, there is no novel mechanistic insight. In fact, the surprisingly small effect of Ex-527 on p53 acetylation (Fig 3A) and reactivation of p53 target genes (compared to untreated controls, Fig 2B) poses the question about the mechanism of apoptosis and proliferation rescue observed in Fig 3D. Also, it remains unclear, if heat can promote UVB induced cancerogenesis, as neither in vivo nor in vitro experiments addressing this question have been attempted. Thus, the novelty of findings in this study remains very modest.

Specific comments:

The authors’ conclusions about differential SIRT1 and p53 activation status in UVB treated and UVB plus heat treated cells are solely based on quantification of immunohistochemistry results. What was the method of signal quantification in immunohistochemistry? Testing protein status by immuno blotting (as partially done in Fig 3A) would allow for independent validation of these results that underlie the whole study.

Figure 1: The resolution of the supplied figure is poor. In addition, the many arrow heads in the figure make it difficult to appreciate immunohistochemistry signals. Also, the last panel (UVB+heat) stained for phosphorylated p53 and CPD shows a lot less CPD positive cells than the UVB only treated cells. Is this a representative picture?

Table 1: In the stainings for phosphorylated SIRT1, it appears that only SIRT1-p/CPD double positive cells were counted. How can the high percentage of double positive cells be explained in heat-only treated NHEK cells in absence of UVB? The percentage of SIRT1-p single positive cells should be separately listed.
Figure 3A: The increase in acetylated p53 in irradiated and heat treated cells treated with the SIRT1 inhibitor is not impressive. At least it is obviously far from UVB irradiated cells, even though quantification of ECL signals suggests otherwise. However, ECL signals are not linear when they come to saturation as shown for the UVB-treated reference.

Figure 3D: The effect of Ex-527 on untreated keratinocytes in terms of apoptosis is not shown.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

Yes

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If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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