Author response 1

Reviewer 1

Comment: In this manuscript the authors have demonstrated decreased SIRT1 expression in peripheral CD8 lymphocytes and correlated this to increased inflammation and features of COPD. The manuscript is an incremental in that the role of SIRT1 as an anti-aging and anti-senescence is well known. It is also well known that senescence leads to secretion of pro-inflammatory cytokines. Also well known that SIRT1 activators can rescue aging and senescence. The authors just take this a step further to demonstrate this in another cell type.

The authors miss an important opportunity to link SIRT1 suppression to other downstream stages like impaired mitophagy and senescence which is something that can be easily done using stains.

Response: We show that SIRT1 suppression is linked to senescence (loss of CD28 and GCR) and increased downstream stages such as increase production of pro-inflammatory cytokines by CD28null CD8+ T and NKT-like cells.

Already in manuscript line 112 “CD8+ T cells have been shown to be the central regulators of the inflammatory network in patients with COPD [11].” Hence it was important to show loss of SIRT1 in these cell types.

Comment: A lot of data mentioned in the manuscript text can be graphed or tabulated to improve the manuscript. For instance, “Increased CD28null CD8+ T and NKT-like cells in COPD” would be better tabulated perhaps as Figure 1A. Likewise, TNF-α production by COPD group should be shown.

Response: We have previously shown increased CD28null CD8+ T and NKT-like cells in COPD and these cells produce increased IFNγ and TNFα (Reference 5, 8, 16). So this data and figures have previously been published with a different cohort of patients/controls.

Comment: While the authors mention that SIRT1 and GCR colocalize in Hela cells, they only shown co-expression in their lymphocyte subset. A simple two color staining would show colocalization in lymphocytes as well. Moreover, co-expression is not very surprising given that SIRT1 is a housekeeping gene and is expressed by most cell types. This data really proves nothing.

Response: Our aim with these experiments was to show a correlation between GCR and SIRT1 expression by CD28null senescent CD8+ T and NKT-like cells and also their CD28+ (non-senescent counterparts). To our knowledge this has never been shown before whereas co-localisation in cells has.

Comment: Figure 8A and B should be plotted as % levels to better understand the outcomes instead of % increase or % decrease.

Response: We believe the graphical representation of the effect of agents that increase SIRT1 and decrease the production of IFNγ is easier to visually comprehend compared with control similar to a previous publication regarding expression of HDAC2 and IFNγ by these cells (reference 21, figure 7 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4619495/pdf/12931_2015_Article_287.pdf).

Comment: On the whole the manuscript provides incremental information for another cell type. Including data that can provide some mechanistic information will significantly improve the manuscript.

Response: We believe our findings of decreased SIRT1 levels adds to our previous findings of decreased HDAC2, GCR, Hsp90 and increased pro-inflammatory cytokines and cytotoxic molecules granzyme b and perforin in CD8+ lymphocytes from COPD patients and provides another therapeutic target to reduce the harmful effects of these cells.

Reviewer 2

Comment: Although the subject is very interesting and the manuscript overall well written, some explanations are required.

o First of all, authors talk about "steroid-resistant senescent cells" but the underlying mechanism is unclear. Probably because these cells express reduced levels of GCR? Please, explain better this key concept.

Response: Added to manuscript line 99 "and are resistant to standard therapeutic dose of prednisolone [6].." Already in manuscript are our previous findings for the mechanisms we have shown in steroid resistance for these cells [6, 9, 19, 24]

Comment: o In the abstract, western blot is used to determinate "expression of CD28, SIRT1 and pro-inflammatory cytokines", but the protocol is not described in the Material and Methods section. What was exactly measured with the western blot?

Response: Removed from abstract line 55 "and western blot."

Comment: o In the line 319 is written "….GCR expression in CD28null and CD28+ CD8+ T cells from a COPD patient…", why from only one patient?

Response: This data was showing GCR and SIRT1 expression in these cells from a representative patient with COPD ie., to show some raw flow data as the grouped data is shown in Fig 5.

Comment: o Why results with EX-527, SRT720 on SIRT1 and intracellular cytokine expression are not showed? Where are results on effect of therapies on GCR expression, as mentioned in the specific paragraph in the material and methods section?

Response: The effects of EX-527 and SRT720 on the percentages of CD8+ T cells producing IFNγ and TNFα are already given line 339 "The presence of the SIRT1 inhibitor, EX-527 (1μM) [15], negated (by 92±12% (median ± SEM)) the effect of the SIRT1 activator SRT720 on the percentage of CD8+ T cells producing IFNγ and TNFα. The percentage of IFNγ+ CD8+ T cells (72 ± 9%) and TNFα (75± 11%); in the presence of SRT720 (IFNγ: 42 ± 5% and TNFα: 35 ± 6%) and in the presence of EX-527 ((IFNγ: 70 ± 10% and TNFα: 71 ± 13%)."

The effects of therapies on GCR expression has been added to the manuscript.

Figure 8C has been added and changes to lines 329 "GCR", line 333 "and GCR (Fig 8C), line 335 "and GCR", and line 337 "and GCR".

Comment: oFurthermore, images and figures are of bad quality and should be revised. They are too small and not sharp. Then, there are some typing errors or punctuation inaccuracies. Please, read again the manuscript.

Response: The figures have been saved as jpeg files with a resolution of 330dpi consistent with the journal's requirements. The manuscript has been further edited for inaccuracies.

Comment: oFurthermore Finally, please revise bibliography about SIRT1 levels in peripheral blood lymphocytes, adding other references (e.g: Oxid Med Cell Longev. 2018;2018:9391261.; Am J Respir Cell Mol Biol. 2015;53(6):782-92….)

Response: These references have been added to the manuscript line 111 "and in peripheral blood mononuclear cells [12,13]."