Telomerase overexpression in MRC-5 fibroblasts induces MnSOD under serum withdrawal

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Introduction

The enzyme telomerase maintains the ends of chromosomes-telomeres, which are protective structures. Telomerase consists of TERT, the protein and catalytic subunit and the RNA part (TERC or TR). However, it recently emerges that telomerase has additional non-telomeric functions. We and others found that telomerase shuttles to mitochondria under oxidative stress where it protects its function and decreases oxidative stress[1,2]. However, the mechanism is poorly understood. We recently found that a mitochondrial enzyme, Manganese superoxide dismutase is induced when telomerase is present in cells and these are grown without serum for a short time. I undertook this project to confirm this finding in different telomerase positive and negative cell pairs (TERT is overexpressed in the positive cells). I found this hypothesis to be proven right in one cell pair. This could contribute to the protective function of telomerase thereby reducing damage by reactive oxygen species, improving cellular longevity by decreasing the rate of cellular apoptosis.

Methods

Cell Culture

- BJ skin fibroblasts overexpressing telomerase (BJ5tE)[3] and parental BJ's =pair 1, MRC-5 lung fibroblasts overexpressing telomerase (T154)[1] and parental MRC-5 (PD22.5) = pair 2 and mouse ear fibroblasts (wild type and telomerase knockouts) were treated with three different conditions:
- The first group was kept in serum-containing medium. This was the control group (1).
- The second group was kept in serum free medium for two hours (2).
- The third group was treated in serum free medium + 500uM hydrogen peroxide also for two hours (3).
- The cells were then trypsinised and collected in pellets and frozen.

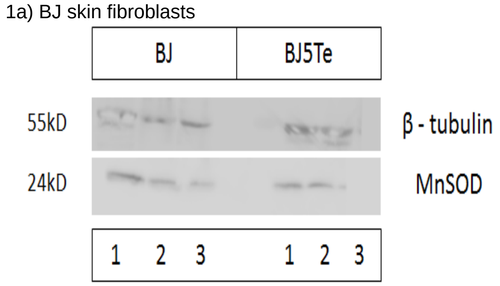
Cell Lysis Protein Determination

- The cells were lysed and the total amount of protein was determined for each sample using a Bradford assay

Western Blot

- After SDS PAGE, the proteins were transferred to a nitrocellulose membrane and probed with an anti-MnSOD antibody from rabbit, and the secondary HRP (horse radish peroxidase) conjugated antibody which was goat anti-rabbit. The membrane was then stripped of the antibodies and re-probed with a loading control. The antibody used for this was anti-beta tubulin. The secondary antibodies were labelled with HRP which was developed with a luminescing solution. And viewed on a chemilluminescence imager.

1a) BJ skin fibroblasts



1b) MRC-5 lung fibroblasts

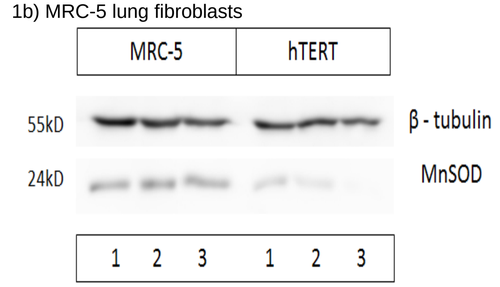
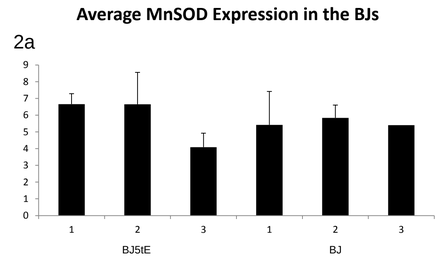
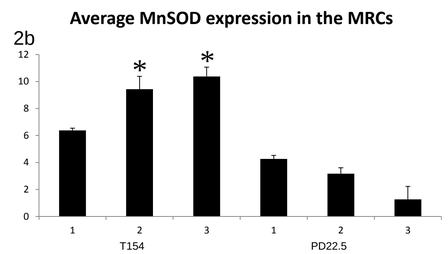


Figure 1 showing results of Western blot in BJ cells (a) and MRC-5 cells (b).

Results

The amount of MnSOD and beta tubulin present in each sample was determined using a computer programme (AIDA) and the expression level of MnSOD for each sample was then calculated using the ratio of the amount of MnSOD to the amount of beta tubulin. The experiment was done twice and the average expression of MnSOD was calculated as is represented graphically in Figures 2a and 2b below.





Figures 2a and b showing the amount of MnSOD expression in the BJ cells and MRC cells respectively

As seen in Figure 2a, There is no increase in MnSOD expression from the BJ cells in serum containing medium (1) to the Bj5te cells in serum free medium (2) or under H_2O_2 (3) in both the immortalised hTERT cells (BJ5tE) and the wild type (BJ).
With the MRC-5 cells, Figure 2b, we saw an increase in MnSOD expression in hTERT overexpressing cells (T154) grown in serum free medium und also under H_2O_2 compared to those grown in serum containing medium. We saw a decrease in MnSOD expression in parental MRC-5 fibroblasts.

Conclusion

With this project, I was able to confirm our hypothesis that there is indeed a significant increase of MnSOD in telomerase overexpressing MRC-5 fibroblasts under serum starvation (using a two sample T test with a p-value of 0.193), while in the other 2 cell pairs (BJ fibroblasts and mouse ear fibroblasts) there were no differences between the treatments. Telomerase expression in fibroblasts does induce MnSOD under serum withdrawal only in the MRC cells. If this MnSOD upregulation under serum withdrawal is specific to MRC-5 fibroblasts, it could be that they, being lung fibroblasts, have adapted to prevent damage due to reactive oxygen species which are abundant in lung tissue. It could also be as a result of a different signalling pathway regulating MnSOD expression under serum withdrawal in MRC-5 cells but this mechanism is not known.

References

1. S Ahmed, J F. Passos, M J. Birket, T Beckmann, S Brings, H Peters, M A. Birch-Machin, T von Zglinicki2, **G Saretzki,** Telomerase does not counteract telomere shortening but protects mitochondrial function under oxidative stress ***J Cell Science 2008*** V 17 p1046-1053
2. Singhapol C, Pal D, Czapiewski R, Porika M, Nelson G , Saretzki G. Mitochondrial telomerase protects cancer cells from nuclear DNA damage and apoptosis ***PloS One*** 2013, 8(1):e52989
3. Bodnar AG, Ouellette M, Frolkis M, Holt SE, Chiu CP, Morin GB, Harley CB, Shay JW, Lichtsteiner S, Wright WE. Extension of life span by introduction of telomerase in normal human cells. Science. 1998 Jan 16;279(5349):349-52

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