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Objective

To investigate whether mutant telomerase decreases oxidative stress and protects mitochondria.

Introduction

Telomerase is a reverse transcriptase best known for telomere maintenance.

- However, it was shown to have a protective role through the shuttling of its catalytic subunit, TERT.
- Therefore, this experiment investigated whether the mutant (D868A) and tagged TERT protein (HA-TERT) can also achieve similar protective effects.

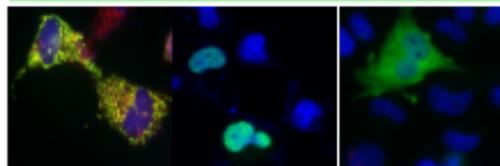
Methods

- DNA of the wild type hTERT, mutant D868A and HA-TERT were expressed in a telomerase-negative cell line MRC5/SV40 using lipofectamin transfection.
- The cells were treated with different concentrations of oxidative stress to induce the shuttling of the TERT protein.
- They were fixed with paraformaldehyde and a double immuno-staining was performed.

- Analysis was performed using a fluorescence microscope (Leica).
- The intensity of the TERT signal was measured using the freehand selection of Image J software.
- The ROS levels were determined using the formula below:

$$\%ROS\ level = \frac{\text{Mitoxox signal in cytoplasmic area}}{\text{Total cytoplasmic area}}$$

Possible cellular localisations of hTERT



hTERT signal
100% outside
the nucleus

hTERT signal
100% inside the
nucleus

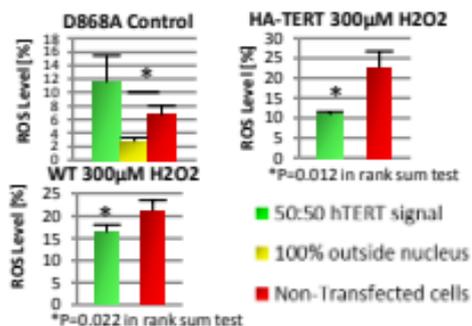
hTERT signal
50:50 inside and
outside the
nucleus

Results

% ROS	50:50 hTERT signal	100% outside nucleus	Non-transfected cells
D868A control	11.58 ± 3.91	2.76 ± 0.52	6.61 ± 1.26
HA-TERT 300 µM H2O2	10.88 ± 0.67	nil	22.66 ± 4.13
WT 300 µM H2O2	16.31 ± 1.71	nil	21.16 ± 2.32

If interested, please contact n.x.y.ong@ncl.ac.uk

Reference: Singhajol et al. (2017) 'Mitochondrial Telomerase Protects Cancer Cells from Nuclear DNA Damage and Apoptosis', *PLoS One*, 8 (5), 1-11.



- WT, HA but and mutant exhibited a protective function of lowering ROS.
- However, this protective function varies and also depends on the localisation of TERT.
- Cells with 100% exclusion of TERT proteins from the nucleus have the lowest oxidative stress signal intensity and vice versa which corresponds with my supervisor's previous findings.
- The protective function of mutated TERT was only exhibited under normal conditions.

Conclusion

Although the mutant telomerase seemed to show a slight protective function, further experiments need to be conducted before a firm conclusion can be made.