How does the telomerase protein TERT travel into the mitochondria - always via the nucleus?

Chinedu Agwu,* Supervisor: Gabriele Saretzki, Institute of Ageing and Health, Newcastle University.

INTRODUCTION

The project aims to address an important question in the field of non-canonical functions of the telomerase protein TERT.
Singhapol et al., (2) have shown that the hTERT protein can stay outside the nucleus for an extended time (up to 5 days) after one initial bolus dose of H_2O_2. Since there is a constant de-novo production of TERT protein, the question is, can it go directly from the cytoplasm into mitochondria or does it always take a detour through the nucleus.

METHODS

Cell Culture

The hTERT overexpressing MRC-5 fibroblast line (1) was grown on coverslips in 12-well plates. Three wells were left untreated while the other 9 were treated with H_2O_2. Six of those wells were treated with the protein synthesis inhibitor Cycloheximide (CHX) for 24h. In three of these wells, CHX was washed out by removing the medium and cells were left for different amounts of time (6h-24h). At the appropriate time points, cells were then fixed with 4% paraformaldehyde and analysed.

Immunostaining:

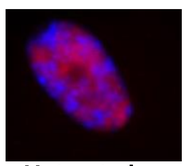
Analysed the localisation of the newly synthesised TERT protein with a TERT antibody (Epitomics) and fluorescence imaging.

RESULTS

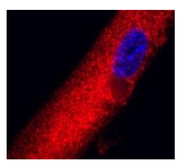
Experiment 1: testing the localisation of hTERT under H_2O_2 treatment

I could confirm that under normal conditions (no H_2O_2) that most of the TERT was localised inside the nucleus and when oxidative stress was applied, TERT moved outside the nucleus.

1a



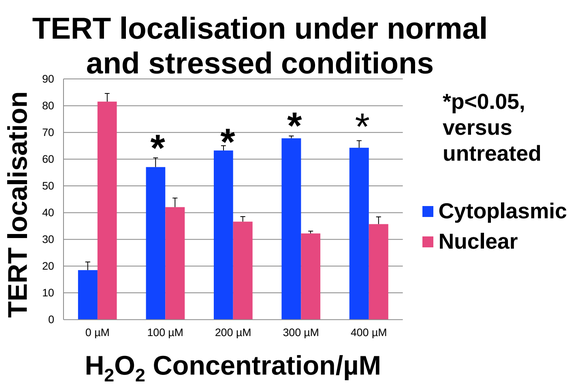
Untreated



100µM H_2O_2

Fig 1a/b Normal and stressed conditions. These figures show the localization of TERT in the hTERT cells, with and without H_2O_2 treatment.

1b

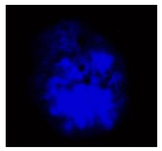


*Chinedu Agwu, Biomedical Science, c.n.agwu@newcastle.ac.uk, 120052437

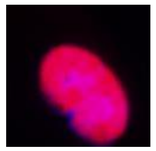
Experiment 2: Analysing the effect of CHX on hTERT levels

According to these results which were collected from the Cycloheximide treatment on hTERT overexpressing cells, the protein synthesis inhibitor was effective by drastically reducing the TERT signal. Once the inhibitor was removed and the cells left in fresh serum containing medium to recover, protein synthesis resumed and the hTERT signal re-appeared (fig. 2b)

2a



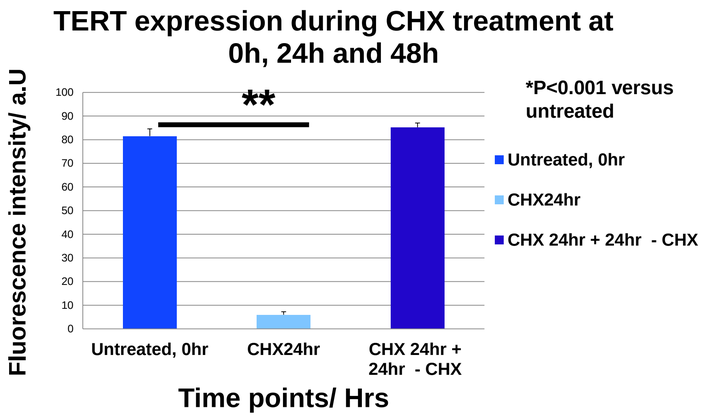
CHX Present



CHX Absent

Fig 2a/b CHX Treatment Showing the effect of the protein synthesis inhibitor Cycloheximide on TERT expression in hTERT cells.

2b



2c

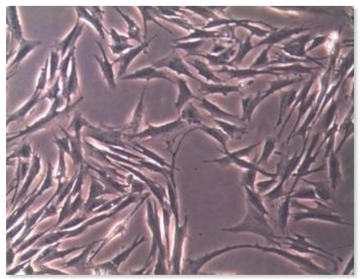
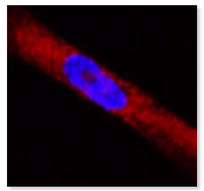


Fig 2c. A Phase contrast image Showing cultured MRC5/hTERT fibroblasts.

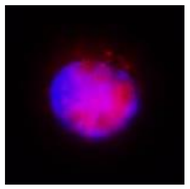
Experiment 3: hTERT localisation after CHX removal

This data indicates that after stress treatment, TERT is excluded from the nucleus (as shown in the first photo in fig.3a) and takes two pathways to reach the mitochondria. At the early stage (6h after CHX withdrawal) TERT is found mainly in the cytoplasm, however there is still some protein present in the nucleus but slightly less compared to the later stage (24h after CHX withdrawal) which appears to show a more 50:50 distribution of TERT in the nucleus and cytoplasm (as shown in the second photo in fig.3b).

3a



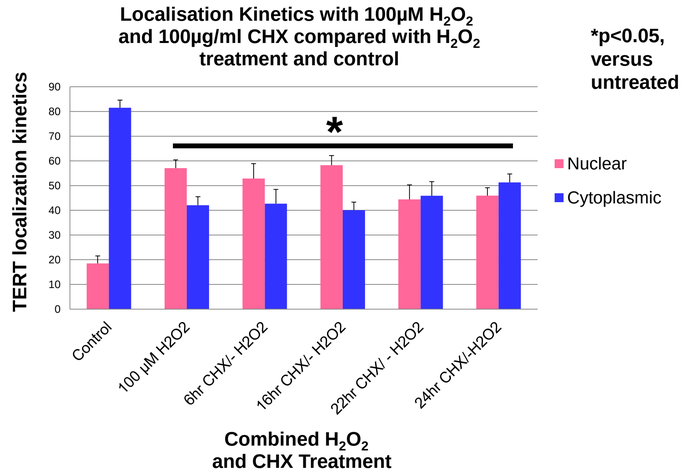
Early exclusion: 6-16 hrs



Late exclusion : 22-24hrs

Fig 3a/b Exclusion kinetics showing the localisation of TERT after H_2O_2 and CHX treatment in MRC/hTERT cells.

3b



CONCLUSION

This project revealed that in the hTERT overexpressing cell line, TERT localization varies under normal and stressed conditions. The extra-nuclear localization could offer protection against stressors such as H_2O_2 treatment as published previously (1, 2). More information could be revealed concerning the localization pathway of TERT under stress using Cycloheximide treatment. However, the results seemed to suggest that even when you suppress TERT protein synthesis in between, the cells still maintain the same exclusion pattern which corresponds to previous findings of a long term effect. Although, it does not seem to give further details about where the protein comes from - the nucleus or the cytoplasm, and this was only proven in one cell line, hence this topic requires further investigative research.

ACKNOWLEDGEMENTS

I want to thank FMS for the funding and Dr. Saretzki and her lab team for all their support and providing me with an invaluable research experience.

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.