Does localisation of DAP3 in the cytosol act as a trigger for apoptosis?

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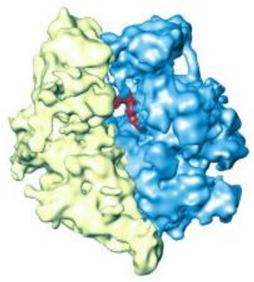
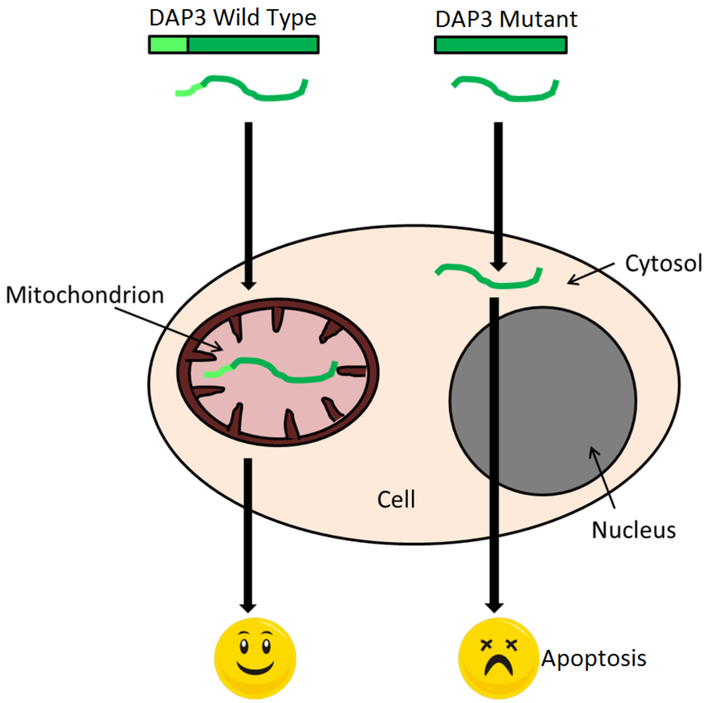
Introduction



Mitochondria contain their own DNA, mtDNA, which needs to be correctly deciphered to allow the mitochondrion and the cell to function correctly. Mitochondria are involved in producing a readily utilisable source of intracellular energy as well as regulating programmed cell death. The vital component behind these cellular processes is the mitoribosomes (Jacques *et al*., 2006).

Death-associated protein 3 (DAP3) is normally targeted into mitochondria. However, it has been predicted that DAP3 without the lethal sequence (DAP3 Mutant) can lead to mislocalisation of this protein, causing cell death.

The aim of this project is to investigate whether the lack of import of DAP3 into mitochondria acts as a signal for apoptosis, or whether are there any other mechanisms that trigger the cell death event.



Methods

Molecular cloning
- Isolation of cells with pcDNA™5/FRT/TO
- DAP3 PCR
- Digestion
- Ligation
- Transformation

Sequencing
- BigDye Terminator v3.1 Cycle Sequencing Kit
- ClustalW2 multiple sequence alignment program

Stable transfection of HEK293T cells
- Selection using Blastocidin S and Hygromycin B
- Expansion of clones

Western blot
- Antibodies detection with monoclonal ANTI-FLAG® antibody

Results and Discussion

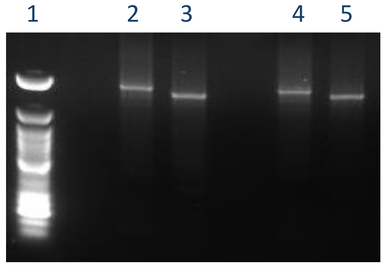


Fig 1. PCR analysis of DAP3
1 = 50bp DNA ladder
2,4 = DAP3 Wild Type(WT)
3,5 = DAP3 Mutant

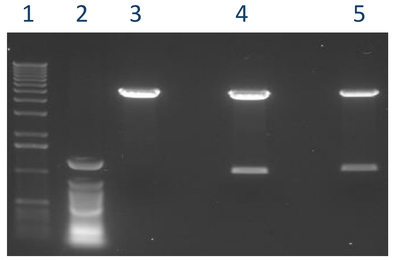


Fig 2. Diagnostic Restriction Digest
1 = 1kb DNA ladder
2 = 50bp DNA ladder
3 = pcDNA5 without insert (Control)
4= pcDNA5 + DAP3 Mutant
5 = pcDNA5 + DAP3 WT

- DAP3 mutant lacks of N-terminal sequence , thus, the product size is smaller than DAP WT
- Diagnostic restriction digest suggested the inserts were successfully cloned into the desired plasmids

Results and Discussion

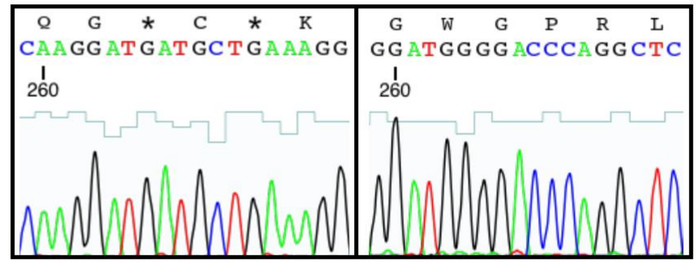
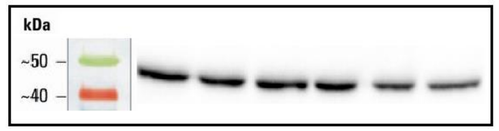


Fig 3. Sequence result at position 260 for DAP3 WT (Left) and DAP3 Mutant (Right)

- DAP3 WT obtained is a variant 5, which is not ideal to be targeted into mitochondrion
- Stable transfection was carried out with DAP3 Mutant



⇦ 45kDA DAP3 Mutant

Fig 4. Western blot of lysates from cell lines

- After expansion of the clones, the mitochondrially targeted and FLAG tagged DAP3 Mutant was inducibly expressed and the cells from individual clones were harvested for western blotting
- A single band with approximately 45kDa is detected
- Western blot analysis of cell lines show that the HEK293T cell lines express detectable DAP3 Mutant protein

Conclusion

- The initial observation suggested that DAP3 which is localised into cytosol does not trigger an apoptosis event
- The experiment would have to be repeated to obtain DAP3 variant 1 clones
- Further studies, such as fluorescence immunocytochemistry can be done to confirm protein's mitochondrial localisation

References

Jacques, C., Chevrollier, A., Loiseau, D., Lagoutte, L., Savagner, F., Malthiery, Y. and Reynier, P. (2006) 'mtDNA controls expression of the Death Associated Protein 3', *Exp Cell Res*, 312(6), pp. 737-45.

Sharma, M.R., Koc, E.C., Datta, P.P., Booth, T.M., Spremulli, L.L. and Agrawal, R.K. (2003) 'Structure of the mammalian mitochondrial ribosome reveals an expanded functional role for its component proteins', Cell, 115(1), pp. 97-108.