How do mitochondrial ribosomal proteins control apoptosis?

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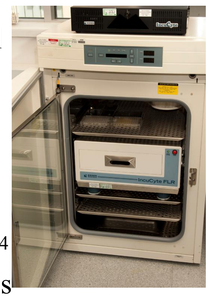
Introduction

- It was discovered that **death-associated protein 3 (DAP3)**, a component of mitoribosome, contains a N-terminal lethal sequence targeting it to mitochondria. It mediates interferon (IFN)-γ, tumour necrosis factor (TNF)-α, and Fas-induced cell death.
- The expression of DAP3 lacking the leader sequence should remain in the cytosol and thus may promote apoptosis.
- I aim to see whether the lack of import /increased cytosolic levels of DAP3 act as a signal for apoptosis, or whether mitochondria need to be damaged in some way to trigger the apoptotic cascade.

Methods

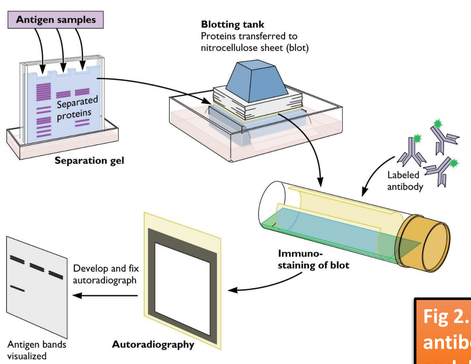
Cell Culture

Human monocytic embryonic kidney cell line, Hek293 maintained at 37°C in 5% CO_2 in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal calf serum (FCS) (Life technologies), 1x non essential amino acid and 50 ug/ml uridine. Growth rate analysis was performed. 18 x 10^4 cells/ml were seeded in 6 wells plate and cells were induced to express the DAP3 variant with tetracycline and incubated in the Incucyte machine for 6 days to monitor cell growth.



SDS Page Western blotting

Samples of cell lysate were electrophoresed on SDS-polyacrylamide gel and transferred to PVDF membrane. Antibody detection was with anti-FLAG, anti-SDHA, anti-β actin and anti-DAP3 antibody ECL+ signal detection.



Conclusion

- DAP3 targeted to the cytosol in Hek293 cells may not trigger apoptosis under normal condition.
- Further studies, such as cell stressing strategy to see in what condition will DAP3 trigger apoptosis.

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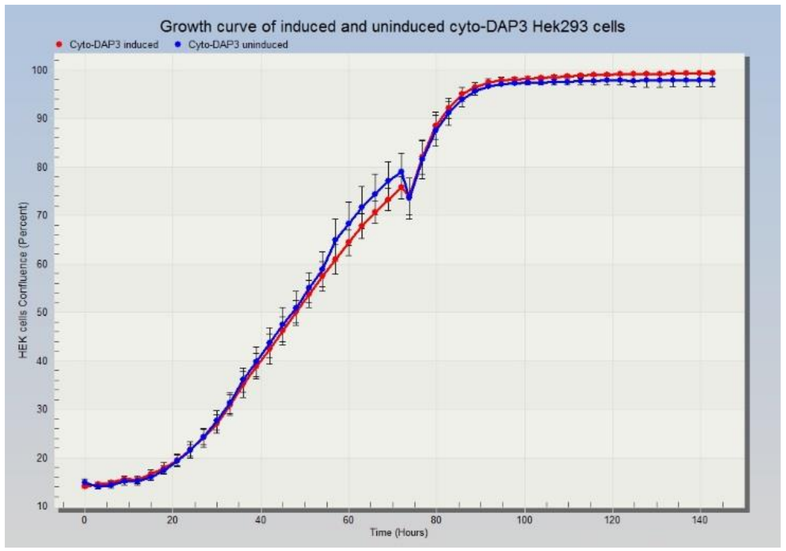
Results and Discussion



Graph 1. Growth curve of induced and uninduced Hek293 cells with tetracycline in terms of confluence(%) over 6 days using Incucyte software.

- The curves for both induced and uninduced cells were growing normally and very similar as shown in **Graph 1** that appeared as sigmoid curves.
- The slightly drop of confluence in day 3 was due to medium change.

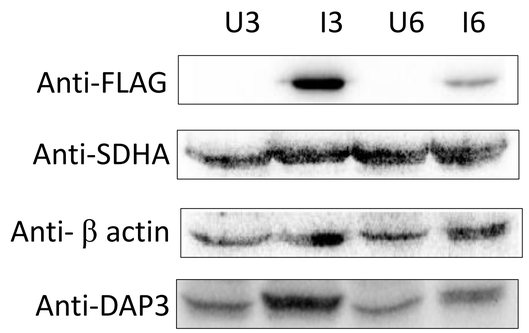
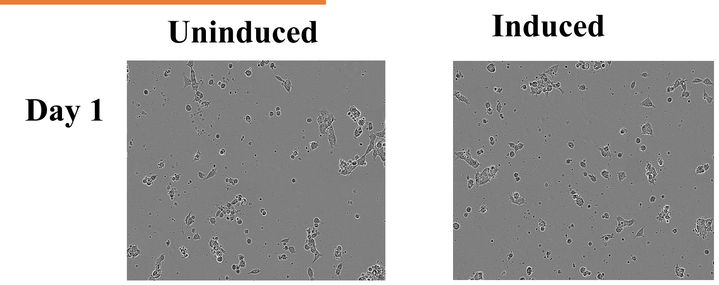


Fig 2. Western blotting analysis of Hek293 cell lines expressing DAP3 for 4 different antibodies shown above. Column U3 and U6 showed uninduced cells collected on day 3 and day 6 respectively. Column I3 and I6 showed induced cells collected on day 3 and day 6 respectively.

- Overexpression of endogenous DAP3 was shown on the blot by anti-FLAG primary antibody in induced cells but not in uninduced cells.
- By using anti-SDHA, it showed equal loading in induced and uninduced cells.
- Anti-beta actin antibody is used as a loading control to show equal loading in induced and uninduced cells.
- Anti-DAP3 antibody showed that the endogenous form has the same size of the overexpressed DAP3.



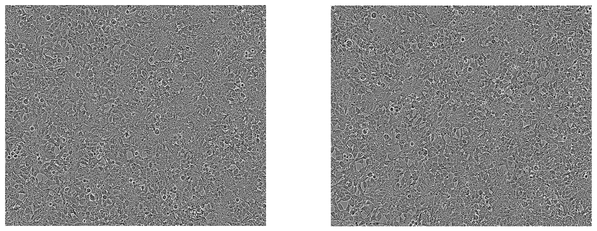


Fig 1. Microscopic photographs taken on day 1, 3 and 5 of induced and uninduced Hek293 cells. All cells were seeded in a 6 well plates and photographs were taken every 3 hours by Incucyte machine.

- The cell morphology between induced and uninduced cells was very similar
- The confluence between induced and uninduced cells was very similar that complied with **Graph 1.**

Reference

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2. Kissil JL, Deiss LP, Bayewitch M, Raveh T, Khaspekov G, et al., (1995) Isolation of DAP3, a novel mediator of interferon-gamma-induced cell death. The Journal of biological chemistry 270(46): 27932-27936.
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