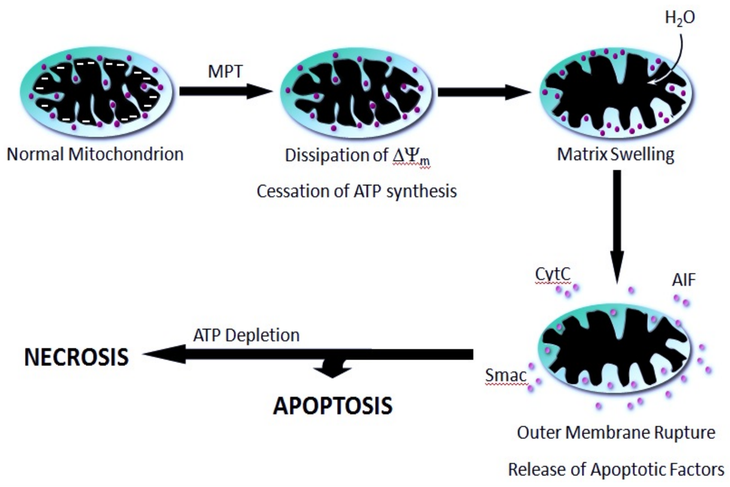
The Role of GSK-3β in Mitochondrial Permeability Transition and Cell Death

C Scudder, K Marshall, M Gutiérrez-Aguilar and CP Baines
Dept. of Biomedical Sciences and Dalton Cardiovascular Res. Ctr.
Univ. of Missouri, Columbia, MO 65211

BACKGROUND

- Mitochondrial dysfunction is a key part in the process of cell death that underlies many pathologies, including myocardial infarction, heart failure, diabetes, and neurodegenerative diseases.
- The Mitochondrial Permeability Transition (MPT) mediates this mitochondrial dysfunction.
- Glycogen synthase kinase-3β (GSK-3β) is a serine-threonine kinase that is involved in a wide variety of cellular functions, such as energy metabolism and development.
- GSK-3β is also thought to be involved in pathways that lead to opening of the pore, making it a promoter of MPT.
- GSK3β is present throughout the cell, but there is a form that appears to localize specifically to mitochondria.

MITOCHONDRIAL PERMEABILITY TRANSITION PORE



HYPOTHESES & OBJECTIVES

- **Specific activation of mitochondrial GSK-3β will induce MPT and cell death.** *Genetic gain- and loss-of-function approaches will allow us to evaluate the role mitochondrial GSK3β in MPT and cell death.*
- **We hypothesize that mitochondrial forms of GSK-3β will localize to the mitochondria only, while normal forms will be distributed throughout the cell.**
- *We will over-express normal forms of GSK-3β (Wild Type, Constitutively Active and Dominant Negative) in 293 cells in chamber slides and co-stain for the HA-tag and ATP synthase.*
- *We will over-express mitochondrially-targeted forms of GSK-3β (Wild Type, Constitutively Active and Dominant Negative) in 293 cells in chamber slides and co-stain for the HA-tag and ATP synthase.*
- **We hypothesize that Ca^{2+} retention capacity will be affected in cells with over-expressed mitochondrial forms of active GSK-3β.** *We will over-express mitochondrial forms of GSK-3β (normal, inactive, active) in 293 cells in 10 cm plates and measure Ca^{2+} retention capacity, an index of MPT, after cell permeabilization.*

RESULTS

Expression of mitochondrially-targeted GFP in transfected cells

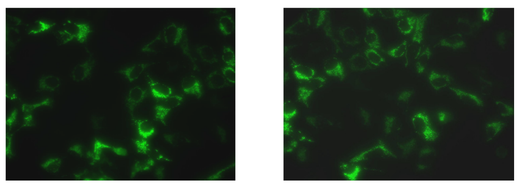


Figure 1. Expression of GFP in transfected cells. *293 cells were transfected for 48 hours with a plasmid encoding the Green Fluorescent Protein with a mitochondrial import signal for use as a control.*

Expression levels of transfected variants of mitochondrially-targeted GSK-3β

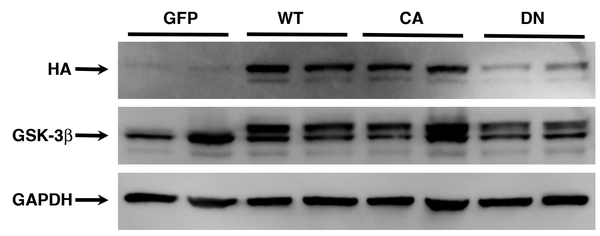


Figure 2. Expression of HA-tagged variants of mitochondrially-targeted GSK-3β. *293 cells were transfected for 48 hours with plasmids encoding either the Green Fluorescent Protein with a mitochondrial import signal (Control), a Wild Type (WT), a Constitutively Active (CA) or a Dominant Negative (DN) version of GSK-3β*

Transfected GSK-3β is mitochondrially-targeted

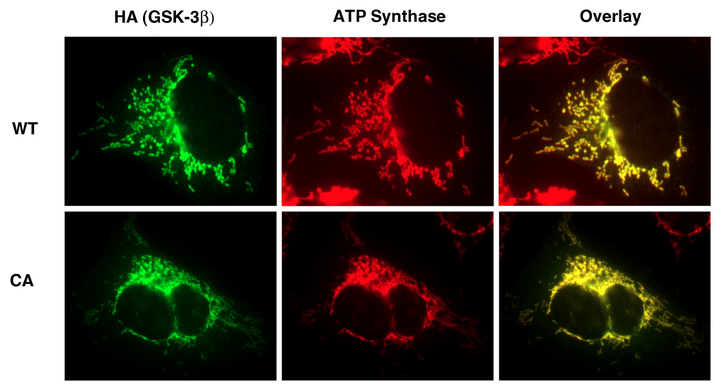


Figure 3. ***Immunocytochemistry of transfected variants of GSK-3β.*** *293 cells were transfected with WT and CA plasmids and incubated with an antibody against HA and ATP synthase to asses co-localization of GSK-3β with the mitochondrial network.*

Mitochondrial calcium retention capacity of cells transfected with mitochondrially-targeted GSK-3β variants

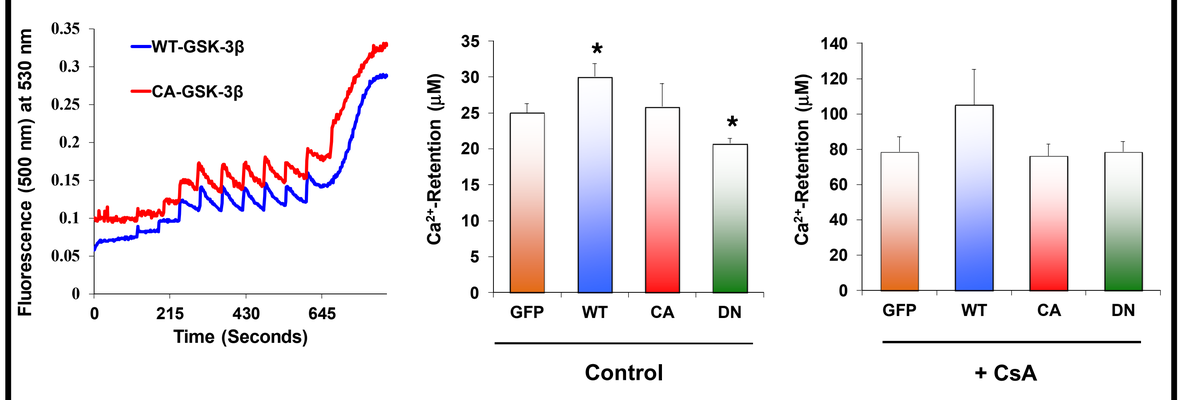


Figure 4. ***Calcium Retention Capacity of digitonin-permeabilized 293 cells.*** *Cells were incubated in buffer containing 2μg/ml digitonin and Calcium Retention was assessed by adding trains of 5μM Ca^{2+} each minute in the presence or absence of 1μM Cyclosporine A to inhibit the MPT pore.*

CONCLUSION

- We successfully over-expressed mitochondrially-targeted forms of GSK-3β (Wild Type, Constitutively Active and Dominant Negative isoforms) in 293 cells.
- These mitochondrial forms of transfected GSK-3β successfully localized to the mitochondria.
- Calcium retention capacity was markedly affected in cells with over-expressed mitochondrial forms of Active (WT) and Dominant Negative (DN) GSK-3β in control conditions (absence of CsA).

FUTURE INVESTIGATION

- Evaluate ROS production in 293 cells with over-expressed mitochondrial forms of GSK-3β. We hypothesize that over-expression of mitochondrial forms of active GSK-3β will result in increased ROS production.
- Evaluate oxidative stress-induced cell death in 293 cells with over-expressed mitochondrial forms of GSK3β. We hypothesize that cell death as a result to exposure H_2O_2 to will be increased in 293 cells with over-expressed mitochondrial forms of active GSK-3β.

ACKNOWLEDGMENTS

- This work was supported by NIH NHLBI grant HL094404 and the University of Missouri Veterinary Research Scholars Program