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

- 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 in 293 cells in chamber slides and co-stain for the HA-tag and ATP synthase.
- We hypothesize that Ca²⁺ 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 Ca²⁺ retention capacity, an index of MPT, after cell permeabilization.

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 in 293 cells in chamber slides and co-stain for the HA-tag and ATP synthase.
- We hypothesize that Ca²⁺ 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 Ca²⁺ retention capacity, an index of MPT, after cell permeabilization.

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 GSK-3β. We hypothesize that cell death as a result to exposure H₂O₂ 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.