

Repeated Glucose Deprivation/Reperfusion Induced PC-12 Cell Death through the Involvement of FOXO transcription Factor



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Conflict of interest disclosure

None

Committee of Scientific Affairs



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INTRODUCTION

- ▶ Diabetes Mellitus is a progressive disease which can result in complications of multiple organs, in addition to vulnerable to dementia, cognitive impairment, and Alzheimer's disease.
- ▶ Most complications resulting from diabetes are related to hyperglycemia, but several recent studies reported that cognitive impairment and brain damage were associated with severe hypoglycemia in animals and humans.

- ▶ Recent studies demonstrated that chronic recurrent hypoglycemia is associated with dementia.
- ▶ Another suggested mechanism of hypoglycemia-induced neuronal cell death and apoptosis is related to reperfusion.
- ▶ Some reported that glucose deprivation/reperfusion produced more oxidative stress and more neuronal cell death than glucose deprivation itself.

- ▶ The rat pheochromocytoma cell line PC-12 is widely used for Alzheimer's dementia (AD) research.
- ▶ The Forkhead box O (FOXO) transcription factors are implicated in the regulation of cell apoptosis and survival, but their role in neuronal cells remains unclear.
- ▶ We examined the role of FOXO transcription factors and the involvement of the phosphatidylinositol 3-kinase (PI3K)/Akt and apoptosis-related signaling pathways in PC-12 cells exposed to repeated glucose deprivation/reperfusion.

METHODS

- ▶ PC-12 cells were exposed to control (DMEM containing 25 mM glucose) or glucose deprivation/reperfusion (DMEM with 0 mM glucose for 1 hour/6 hours and then DMEM with 25 mM glucose for 23 hours/18 hours) for 5 days.
- ▶ **MTT assay and Western blot analysis** were performed for cell viability, apoptosis, and the expression of survival signaling pathways.

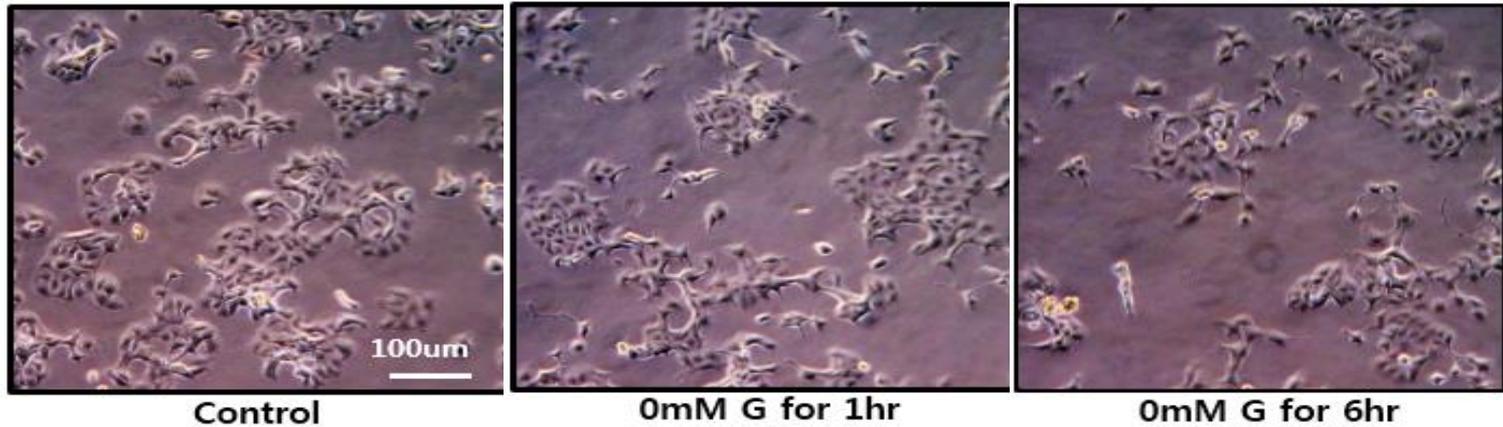
- ▶ FOXO3/4',6-diamidino-2-phenylindole (DAPI) staining was done to ascertain the involvement of FOXO transcription factors in glucose deprivation/reperfusion conditions.
- ▶ We chose the glucose deprivation for 6 hours/reperfusion for 18 hours to emphasize the relationship of FOXO in repeated glucose deprivation/reperfusion in PC-12 cells.



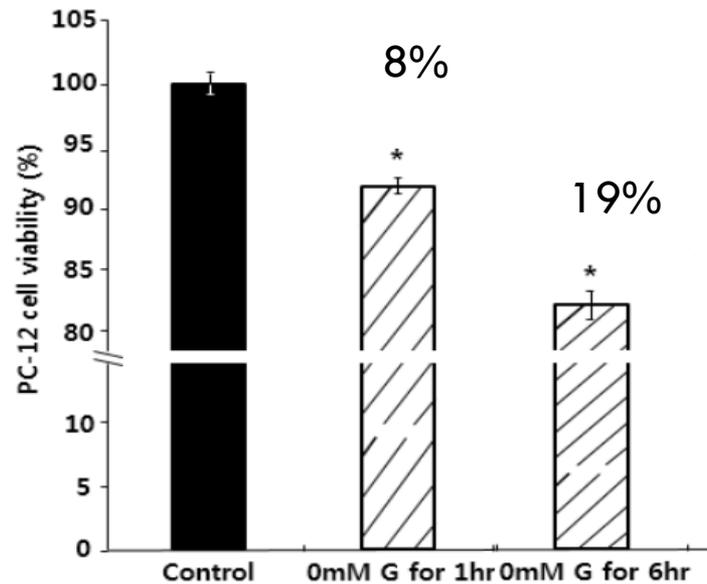
RESULTS

Cell growth and cell viability in glucose deprivation for 1 h/reperfusion for 23 h
and glucose deprivation for 6h/reperfusion for 18 h for 5 days

A

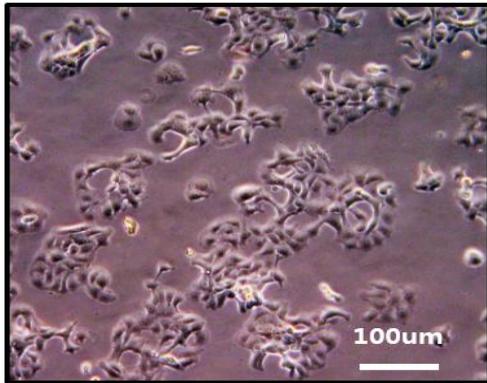


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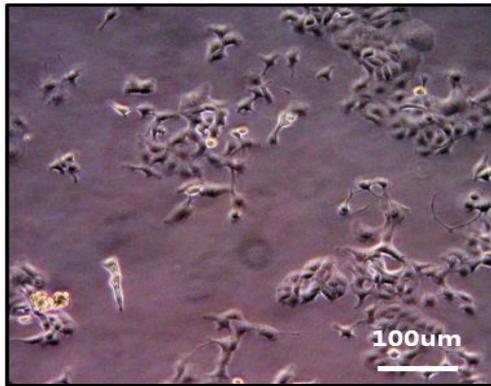


Cell growth and cell viability in PC-12 cells exposed to glucose deprivation/reperfusion

A

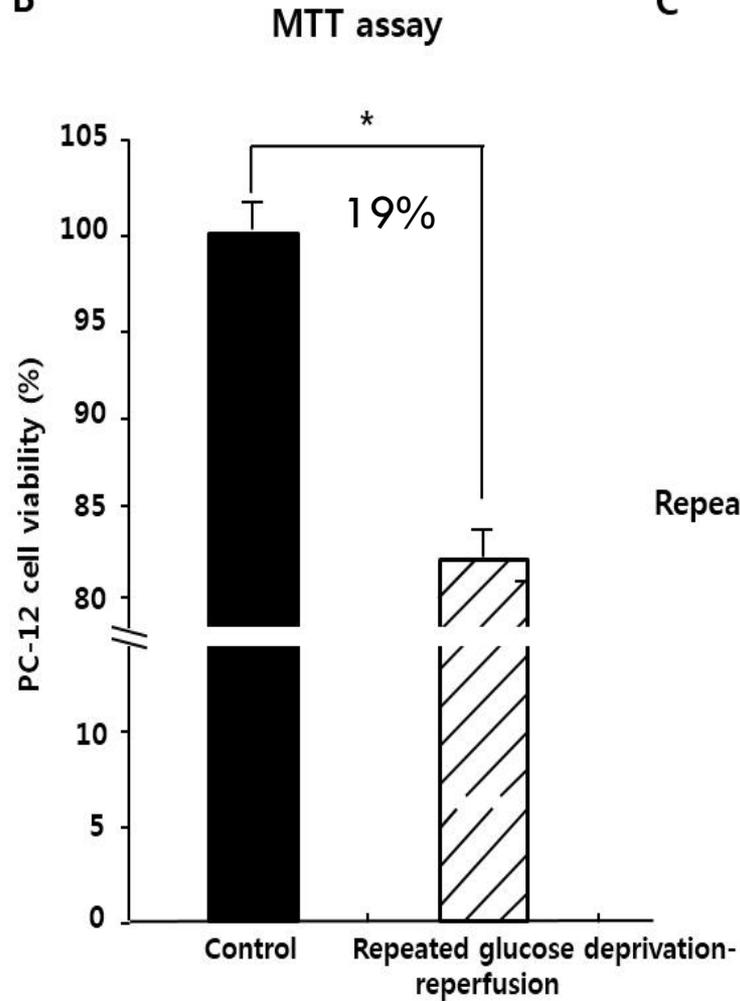


Control



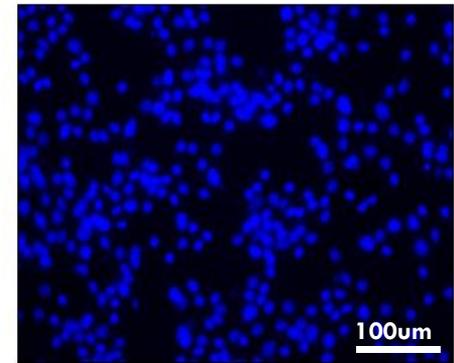
Repeated glucose deprivation-reperfusion

B

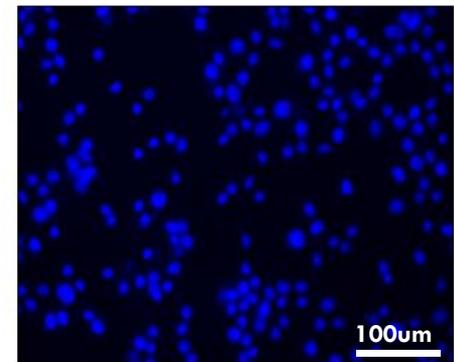


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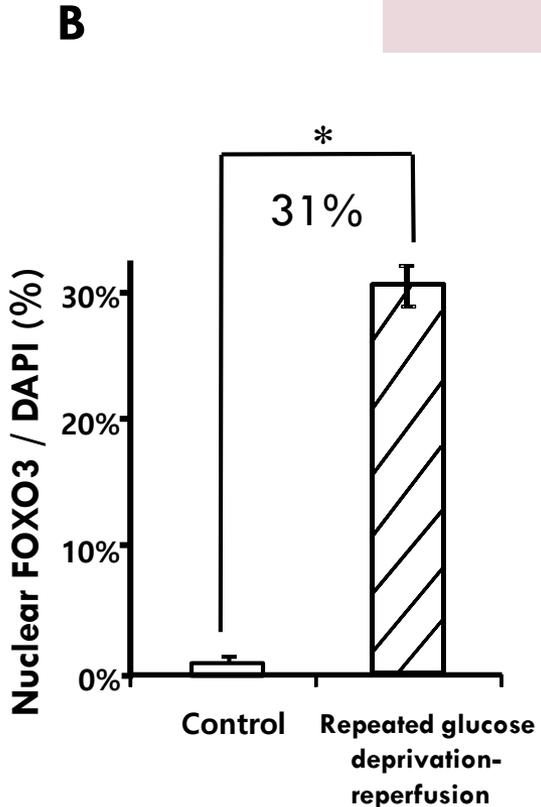
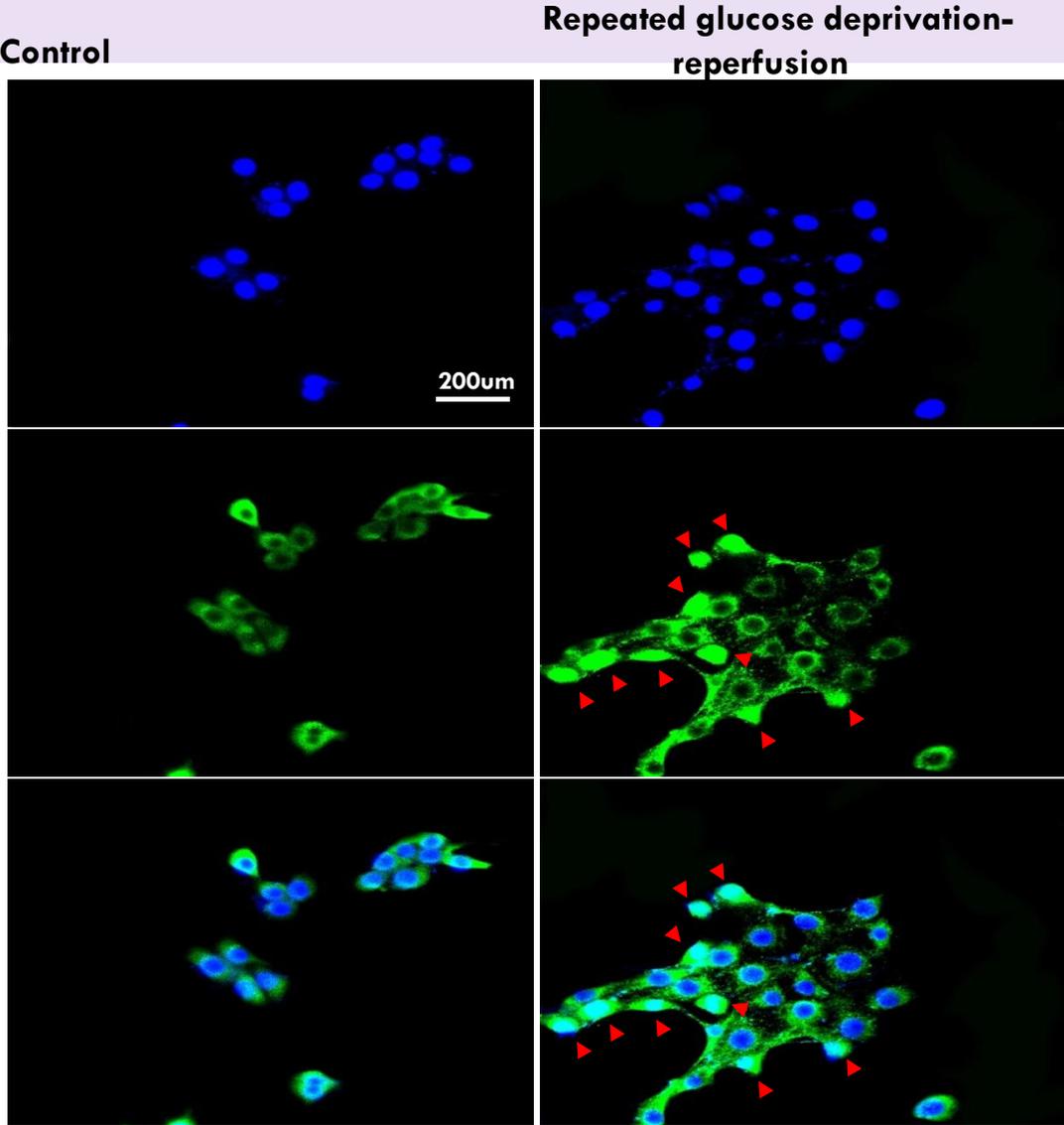
Control



Repeated glucose deprivation-reperfusion

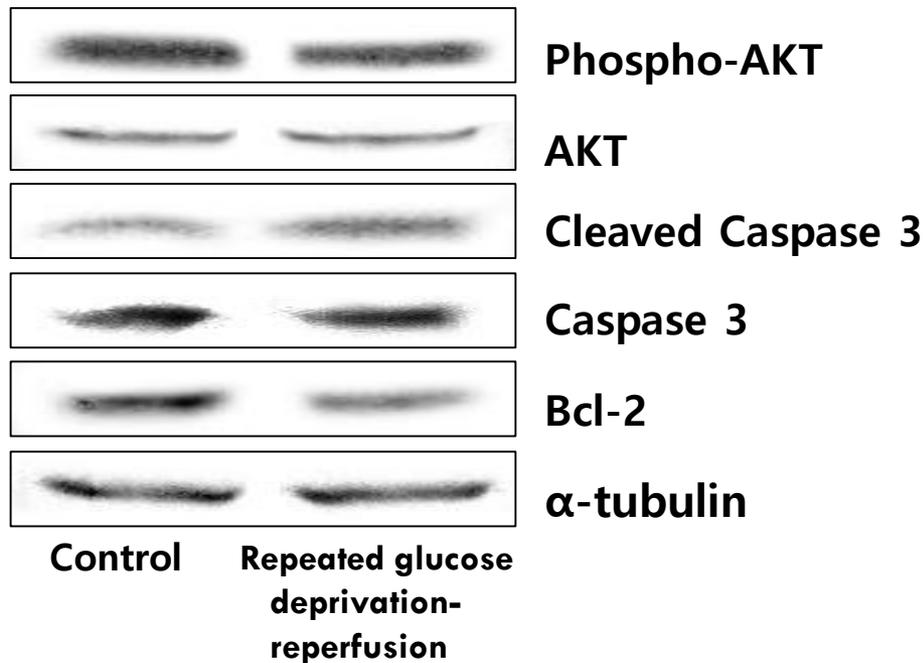


The nuclear localization of forkhead box O3(FOXO3) in PC-12 cells

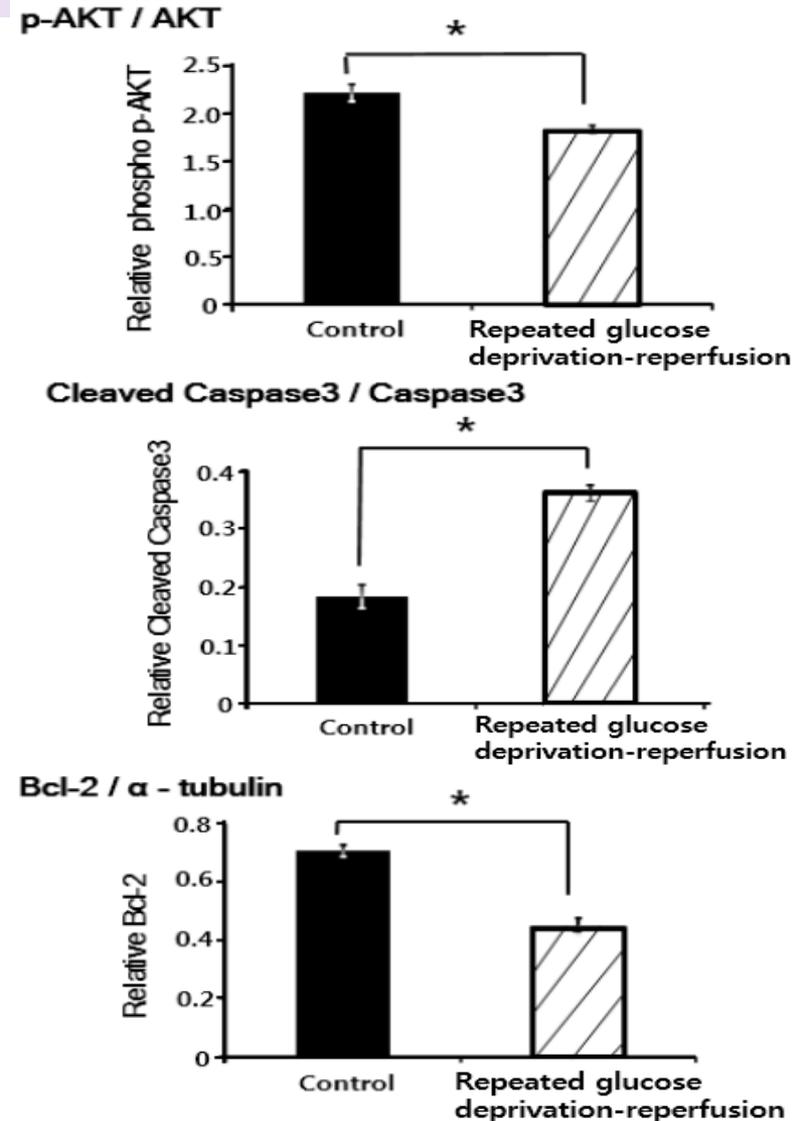


Protein expressions of Akt, phosphorylated Akt (phospho-Akt), caspase-3, cleaved caspase-3, and Bcl-2 in PC-12 cells exposed to repeated glucose deprivation/reperfusion condition

A



B



DISCUSSION

- ▶ Hypoglycemia itself needs severe and prolonged exposure time for inducing neuronal injury, but reperfusion/hypoglycemia caused neuronal damage within a relatively shorter hypoglycemic period.
- ▶ One study showed superoxide production occurred mainly during the period of glucose reperfusion, rather than during hypoglycemia itself.

- ▶ FOXO transcription factors are activated upon inhibition of PI3K-Akt signaling and involved in diverse cellular processes such as glucose metabolism, cell cycle, and apoptosis. On inhibition of the PI3K-Akt pathway, FOXO transcription factors are localized in the nucleus, which cause cell cycle arrest, stress resistance, and cell death.
- ▶ FOXO proteins are expressed to varying degrees in all tissues in mammals. FOXO1 mRNA is predominantly expressed in adipose tissues, FOXO3 mRNA in the brain, FOXO4 mRNA in the heart, and FOXO6 mRNA in the developing brain.

- ▶ Sustained hypoglycemia induced apoptosis through involvement of FOXO transcription factors and caspase-3 activation in the brain of the silkworm *Bombyx mori*.
- ▶ Several studies have used experimental models, so called 'ischemia induced oxygen-glucose deprivation.' PC-12 cells were exposed to culture medium without glucose and placed in a controlled atmosphere chamber for 4-10 hours in that model. They reported that reperfusion after oxygen-glucose deprivation caused neuronal cell apoptosis.

- ▶ We modified the experimental conditions from previous reports for reperfusion/hypoglycemic condition. The previous studies used oxygen-glucose deprivation for 4 hours/reperfusion over a 24 hour period, serum and glucose deprivation for 6 and 18 hours, and 2.5 hours oxygen-glucose deprivation followed by a 24 h reoxygenation period to study PC-12 cell apoptosis.
- ▶ We used glucose deprivation for 1 hour/reperfusion for 23 hours and glucose deprivation for 6 hours/reperfusion for 18 hours, and continued for 5 days.

▶ The limitations of this study

- ▶ We did not apply FOXO inhibitor or PI3K inhibitor to evaluate causal relationship between FOXO related transcription factors and neuronal cell death. But, previous studies already showed that FOXO3 is closely related to the PI3K/Akt pathway, apoptotic signals and cell death.
- ▶ The second limitation is we did not compare glucose deprivation/reperfusion stimuli to hypoglycemia only or high glucose treatment in PC-12 cells. Our major interest was to determine if FOXO3 and related proteins could change under reperfusion/hypoglycemia stimuli in neuronal cells.

- ▶ A third limitation is that we just calculated nuclear FOXO3 localization ratio by imaging, and did not perform Western blots on FOXO3 with the cytosolic fraction or the nuclear fraction of cell extracts. However, we repeated counting the nuclear FOXO3 staining by 3 different investigator to reduce bias of interpreters.
- ▶ Finally, our fourth limitation is that we did not evaluate the relationship between FOXO and oxidative stress under reperfusion/hypoglycemia stimuli. The previous studies already showed that superoxide is produced at the time of glucose reperfusion after hypoglycemia and the degree of superoxide production and neuronal death increased with increasing glucose concentration during the reperfusion period.

▶ In conclusion

- ▶ Cell growth and viability of PC-12 cells were decreased in repeated glucose deprivation/reperfusion.
- ▶ FOXO3 nuclear localization was observed in PC-12 cells against repeated glucose deprivation/reperfusion and activated FOXO proteins were related to a decrease of Bcl-2 and an increase of cleaved caspase 3 expression.
- ▶ We showed that FOXO transcription factors via the PI3K/Akt pathway may be involved in repeated glucose deprivation/reperfusion induced-neuronal cell death.



Thank you for your
attention

