

The role of Parkinson's disease-related gene, DJ-1 protein, in the unfolded protein response (UPR)



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Introduction

Parkinson's disease (PD) is the most common degenerative disorder of the nervous system that affects movement. It results from the death of dopamine generating cells in the substantia nigra of the brain. Although the origin of the disease is unknown, some cases of PD have genetic causes due to mutations in several genes, including DJ-1. DJ-1 acts as a mild antioxidant, a transcriptional factor and a RNA binding protein, and localizes in the nucleus, cytoplasm and mitochondria. However, the precise role of DJ-1's function on the pathogenesis of PD remains unclear.

Many PD patients and PD related mouse models display activation of the unfolded protein response (UPR) in the substantia nigra pars compacta (SNpc), that is induced by endoplasmic reticulum (ER) stress (see Figure 1), and that the UPR is strongly involved in many neurodegenerative diseases including Alzheimer's disease, Huntington disease, and Parkinson's disease. Regulation of the UPR may lead to either cell survival or death.

Interestingly, past studies have indicated that DJ-1 has a role in the unfolded protein response (UPR) pathway that is activated by endoplasmic reticulum stress. Whether/how DJ-1 contributes to the regulation of UPR in PD remains unclear. Previous studies have also shown that DJ-1 null mouse embryonic fibroblasts (MEF) and primary cortical neuron cells have reduced transcriptional and translational levels of activating transcription factor 4 (ATF4), an important UPR factor, compared to wild type (WT) basally and following ER stress. The activating transcription factor 4 (ATF4) plays important roles in promoting either neuronal cell death or survival.

In this study, the regulation of the ATF4 expression by DJ-1 and its activity in the cellular stress conditions will be examined. We hypothesize that DJ-1 up-regulates ATF4 protein expression in non-stress condition and induction of ATF4 in response to ER stress.

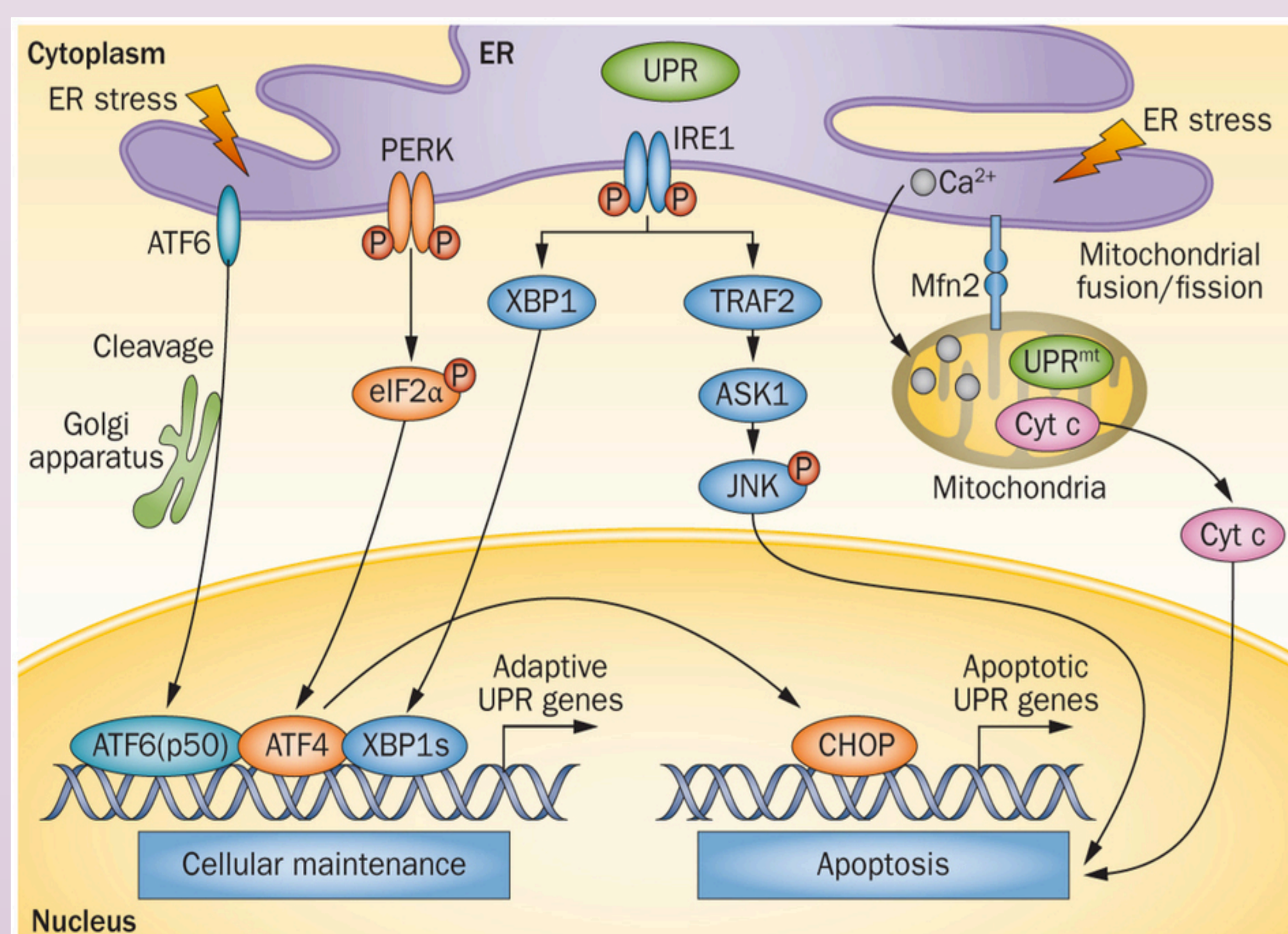


Figure 1. Model illustrates the unfolded protein response pathway (UPR). UPR is an integrated intracellular signalling pathway that transmits information about the protein folding status in the ER lumen to the cytoplasm and the nucleus.

Methods and materials

SHSY5Y cells were cultured in DMEM containing 10% fetal bovine serum, 1% antibiotic/antimycotic solution (penicillin, streptomycin and amphotericin) at 37°C. Cells were grown to 80% confluency prior to transfection using Lipofectamine 2000. SHSY5Y cells were transfected with DJ-1 WT and pcDNA3 (control). Cells were treated with DMSO and Tm (3h and 6h). Western blot analysis, using 12% polyacrylamide gel, was used to distinguish the protein expressions of ATF4 in the transfected SHSY5Y cells. The resulting bands were viewed by development of the membranes.

Results

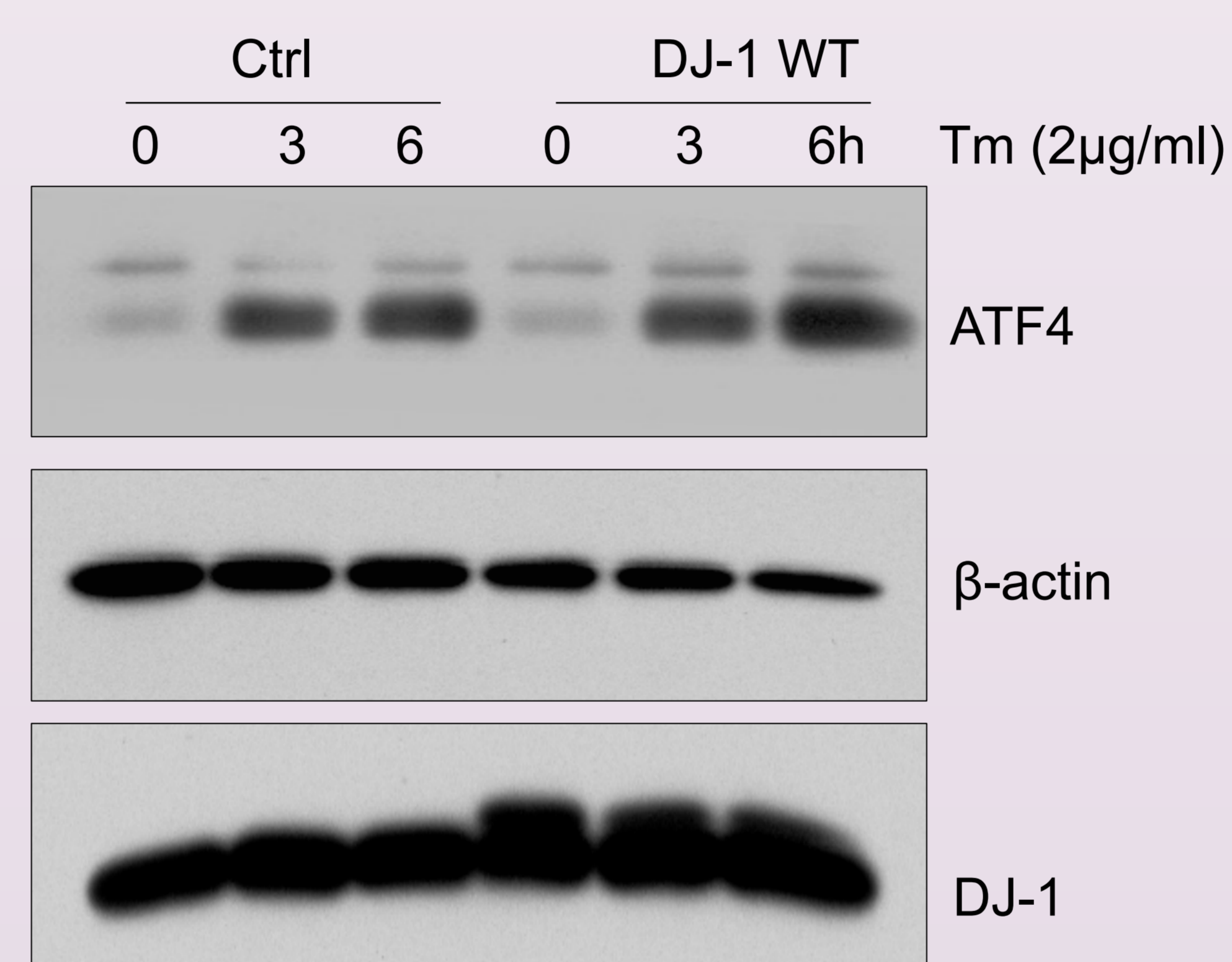


Figure 2. Extracts of SHSY5Y cells, transiently transfected with Flag-DJ-1 WT and pcDNA 3 (control) for 40 hrs and treated with Tm (2μg/μl) from 3h to 6h, were used for immunoblotting with an antibody against ATF4, β-actin and DJ-1. β-actin was used as a loading control. (n = 1)

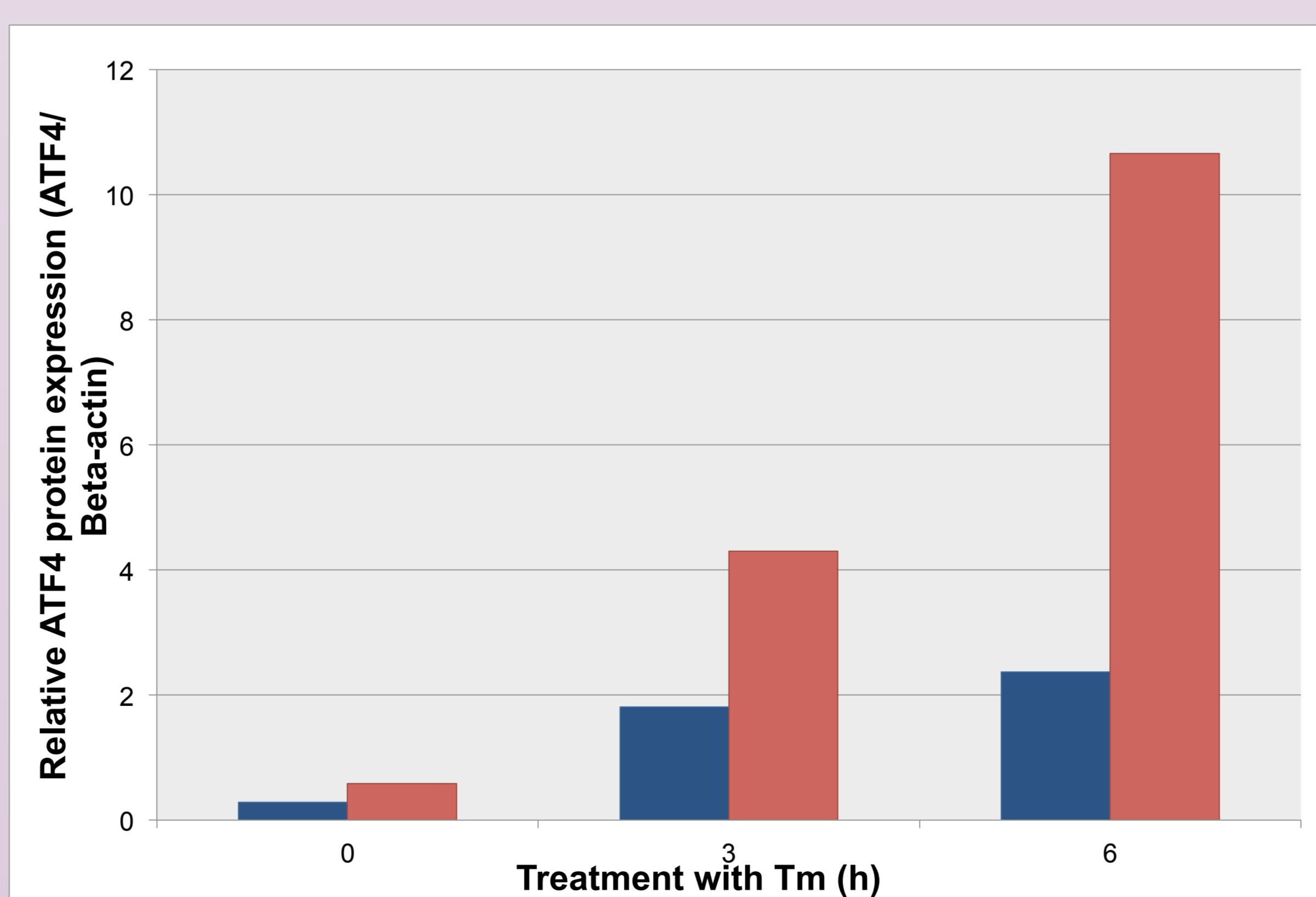


Figure 3. Relative ATF4 protein expression levels at different hours of treatment with Tm. Extracts of SHSY5Y cells, transiently transfected with Flag-DJ-1 WT (■) and pcDNA 3 (■) (control) for 40 hrs and treated with Tm (2μg/μl) from 3h to 6h, were used for immunoblotting with an antibody against ATF4, β-actin and DJ-1. β-actin was used as a loading control. (n = 1)

Conclusion

1. Over expression of DJ-1 up-regulates ATF4 protein expression in non-stress condition and induction of ATF4 in response to ER stress.
2. DJ-1 plays an important role in ATF4 expression basally and in ER stress.
3. DJ-1 seems to be involved in the UPR signalling pathway through regulating ATF4 expression and is an important molecule in cell death.

However, further studies and experiments must be performed to support the results. A larger sample size should be used for more accuracy. RT-PCR analysis must be performed to demonstrate whether DJ-1 regulates the transcription level of ATF4. Moreover, to test whether DJ-1 forms a complex with ATF4, co-immunoprecipitation should be performed with the use of anti-DJ-1 and ATF4 antibodies. The experiment should be replicated, along with knocked down DJ-1 conditions, in MEF cells and primary cortical neurons.

References

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