主論文の要旨

# Unfolded protein response in hypothalamic cultures of wild-type and ATF6α-knockout mice

（野生型およびATF6α欠損マウスの視床下部器官培養における小胞体ストレス応答）

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**<Background>**

In the endoplasmic reticulum (ER), newly synthesized and transmembrane proteins are folded and assembled. This process can be affected under various stress conditions. When the load of unfolded and misfolded proteins surpasses the folding capacity of the ER, unfolded proteins accumulate in the ER lumen, consequently leading to ER stress. In order to cope with ER stress and maintain homeostasis of the protein quality control system, the unfolded protein response (UPR) occurs through three distinct types of ER stress transducers localized in the ER membrane: inositol requiring enzyme 1 (IRE1), protein kinase RNA-like ER kinase (PERK), activating transcription factor 6α (ATF6α). ATF6α increases the expression of ER chaperones and ER-associated degradation (ERAD) components under ER stress.

The hypothalamus is responsible for various systemic homeostatic mechanisms such as energy and water balance, circadian rhythm, sleep-awake cycle, thermoregulation, and the sympathetic and parasympathetic nervous system. Recent studies including those from our laboratory have provided evidence that ER stress in the hypothalamus could affect its function, suggesting that UPR in the hypothalamus plays a crucial role in homeostasis and pathophysiology. However, the detailed regulation of UPR in the hypothalamus under ER stress is not yet clear.

**<Materials and methods>**

Seven-day-old wild-type (WT) and ATF6α$^{-/-}$ mice were sacrificed by decapitation, and three hypothalamic slices containing arcuate nucleus, ventromedial hypothalamic nucleus, and supraoptic nucleus were incubated with thapsigargin (TG) to induce ER stress. Total RNA was extracted from the hypothalamic slices and subjected to real-time qRT-PCR analyses. Statistical significance of differences between groups was analyzed by one- or two-way ANOVA followed by Bonferroni's test as appropriate. Results are expressed as means ± SE, and differences were considered statistically significant at $P < 0.05$.

**<Results>**

Incubation of hypothalamic explants of WT mice with 0.1-10 μM TG, but not 0.01 μM TG, significantly increased the expression levels of BiP, spliced XBP1, ATF4, and CHOP mRNA (Fig. 1), as well as ERAD-related genes (HRD1, EDEM1, HERP1, and Derlin-1, 2, 3) (Fig. 2) at 12 h. Incubation of hypothalamic explants of WT mice with 1 μM TG significantly increased the expression levels of BiP, spliced XBP1, ATF4, and CHOP mRNA (Fig. 3), as well as ERAD-related genes (HRD1, EDEM1, HERP1, and Derlin-1, 2, 3) (Fig. 4) at all time points (6, 12, 24 h) examined. There were no significant differences in basal expression levels of UPR-related genes examined between genotypes except Derlin-3, which had a significantly lower expression in ATF6α$^{-/-}$ mice (Fig. 3 and Fig. 4).

TG-induced upregulation of BiP mRNA was significantly attenuated in ATF6α$^{-/-}$ mice (Fig. 3A). On the other hand, expression levels of spliced XBP1 and ATF4 were significantly higher at 24 h in ATF6α$^{-/-}$ mice compared with WT mice (Fig. 3B and C). Of note, the upregulation of CHOP mRNA was significantly attenuated in ATF6α$^{-/-}$ mice (Fig. 3D). TG-induced upregulation of ERAD-related genes was significantly attenuated in ATF6α$^{-/-}$ mice compared with WT mice with the exception of Derlin-1 and 2, which had similar expression levels between genotypes (Fig. 4).

**<Discussion>**

In the present study, we investigated the regulation of UPR-related gene expression in mouse hypothalamic organotypic cultures treated with an ER stress inducer TG. Our data showed that UPR-related genes examined were all upregulated similarly in hypothalamic cultures of WT mice. We also showed that TG-induced upregulation of BiP, CHOP, and most ERAD-related genes was attenuated in the hypothalamus of ATF6α$^{-/-}$ mice compared with WT mice.

There is evidence to indicate that BiP expression is regulated by ATF6α. Consistent with this, our data showed that BiP mRNA expression induced by TG was attenuated in hypothalamic cultures of ATF6α$^{-/-}$ mice. The upregulation of spliced XBP1 and ATF4 at later time points could be interpreted as compensated UPR in the absence of ATF6α. On the other hand, CHOP is supposed to be downstream of PERK, our data showed that the upregulation induced by TG was attenuated in ATF6α$^{-/-}$ mice, indicating that CHOP is at least in part regulated by ATF6α under ER stress in hypothalamus, as suggested in previous studies using cell lines.

The upregulation of most ERAD-related genes examined were attenuated in hypothalamic cultures of ATF6α$^{-/-}$ mice, as in ATF6α$^{-/-}$ MEFs. These data suggest that ATF6α plays a pivotal role in ERAD in the hypothalamus. In particular, the expression of Derlin-3 in ATF6α$^{-/-}$ mice was significantly decrease compared with that in WT mice even under basal condition, and ER stress-induced upregulation was almost completely suppressed in ATF6α$^{-/-}$ mice. On the other hand, there were no differences in expression levels of Derlin-1 and 2 between WT and ATF6α$^{-/-}$ mice. These data suggest that among the Derlin family, only Derlin-3 is downstream of ATF6α, as was reported previously in MEFs and heart.

**<Conclusion>**

Our data revealed that all the UPR arms (ATF6α, IRE1, and PERK) are activated similarly, and that ATF6α regulates the expression of ER chaperones, CHOP, and ERAD components under ER stress in the mouse hypothalamus.