論　文　内　容　要　旨

Neuronal activity-dependent local activation of dendritic unfolded protein response promotes expression of brain-derived neurotrophic factor in cell soma.

（樹状突起における神経活動依存的な小胞体ストレス応答の活性化は細胞体における brain-derived neurotrophic factor の発現を誘導する）

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主指導教員：今泉　和則　教授
（医系科学研究科　分子細胞情報学）
副指導教員：相澤 秀紀　教授
（医系科学研究科　神経生物学）
副指導教員：橋本　浩一　教授
（医系科学研究科　神経生理学）

蔡龍杰

（医歯薬保健学研究科　医歯薬学専攻）

Introduction

The endoplasmic reticulum (ER) is a cellular organelle, responsible for synthesis, folding and posttranslational modifications of secretory proteins. Various cellular stress conditions lead to accumulation of unfolded proteins in ER lumen. These abnormalities are collectively termed ER stress. Eukaryotic cells adapt to ER stress by producing signals dealing with unfolded proteins, referred as the unfolded protein response (UPR). In mammalian cells, the three major canonical branches of UPR are activated by ER-resident transducers inositol-requiring kinase 1 (IRE1), protein kinase R-like ER kinase (PERK) and activating transcription factor 6 (ATF6). Of the three transducers, IRE1 has endonuclease activity in its cytoplasmic domain and is phosphorylated in response to ER stress. The phosphorylated IRE1 triggers non-conventional splicing of *x-box binding protein 1 Xbp1* mRNA to mature *Xbp1* mRNA (spliced *Xbp1*; *Xbp1s*), generating XBP1s transcription factor. Recent reports suggest that XBP1 plays roles not only in adjusting ER dysfunctions via expressions of target genes including molecular chaperones, but also in driving neurite outgrowth and memory formation through the expression of neuronal activity-related genes such as brain-derived neurotrophic factor (BDNF). However, the activation mechanisms for XBP1 to manage neuronal homeostasis in physiological conditions, and the neuronal activity-dependent transcriptional regulation of BDNF are unexplored. Here, we demonstrated that glutamate induced excitatory synaptic activation accelerates local activation of IRE1-XBP1 pathway in distal dendrites, followed by increased BDNF transcription in soma. BDNF in turn drove positive feedback loop, eventually orchestrating dendritic outgrowth.

Results and Conclusion

Previous studies have reported that treatment of neurons with glutamate, an excitatory neurotransmitter, induces ER stress. Thus, we tested whether IRE1-XBP1 pathway is activated in response to excitatory synaptic activation. Primary cultured mouse hippocampal neurons treated with glutamate exhibited the temporal increase in phosphorylated IRE1 and *Xbp1s* mRNA. Immunofluorescence staining analysis showed that IRE1 localized in microtubule-associated protein 2 (MAP2)-positive dendritic neurites and in postsynaptic density protein 95 (PSD95; postsynaptic marker)-positive punctate sites existing neighboring dendritic neurites. Although phosphorylated IRE1 was undetectable under normal conditions, the level was drastically increased in the punctate sites overlapped with immunoreactivities of PSD95 after glutamate stimulation. Significant increase in immunoreactivities of phosphorylated IRE1 was not found in MAP2-positive neurites, suggesting that IRE1 is activated in postsynaptic sites in response to glutamate stimulation. Next, we examined whether the activated IRE1 in postsynaptic sites promotes accumulation of XBP1s in the nucleus. In hippocampal neurons infected with lentiviruses expressing scrambled shRNA, XBP1s accumulated into the nucleus following glutamate treatment. The accumulation was canceled by the infection of lentiviruses expressing *Ire1* shRNA, indicating that glutamate enhances XBP1s accumulation in the nucleus via IRE1 phosphorylation.

We further investigated the roles of glutamate-induced XBP1s in regulating neuronal functions. It has reported that XBP1 is involved in expression of BDNF, a key component in

construction of neural network. Other studies have suggested that excitatory synaptic activation fine-tunes neurite outgrowth and synaptic plasticity, responsible for construction of neural network. Therefore, we speculated that IRE1-XBP1 cascade induced by excitatory synaptic activation orchestrates neuronal activities via expression of BDNF. We noticed that putative XBP1s-binding site (unfolded protein response element; UPRE) is included in *Bdnf* promoter region. Neuro-2A cells (murine neuroblastoma cells) transfected with *Bdnf* promoter-luciferase construct (BDNF-Luc) showed the increase in the luciferase reporter activities after glutamate stimulation. The induction was inhibited by the knockdown of *Ire1*. Transfection with BDNF-Luc lacking UPRE core also canceled the induction of luciferase activities. These data indicate that glutamate-induced IRE1-XBP1 pathway directly up-regulates BDNF expression through the binding of XBP1s to UPRE core in *Bdnf* promoter.

BDNF is known to drive its own expression during construction of neural network. Our data and the previous reports let us hypothesize that BDNF drives positive feedback loop via IRE1-XBP1 pathway to acquire substantial effects on regulation of neuronal functions. Hence, we examined whether BDNF activates IRE1-XBP1 signaling in hippocampal neurons. Treatment with BDNF induced IRE1 phosphorylation and splicing of *Xbp1* mRNA. The other canonical UPR branches were not activated after BDNF treatment. It is known that BDNF activates diverse signaling modules including protein kinase A (PKA) signaling, which maintains neuronal activities. Previous studies have shown that PKA directly binds to the cytosolic sites of IRE1, subsequently promoting phosphorylation of IRE1 without luminal events of ER. We found that the phosphorylation of p38 MAPK, which is phosphorylated by PKA, was elevated in hippocampal neurons exposed to BDNF. The treatment with PKA inhibitor H89 attenuated the BDNF-induced phosphorylation of p38 MAPK. The increase in phosphorylated IRE1 and *Xbp1s* was also canceled by H89, suggesting that BDNF induces IRE1-XBP1 pathway via PKA activation. Neuro-2A cells transfected with BDNF-Luc showed the induction of luciferase activities by BDNF treatment. Treatment with H89 reduced the luciferase activities in these cells. Knockdown of *Ire1* inhibited the binding of XBP1s to UPRE core in *Bdnf* promoter in the cells treated with BDNF. Collectively, BDNF drives positive feedback loop via PKA-IRE1-XBP1 cascade. We next monitored the dendritic expansion in hippocampal neurons treated with BDNF and H89. Sufficient expansion of MAP2-positive neurites was observed in neurons treated with BDNF. The expansion was suppressed by treatment with H89 or *Ire1* knockdown. In conclusion, the synergistic augmentation of BDNF signaling through PKA-IRE1-XBP1 cascade is critical for substantial dendritic outgrowth.

Our findings reveal a novel mechanism for neuronal activity-dependent activation of IRE1-XBP1 pathway in distal dendrites. The output retrogradely propagate into the soma for inducing *Bdnf*. BDNF in turn drive a positive feedback loop via PKA-IRE1-XBP1 cascade to orchestrate dendritic outgrowth.