CHAPTER 10

Pathophysiological Relevance of Forkhead Transcription Factors in Brain Ischemia

Kohji Fukunaga* and Norifumi Shioda

Abstract

Forkhead box transcription factor, class O (FOXO) is a mammalian homologue of DAF-16, which is known to regulate the lifespan of Caenorhabditis elegans and includes subfamilies of forkhead transcription factors such as FOXO1 (FKHR), FOXO3 (FKHRL1), FOXO4 (AFX) and FOXO6. All these FOXO members are expressed in the brain with different spatial patterns. FOXO1 is phosphorylated on three sites (Thr-24, Ser-256 and Ser-319) in phosphati-dylinositol 3-kinase (PI3-K)/Akt-dependent manner, thereby inhibiting apoptosis signals. We here documented dephosphorylation of FOXO1, FOXO3 and FOXO4 following transient forebrain ischemia with its concomitant translocation into the nucleus in neurons in the gerbil and mouse brains. The dephosphorylation of FOXO1 following brain ischemia is in part mediated by constitutively active calcineurin in the mouse hippocampus. The activation of FOXOs preceded delayed neuronal death in the vulnerable hippocampal regions following ischemic brain injury. The FOXO1 activation is accompanied by an increase in DNA binding activity for FOXO1-responsive element on the Fas ligand promoter. Thus, downstream targets induced by FOXO1 include Fas ligand and Bcl-2-interacting mediator of cell death (Bim) in the brain ischemia. Accumulating evidence documented how FOXO activation is involved in the mechanisms of ischemic cell death. In this chapter, we document the activation mechanism of FOXO factors following brain ischemia and define their downstream targets underlying neuronal death. The pathophysiological relevance of crosstalk between FOXOs and calcineurin pathways is also discussed. Finally, we propose therapeutic perspectives to rescue neurons from delayed neuronal death by promoting the Akt signaling. Vanadium compounds, protein tyrosine phosphatase inhibitor, up-regulates Akt activity in the brain and thereby rescues neurons from delayed neuronal death by inhibiting FOXO-dependent and -independent death signals in neurons.

FOXO Phosphorylation Regulating Shuttling between Nucleus and Cytoplasm

The forkhead box transcription factor, class O (FOXO) is mammalian homologue of DAF-16, which is known to regulate life span of Caenorhabditis elegans and includes subfamilies of forkhead transcription regulators such as FOXO1 (FKHR), FOXO3 (FKHRL1), FOXO4 (AFX) and FOXO6. The FOXO factors share DNA-binding specificity to a core consensus site named as Forkhead-responsive element and downstream targets of diverse protein kinases stimulated

*Corresponding Author: Kohji Fukunaga—Department of Pharmacology, Graduate School of Pharmaceutical Sciences, Tohoku University, Aramaki-Aoba, Aoba-ku, Sendai 980-8578, Japan. Email: fukunaga@mail.pharm.tohoku.ac.jp

Figure 1. Possible phosphorylation sites for Akt and other possible kinases in FOXOs. The FOXO members are phosphorylated by several protein kinases. Phosphorylation of T1, S1 and S2 by Akt activity through PI3-K underling growth factor signaling is crucial step of the FOXO function. Serum- and glucocorticoid-inducible kinase (SGK) underlying PI3-K pathway also phosphorylates T1 and S2. The S2 phosphorylation by Akt is required for subsequent phosphorylation of Ser 322 and Ser325 in FOXO1 by casein kinase 1 (CK1). Together with these phosphorylation, the S3 phosphorylation by the dual-specific tyrosine-regulated kinase 1a (DYRK1a) may contribute the nuclear export of FOXO. In addition, T2 in the C terminal regions is also phosphorylated by JNK underlying the Ras-Ral pathways as described in the text.

by various cellular stresses such as DNA damage, nutrient deprivation, cytokines and hypoxia. FOXO factors are phosphorylated in vivo on multiple threonine and serine residues (T1, T2, S1, S2 and S3) as shown in Figure 1. Protein kinase B (Akt) in response to growth factor and insulin stimulation directly phosphorylate FOXO1 at three specific sites (Thr-24, Ser-256 and Ser-319, labeled T1, S1 and S2, respectively). Serum- and glucocorticoid-inducible kinase (SGK) underlying PI3-K pathways also phosphorylates T1 and S2 of FOXO3. The functions of FOXO factors negatively regulated by Akt- and SGK-dependent phosphorylation on these sites, thereby promoting maintenance of cell survival. Akt phosphorylates Ser-256 and SGK phosphorylates Ser-319 preferentially. Reporter assays for transcriptional activity and mutational analysis of the phosphorylation sites T1, S1 and S2 show that Akt-induced phosphorylation inhibits the transcriptional activity of FOXO factors. The Akt/SGK-dependent phosphorylation regulates the shuttling of FOXO factors between the nucleus and the cytoplasm. Homologous sequences for nuclear localization signal (NLS) and nuclear export signal (NES) have been identified within the FOXO factors as shown in Figure 2. The shuttling of FOXOs between nucleus and cytoplasm is regulated by accessory proteins such as importins or exportins (Crm1). The phosphorylation of murine FOXO1 at Ser-253 (corresponding to Ser-256 in human FOXO1) is required for phosphorylation of the other two sites for Akt. In the case of FOXO1, one of the NLS lies near the Akt-dependent phosphorylation site S1. Upon phosphorylation on S1 by Akt, the NLS in FOXOs is inactivated. The 14-3-3 protein possibly recognized the S1 phosphorylated form of FOXO1 and export it to the cytoplasm by masking the NLS and/or by promoting nuclear export. However the importin that binds to the NLS remains unidentified. The function of additional Akt-dependent phosphorylation sites of T1 and S2 also remains unclear. Since nuclear export of FOXOs is inhibited by leptomycin B treatment, implying involvement of Crm1 in the nuclear export. Zhao et al proposed two putative NES in the C terminal region and one NES near phosphorylation site S3. Although exportin (Crm1) may not directly bind to the NES, the phosphorylation of T1 and S3 possibly affects the Crm1 binding to the NES. The phosphorylation of T1 and S2 dose not affect the binding between FOXOs and Crm1. The association between FOXO and exportins is also regulated by the small GTPase Ran. Phosphorylation of Ser-322 and Ser-325 by casein kinase 1