Abstract

This work deals with the study of protein-protein interaction of human transcription factors FOXO4 and p53. Under stress conditions, p53 and FOXO4 interact in the nucleus. Their interaction is associated with increased transcription of p21 protein, an inhibitor of cyclin-dependent kinases, which is responsible for cell cycle arrest and the transition of cells to a senescent state. Senescent cells are excluded from the cell cycle and are unable to perform their physiological function, yet they remain metabolically active and secrete pro-inflammatory signals to the environment. The accumulation of senescent cells is considered one of the main causes of aging and is associated with the development of several diseases. It is known that the formation of the FOXO4:p53 complex leads to a preference of the senescence process over apoptosis, whereas disruption of this interaction leads to unblocking of the apoptosis process. As a result, senescent cells are removed, replaced and tissue homeostasis is restored. The molecular mechanism by which this protein-protein interaction regulates p21 protein transcription is unknown. The activity of both proteins is regulated by a variety of post-translational modifications. One of these posttranslational modifications is the phosphorylation of FOXO4 by AKT kinase at residues T32, S197 and S262, which occurs after activation of the PI3K/AKT signalling pathway, also related to senescence. The first two of these phosphorylation sites form binding sites for dimeric regulatory proteins 14-3-3. However, it is still unclear whether phosphorylation and subsequent binding of 14-3-3 proteins somehow affect the interaction of FOXO4 with p53. Therefore, the effect of FOXO4 phosphorylation and subsequent binding of dimeric 14-3-3 protein on the ability to interact with p53 was studied using sedimentation velocity analytical ultracentrifugation method. The results of this work show that FOXO4 phosphorylation alone does not affect the interaction with p53, whereas binding of 14-3-3 protein prevents the formation of the FOXO4:p53 complex.

Keywords: FOXO, p53, cellular senescence, protein-protein interaction, posttranslational modifications, 14-3-3