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Silencing of the CKIIα and α′ Genes via DNA Methylation during Cellular Senescence

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Protein kinase CKII is a ubiquitous and highly conserved protein serine/threonine kinase, which plays a significant role in the control of cell proliferation and transformation. The holoenzyme of CKII is a heterotetramer, composed of two catalytic (α and/ or α′) and two regulatory (β) subunits. Previously we reported that down-regulation of CKII activity is tightly associated with cellular senescence and that the mRNA and protein levels of CKIIα decrease during senescence. The present study demonstrates that the mRNA and protein levels of CKIIα′ also decrease during senescence. Knockdown of CKIIα′ in IMR-90 cells by RNA interference induced the senescent phenotype. Treatment of IMR-90 cells with a demethylating agent 5-aza-2′-deoxycytidine induced CKIIα and CKIIα′ expression, suggesting that DNA hypermethylation might be involved in the silencing of CKIIα and CKIIα′ genes in senescent cells. However, bisulfite sequencing analysis revealed that the methylation status of the CpG islands within the reported CKIIα and CKIIα′ promoters was not associated with senescence. Instead, senescence-dependent hypermethylation was observed in the region ranging from position +1112 to +1128 of the CKIIα gene and at positions -527 and +829 of the CKIIα′ gene. In addition, this study indicates that DNA methylation-dependent down-regulation of transcription factors Sp1, Ets1 and NF-κB might be involved in silencing of the CKIIα and CKIIα′ genes during cellular senescence.

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Regulation of Protein Kinase CKII by Phospholipase D2 in Human Colon Cancer Cells

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Phospholipase D (PLD) catalyzes the hydrolysis of phosphatidylcholine to form phosphatidic acid and choline. Protein kinase CKII (CKII) is a ubiquitous protein serine/threonine kinase and catalyzes the phosphorylation of a broad spectrum of substrates, which are involved in cell growth and proliferation. In the present study, we investigated the physiological significance of the interaction between CKII and PLD in HCT116 human colon cancer cells. CKII interacted with phospholipase D2 (PLD2) in HCT116 cells. The N-terminal domain of CKIIβ and the C-terminal domain of PLD2 were necessary for the interaction. PLD2 and CKIIβ colocalized in the perinuclear region of HCT116 cells. The overexpression of PLD2 reduced the protein level of CKIIβ. The role of PLD2 was further elucidated using PLD2-small interfering RNA-transfected cells. PLD2-induced CKIIβ reduction was mediated by ubiquitin-dependent degradation and the catalytic activity of PLD2 was not involved in the CKIIβ degradation. Despite of CKIIβ reduction, the overexpression of PLD2 stimulated CKII activity in HCT116 cells through protein kinase C-mediated CKII phosphorylation. Decrease of CKIIβ protein and stimulation of CKII and PKC activities were observed also in PLD2-overexpressed HEK293 cells. Taken together, these data suggest that PLD2 regulates CKII activity through the acceleration of CKIIβ degradation and stimulation of protein kinase C activity.

Key words: CKII, PLD2, Cancer