

SECTION 2. Recombinant protein and antibody engineering

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GENERATION OF S6K1 CYSTEINE MUTANTS FOR THE FURTHER RESEARCH OF REDOX-DEPENDENT KINASE REGULATION

Background/Aim. S6K1 contains six cysteine residues, two of which (Cys217 and Cys231) are located within the activation loop at the conserved DFG+2 and T+2 positions, respectively. Evidence suggests that, in response to changes in cellular redox status, these conserved cysteine residues may regulate kinase activity through the formation of intra- and/or intermolecular autoinhibitory disulfide bonds, thereby restricting ATP access to the catalytic site. This study aimed to generate plasmid constructs and stable cell lines carrying mutations of these cysteine residues to enable future investigation of redox-dependent S6K1 regulation. **Methods.** Wild-type S6K1 was cloned into the pcDNA4TO-myc expression vector, followed by site-directed mutagenesis to generate cysteine-to-alanine substitutions. The resulting constructs were transfected into PANK1 β -overexpressing HEK293 cells to establish stable cell lines. Protein expression was confirmed by Western blot analysis. **Results.** We successfully generated plasmids encoding wild-type S6K1 and three cysteine mutants: C217A, C231A, and the double mutant C217A/C231A. Stable cell lines expressing each construct were established and validated by Western blotting. The cell lines exhibited distinct proliferation rates, with wild-type p70S6K1 overexpression cell line proliferating the fastest, followed by C231A, the double mutant, and C217A, which showed the slowest growth. Notably, the C217A mutant displayed a density-dependent lethal phenotype. While cells remained viable below approximately 70% confluence, the establishment of cell-to-cell contacts triggered cell detachment and death. **Conclusions.** The generated plasmids and stable cell lines provide useful tools for studying the role of redox-sensitive cysteine residues in S6K1 regulation. These models will allow us to investigate whether oxidative stress induces kinase autoinhibition through disulfide bond formation between Cys217 and Cys231. The reduced proliferation rate and confluence-induced cytotoxicity observed in the C217A mutant suggest that Cys217 plays an important role in the proper regulation of S6K1 and normal cell growth. **Funding.** This work was supported by the National Research Foundation of Ukraine within the framework of the "Competition for a grant from the President of Ukraine for young scientists and doctors of sciences" (No. 308.05/0042).

Keywords: S6K1, protein kinase, redox regulation, cysteine residues, site-directed mutagenesis.

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