89 Defective Accumulation of p53 Protein In X-irradiated Human Tumor Cells With Low Proteasome Activity

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Because the loss of p53 function is the most common event in human cancers, p53 gene therapy is now under clinical trial. Here, we examined whether X-irradiation potentiated the function of the exogenous p53 protein induced in H1299 cells, human non-small cell lung carcinoma. We found that the induced p53 protein was not accumulated after X-irradiation, although both phosphorylation of p53 protein at Ser15 and Ser20, and phosphorylation of CHK2/Cds1 and MDM2 were observed normally. We next examined the kinetics of degradation of p53 protein in the presence of cycloheximide, a translation inhibitor. The level of p53 protein in HE49 cells decreased rapidly, but there was no change in H1299 cells. Furthermore, significant accumulation of p53 protein was observed only in HE49 cells after 2hr treatment of ALLN, a proteasome inhibitor. These results indicate that low proteasome activity in H1299 cells cause defective accumulation of p53 protein. It is possible that proteasome activity in cancer cells may determine the prognosis of p53 gene therapy.

90 Characterization and purification of PE21 binding factor(s) that determines human p53 gene transcription and stress response.

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In human cells p53 gene expression is regulated by a novel promoter element (PE21) that located about 50 base upstream from the putative transcription start site. Previously, I have analyzed the role of PE21 on p53 gene transcription and stress response, and found that the PE21 sequence is indispensable for basal expression as well as induced response by stress. In the present study, nuclear factors (putative p53 gene transcription factors) are partially purified by standard biochemical methods followed by PE21-affinity column, from HeLa and Molt4 nuclear extracts. Final preparations showed single (HeLa) or two distinct (Molt 4) proteins by SDSPAGE analyses. The protein(s) recovered from the gel exhibited PE21 binding, and the lower molecular weight band was assumed to be a common protein in HeLa and Molt4 cells.

91 Radiation-inducible hSNK Gene and Protein Regulation in Cultured Thyroid Cells

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Polo-like kinase (PLKs) family plays an important role in several stages of mitosis. Using cDNA subtraction method, we recently identified a radiation-inducible gene belonging to PLKs family, a human homologue of mouse serum-inducible kinase (hSNK) whose mRNA expression was rapidly upregulated in irradiated cultured human thyroid cells.

Analysis of 0.7 kb of 5'-upstream region of the gene revealed a number of motifs comprising putative binding sites for several transcription factors and, notably, p53 binding homology sequence.

As the intrinsic hSNK protein was hardly detectable, we introduced hSNK expression vector into Cos 7 cells and found several sized bands of hSNK protein by Western blotting, indicating the discrepancy between low hSNK protein and corresponding mRNA levels. To detect intact or cleaved type of hSNK polypeptide, therefore, proteasome inhibitor was used. LLnL up-regulated the expression of long hSNK protein in transfected cells, suggesting that radiation-induced suppression of proteasome action mediated via DNA damage may influence the increased amount of hSNK protein.