SP-I-3 Sensors and signal transduction pathways involving p53 protein accumulation in normal human embryonic cells irradiated with X-rays

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p53 protein is a nuclear phosphoprotein, whose level is accumulated and activated by various cellular stresses, such as ionizing radiation, UV-irradiation, and heat shock treatment. Recently, we have suggested that both nuclear- and membrane-originated signals are responsible for the regulation of the p53 function in X-ray-irradiated normal human cells. Here we examined the role of ATM protein and the involvement of growth factor receptor in signal transduction activating p53. We found that the amount of ATM protein in the nucleus did not change after X-irradiation. However, using *in vitro* pull-down assay, ATM protein showed increased affinity to both X-irradiated DNA and restriction enzyme-treated DNA, but not to UV-irradiated DNA. Thus it can be hypothesized that increased recognition of damaged DNA by ATM protein activates the nuclear signal transduced to p53 protein. We next examined the effect of the EGFR inhibitor, Tyrophostin AG 1478, on the accumulation of p53 after X-ray irradiation. It was found that 200 $\mu$ M of AG1478 decreased p53 accumulation by approximately 35%. Because treatment of cells with 20 $\mu$ M of PD98059, an inhibitor for MEK, suppressed p53 accumulation as well, it is likely that membrane signal mediated by EGFR is transduced to p53 though activation of MAP kinase pathway.

SP-I-4 A mutant human *p53*, 123A, which led to apoptosis but not Waf-1 in response to ionizing radiation in a human osteosarcoma cell line, Saos-2

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To investigate differential transcriptional activity on human *p53* mutants, stable transformants with mutants or wild type *p53* were made by introducing cDNA into the human osteosarcoma cell line, Saos-2, which lacks an endogenous *p53*. Accumulation of p53 protein in response to irradiation occurred in transformants with wild type *p53* and the 123A mutation. Waf-1 induction by ionizing radiation was occurred in the wild type *p53* transformant, but not in the 123A transformant. Ionizing radiation induced apoptosis as assessed with DNA laddering from 36 h in the wild type transformant and from 24 h in the 123A transformant. Waf-1 expression seems to prevent the earlier induction of apoptosis in the wild type *p53* transformant.

SP-I-5 NO is a mediator of the intercellular signal transduction for stress response in human glioblastoma cells

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The accumulation of inducible nitric oxide synthase (iNOS) was observed after heat treatment in human glioblastoma mutant *p53* (m*p53*) cells but not in wild-type *p53* (w*tp53*) cells. The accumulations of hsp72 and p53 in the non-treated w*tp53* cells were induced by the co-cultivation with the heated m*p53* cells and these protein accumulations were suppressed by the addition of a specific iNOS inhibitor, aminoguanidine to medium. The w*tp53* cells were cultured in the conditioned medium by pre-culture of the heated m*p53* cells. As a result, the accumulations of hsp72 and p53 were induced in the w*tp53* cells. In contrast, these accumulations were completely blocked the addition of a specific NO scavenger, 2-(4-carboxyphenyl)-4,4,5,5-tetramethyl-imidazoline-1-oxyl-3-oxide (c-PTIO), to the conditioned medium. To confirm the effects of NO, the w*tp53* cells were treated with NO generating agent, *S*-nitroso-*N*-acetylpenicillamine (SNAP). It resulted in the increase of hsp72 and p53 in the cells. Finally, the NO production in the m*p53* cells also affected the thermosensitivity of the w*tp53* cells. The thermosensitivity of the w*tp53* cells was reduced in the conditioned medium by pre-culture of the heated m*p53* cells as compared with that in a conventional fresh growth medium. Our finding that accumulations of p53 and hsp72 in the co-cultivated NO donee cells with the heated NO donor cells provides the first evidence for the intercellular signal transduction pathway via NO as intermediate, without cell-to-cell interactions such as gap junctions.