SP-II-3 p53-dependent cell cycle regulation is not mediated by RB in normal human cells treated with heat shock

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p53 protein is accumulated following exposure to various stresses. The activated p53 protein transactivates genes, such as *p21*, *gadd45*, *bax* and *bcl-2* genes, and regulates cell cycle at G1/S and apoptosis. We examined here the accumulation of p53 protein by heat-shock treatment in normal human embryonic cells. Cells were heat shocked at 43°C for 2 hours and recovered at 37°C subsequently. The level of p53 protein gradually increased after 1 hour of recovery and reached maximum at around 4 hours. The accumulated p53 induced p21 and the maximum level of p21 protein was at around 5 hours after heat shock, followed by the gradual decrease of the S-phase. We found that G1 and G2 arrest was evident after 12 hours. p21 can bind to and inhibit cyclin-dependent kinases, causing hypophosphorylation of RB, thus preventing the release of E2F, and blocking the G1-S transition. So we examined whether heat shock also promoted RB dephosphorylation and thus arrested cell cycle at G1/S. While the phosphorylation pattern of RB protein was not affected by 12 hours after heat shock, remarkable change was observed at 24 hours. The results suggest that there is little possibility that RB is associated with cell cycle arrest observed by 12 hours. Therefore it can be concluded that the activation of p53-p21 pathway induced by heat shock arrests cell cycle at G1/S in a RB-independent manner.

SP-II-4 Hypermutability of xeroderma pigmentosum variant cells is caused by deficiency in induction of p21 after UV irtradiation

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Xeroderma pigmentosum variant (XPV)patients show the clinical characteristics of the desease, with increased frequencies of skin cancer, but their cells have a nearly normal rate of nucleotide excision repair of UV-induced DNA damage and are only slightly more sensitive than normal cells to the cytotoxic effect of UV radiation.However, they are significantly more sensitive to its mutagenic effect. To examine the mechanisms responsible for this hypermutability, we mesured the level of p53, GADD45 and p21 proteins after UV-irradiation in normal and XPV cells. After UV irradiation, the level of p53 and GADD45 proteins increased in both normal and XPV cells. By contrast, the p21 increased in normal cells, but did not in XPV cells.In addition, XPV cells are deficient in cell cycle checking at G1-S after UV-irradiation.This could account for the hypermutability of XPV cells with UV irradiation.

SP-II-5 Role of *Gadd45* gene in X-ray-induced G_1 arrest in human cells

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The expression of *Gadd45* gene is dependent on the p53 function and suboptimal in ataxia telangiectasia (AT) cells after exposure to radiation. To know the role of *Gadd45* gene in the p53 dependent G_1 checkpoint pathway, we introduced *Gadd45* cDNA into a SV40-transformed AT cell line and established a cell line whose expression of the introduced *Gadd45* gene was inducible by treatment with isopropyl thiogalactoside (IPTG). The treatment of IPTG induced 5- to 8-fold accumulation of GADD45 within 24 hr. Using this AT cell line, we studied the effect of GADD45 expression on the G_1 checkpoint and found that the induction of GADD45 with IPTG treatment caused neither reduced DNA synthesis nor cell cycle arrest, suggesting that *Gadd45* gene may not be involved in radiation-induced G_1 arrest. To clarify the more defined function of *Gadd45* gene, we investigated the effect of GADD45 expression on growth rate and colony forming ability in the AT cells and found that the elevated expression of GADD45 delayed, but not completely inhibited, the cell growth and caused 30% reduction of the cloning efficiency. Because GADD45 did not reduce the cell adhesion ability, our results suggest that GADD45 could function as a negative regulator for cell growth.