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p53 MUTATION AND NEOPLASTIC TRANSFORMATION IN CULTURED HAMSTER EMBRYO CELLS

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In vitro transformation systems using primary cultured hamster cells are useful for analyzing the mechanism of neoplastic development, because they have a diploid karyotype and show normal growth properties in culture. For example, we examined the acquisition of transformed phenotypes in Syrian/golden hamster embryo (SHE) cells following exposure to X-rays and demonstrated that stepwise changes in karyotypes are necessary for malignant progression of X-ray-induced transformation. Similar progressive phenomena have also been observed in spontaneous neoplastic transformation in Chinese hamster embryo (CHE) cells transferred successively. We found that CHE cells transferred *in vitro* can easily transform to permanent stages, but only 2 (CHE A1 and CHE A2) out of 9 CHE strains exhibit tumorigenicity at later passages.

Since various mutations in *ras* or *p53* genes have been detected in a wide variety of human tumors, we analyzed genetic alterations of these genes in the process of spontaneous transformation in CHE A1 and CHE A2 cells. Although no mutation in *p53* cDNA was detected in tumorigenic CHE A2 cells, their tumor-derived cell lines had one mutation in the second position of N-*ras* codon 61. It was found, on the contrary, that G to A transition in *p53* at codon 245 was detected in CHE A1 cells cultured for 40 passages and that this mutant allele in passaged cells became predominant with increasing passages thereafter. Because tumor-derived cell lines from CHE A1 cells contained only the mutant allele and showed a high degree of aneuploidy with various abnormal chromosomes, this *p53* mutation may have created genetic instability, providing the means for the expression of malignant phenotypes in transformed CHE A1 cells.

Increased expression of normal *p53* protein in response to DNA damage is known to induce cell cycle arrest at the transition from G1 to S phase by stimulating the synthesis of inhibitor of cycline-dependent kinase. A decreased chromosome stability has been demonstrated in mutant *p53* cells following irradiation with ionizing radiation. To confirm to this possibility, we isolated various clonal cell lines having normal or mutant *p53* genes from 40 passaged CHE A1 cells and examined their responses to X-rays. Although X-ray dose survival curves showed a slight variation among different clonal cell lines, there was no tendency for X-ray sensitivity between normal and

mutant *p53* cell lines. Both normal and mutant *p53* cell lines also showed almost the same cell cycle progression from G1 to S phase after X-irradiation. However, when the cells were irradiated with 2 Gy of X-rays, two times lower mitotic indexes were observed within a few hours in all normal *p53* cell lines than in mutant *p53* cell lines, indicating a lack of radiation-induced G2 arrest in mutant *p53* cells. Interestingly, all X-irradiated mutant *p53* cell lines exhibited a higher proportion of abnormal cells containing 10 or more chromosome aberrations than X-irradiated normal cell lines. Furthermore, when 6-thioguanine resistant mutation and chromosome aberration were examined in the cells cultured for 9 days after X-irradiation, all mutant *p53* cell lines exhibited much higher frequencies than normal cell lines. These results suggest that CHE cells having mutant *p53* cause an enhanced genetic instability when irradiated with X-rays.

p53 is considered to have various biological functions, such as a checkpoint regulation that serves to cause cell cycle arrest in response to DNA damages, a signal transduction for apoptosis following DNA damages and a gene conservation that regulates correct DNA synthesis. I propose that the disruption of cell cycle checkpoint in G2/M phase by *p53* mutation plays an important role as a trigger to cause genetic instability in X-irradiated CHE cells.

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