ANTICANCER RESEARCH *28*: 2687-2690 (2008)

Variations in Sensitivity to Ionizing Radiation in Relation to *p53* Mutation Point

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Abstract. *Mutation of p53 is the most common genetic alteration observed in human tumours and is reported to lead to variations in cell radiosensitivity. However, the relationship between the mutation point and the degree of radiosensitivity is unclear. Saos-2 cells with different mutations of p53 were prepared and examined for radiosensitivity. Cells with p53 mutations at codons 175, 244, 245, 273 and 282 were radioresistant, whereas those with mutations at codons 123, 195, 238 and 242 were radiosensitive. Mutations at codons 130, 143, 157, 168, 277, 280 and 286 resulted in medium radiosensitivity. Thus the sensitivity of Saos-2 cells to ionizing radiation varies with the mutation point of the p53 gene.*

Normal p53 protein accumulates after exposure to ionizing radiation and causes arrest of the cell cycle or promotion of apoptosis (1-3). Normal p53 in irradiated cells can increase cell survival by DNA repair during growth arrest, or decrease it by induction of apoptosis. The tumour-suppressor gene *p53* is frequently mutated in a variety of human tumours (4). The positions of *p53* mutations in human tumour cells are spread widely throughout the gene, especially between the DNA binding domain-containing hot spot (at codons 175, 245, 248, 249, 273 and 282). It has been widely reported that cells with mutant *p53* are more resistant to radiation (5-8). On the other hand, there have been reports of cells with mutant *p53* that are sensitive to radiation (9, 10). However, these studies did not examine the position of mutation in the *p53* gene (11).

The radiosensitivity of tumour cells is an important consideration in radiotherapy. We have succeeded in transferring mutant *p53* DNA (T123A, Q143A, R175H, R273H) to the human osteosarcoma cell line, Saos-2, which is null for *p53*, and examined the radiosensitivity of four *p53* mutations (12). The T123A mutant became very radiosensitive whereas the other mutants were radioresistant. This result indicated that the radiosensitivity of the cells was dependent on the position of the mutation in the *p53* gene. In the present study, we prepared another twelve types of *p53* mutant cell (L130V, V157F, H168R, I195T, C238Y, C242F, G244C, G245S, C277F, R280T, R282W, E286K) and examined their radiosensitivity.

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Key Words: Ionizing radiation, mutant *p53*, radiosensitivity, Saos-2.

Materials and Methods

Cell culture. The Saos-2 cell line (RCB0428: Riken Cell Bank, Tsukuba, Japan) was cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS). The transformants were selected in medium containing 100 μg/ml hygromycin or 400 μg/ml G418.

Plasmids. The LacSwitch-inducible mammalian expression system (Stratagene, La Jolla, CA, USA) was used. The expression plasmid was pOPI3CAT and the CAT gene was replaced by genes of a mutant or wild-type *p53* at the Not I site. Saos-2 cells were transfected with 2 μg of expression plasmid using the calcium phosphate precipitation method (13). A plasmid of p3'SS, expressing the *lacI* gene encoding the lac repressor protein, was used as a regulation plasmid. The expression plasmid and regulation plasmid were then cotransfected into the cells. The expression of each mutant *p53* gene was suppressed by the lac repressor in this LacSwitch system.

In vitro mutagenesis. Mutagenesis was performed with a QuikChange site-directed mutagenesis kit (Stratagene) in accordance with the manufacturer's protocol. Wild-type *p53* cDNA (pPro Sp53: CD 104; Japanese Cancer Research Resources Bank, Totyo, Japan) was introduced into the M13 vector. Oligonucleotides for the mutations were ordered from Hokkaido System Science, Japan. The mutant *p53* DNA sequences were checked with a DNA sequencing kit and a model 373A DNA sequencer (Applied Biosystems, CA, USA).

Western blotting of p53 protein. Total protein was extracted as described elsewhere (14) and 30 μg of protein were subjected to Western blotting analysis. Anti-p53 monoclonal antibody (Pab 1801:

Santa Cruz Biotechnology, CA, USA) was used. Protein levels were analyzed using enhanced chemiluminescence (Amersham, Piscataway, NJ, USA).

Radiation treatment and survival studies. Cells were irradiated with a PS-3100S Cs-137 γ-ray machine (Pony Industry Co. Ltd., Osaka, Japan) at a dose of 0.993 Gy/min at room temperature. Growth cultures incubated in 10-cm tissue culture dishes were trypsinized for irradiation. After irradiation, the cells were plated onto 10-cm dishes at densities expected to yield about 50 clonogenic cells. Colonies were examined after about one month to calculate the surviving fraction. Three replicate plates per experiment were used for each survival point and the experiments were repeated at least three times. The results for survival were calculated as mean±SE, and comparisons between values were analyzed statistically by Student's *t*-test.

Results

We had already cloned the cells harbouring mutant *p53* (T123A, Q143A, R175H, R273H) and wild-type *p53* (12). We selected another 12 *p53* mutation positions present in human tumours and available in the *p53* mutation database (15). These *p53* mutations were those most frequently affecting the codons of amino acids among the positions recorded in the database. The mutant *p53* plasmids were introduced into Saos-2 cells and clones were obtained for each mutant as described in Material and Methods. Western blotting was performed to determine the expression of p53 protein, and finally we obtained a total of 16 transformants of mutant *p53* (T123A, L130V, Q143A, V157F, H168R, R175H, I195T, C238Y, C242F, G244C, G245S, R273H, C277F, R280T, R282W, E286K).

Figure 1 shows the survival fractions of each of the mutant cell types after exposure to 6 Gy of radiation. The parental Saos-2 cells and control pOPI3 vector transformants were radioresistant, but the wild-type *p53* transformant was radiosensitive. The mutant *p53* transformants showed a range of radiosensitivities. The 175H, 244C, 245S, 273H and 282W transformants exhibited radioresistance much like the parental Saos-2 cells and the control transformants. Their survival fractions exceeded 0.01, which was significantly higher ($p<0.005$) than that of the wild-type *p53* cells. On the other hand, the 242F transformants exhibited almost the same radiosensitivity as the wild-type *p53* cells ($p>0.05$). The 123A, 195T and 238Y transformants were significantly ($p<0.005$) more radiosensitive than the wild-type *p53* cells. The survival fractions of these radiosensitive mutants were less than 0.005. The remaining transformants, 130V, 143A, 157F, 168R, 277F, 280T and 286K, showed medium radiosensitivity, with survival fractions of between 0.005 and 0.01. They were significantly ($p<0.005$) more radiosensitive than Saos-2 cells. Furthermore, they were significantly (130V, 143A, 168R and 286K: $p<0.005$; 157F, 277F and 280T: $p<0.025$, respectively) more radioresistant than wild-type *p53* cells.

To clarify whether the radiosensitivity of the various mutants merely represented clonal variation, we examined two clones of each of the transformants 277F, 280T and 286K, which exhibited medium radiosensitivity (Figure 2). The radiosensitivity of both clones of each mutant was similar ($p>0.05$, respectively) and in all cases the survival fractions were still between 0.005 and 0.01. Thus the variation of radiosensitivity observed among the transformants was likely a specific property of the *p53* mutation in each case.

The most significant finding was that all hot spot mutations (175H, 245S 273H and 282W) conferred radioresistance. As hot spot mutations are frequent in human tumour cells, this may have some relation to tumour radioresistance. We calculated the frequency of mutation at the codon in human tumour cells by reference to the TP53 website (http://p53.free.fr/index.html) and plotted the relationship between mutation frequency and radiosensitivity (Figure 3). However, there was no clear correlation between mutation frequency in human tumour cells and the radiosensitivity of mutants, other than that of hot spot mutants.

Discussion

p53 plays an important role in cellular response to radiation (1). Normal p53 acts as a guardian of the genome by promoting G1 arrest and repairing DNA damage (16), and also kills cells by apoptosis (17) in irradiated cells. The *p53* gene is mutated in over half of all human tumours and the mutation points are widespread throughout the gene (18). It is important to determine which mutations affect the radiosensitivity of tumour cells because tumour cell radiosensitivity has substantial clinical relevance in tumour radiotherapy.

We prepared a total of 16 stable *p53* mutant transformants to determine which p53 mutations affect radiosensitivity. The radiosensitivity of the transformants varied with the mutation point of the *p53* gene (Figure 1). Four of the radioresistant mutations (175H, 245S 273H and 282W) were hot spot mutations and one (244C) was adjacent to the hot spot. Hot spot points in wild p53 protein bind directly to DNA or play an important role in DNA binding (19). It is reasonable to assume that if these hot spot points are mutated, then the mutant protein will lose its ability to bind the consensus DNA sequence for p53 binding and fail to induce p53 downstream gene expression. In this case, hot spot mutation is almost equivalent to loss of the *p53* gene, as in Saos-2 cells, except for the gain-of-function (20). Thus hot spot mutants might show radioresistance similar to that of Saos-2 cells. There are three loops (loops 1, 2 and 3) in the protein structure of the DNA-binding domain of p53 (21), and the mutations conferring radiosensitivity are localized in loop 1 (123A), loop 2 (175H and 195T) and loop 3 (242F).

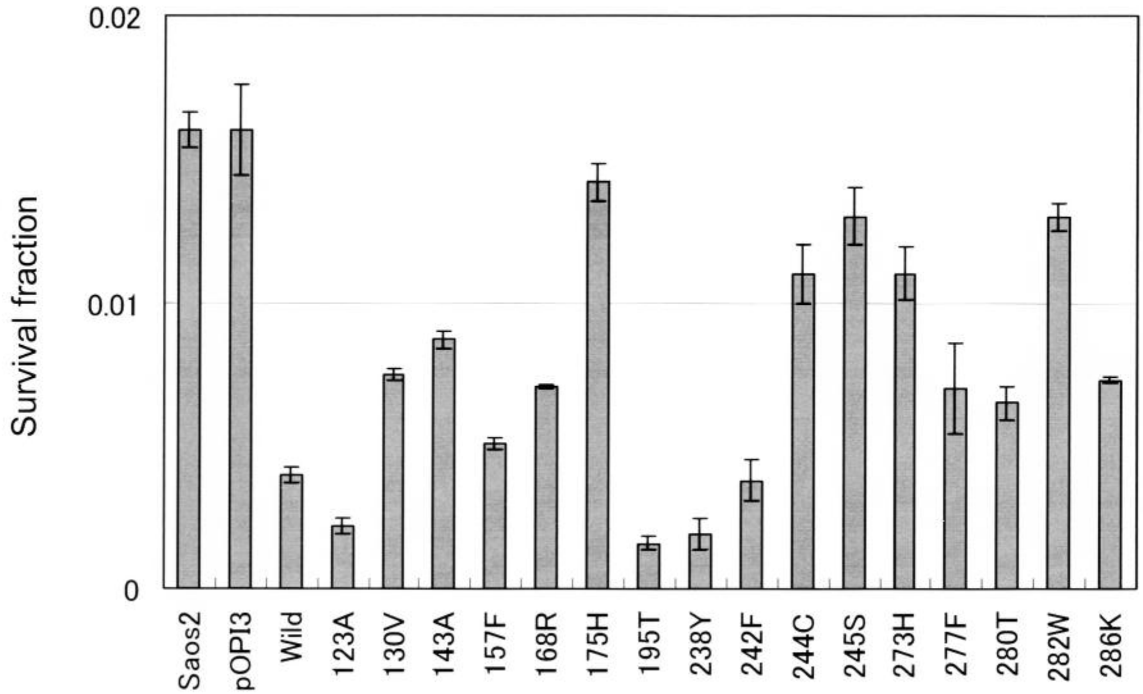

Figure 1. *Radiosensitivity of the mutant p53 cells. Saos-2, the pOPI3 transformant, the wild-type p53 transformant and 16 mutant p53 transformants were exposed to 6 Gy of radiation. Error bars represent standard deviation.*

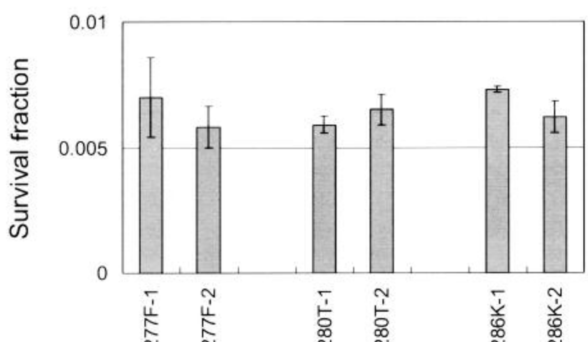

Figure 2. *Radiosensitivity of clones. Two clones of each mutant p53 transformant (277F, 280T and 286K) were exposed to 6 Gy of radiation. Error bars represent standard deviation.*

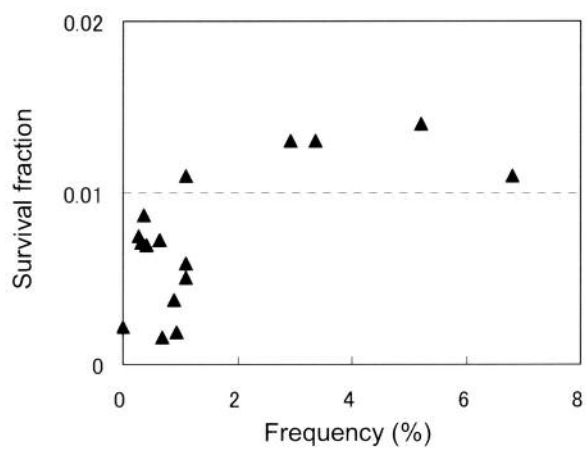

Figure 3. *Relationship between the frequency of p53 mutation in human tumour cells and radiosensitivity of the p53 mutants.*

Mutations in these loop domains may not change in the conformation of p53 protein and may thus allow p53 to retain some properties of the wild-type.

p53 also plays an important role as a tumour suppressor by inducing death or growth arrest in cells (22, 23). *p53* is frequently mutated in human tumour cells and the alteration of *p53* is very important in cells becoming tumourous. The frequency of *p53* mutations vary with mutation points, the most frequent mutations being the hot spot mutations. The frequency of the mutation at specific points might have a correlation with the tendency for cells to become tumourous. We wondered if the frequency of mutation at such points may also have some correlation with the radiosensitivity in the cells. Indeed, all hot spot mutants were radioresistant. However, in the present study, except for hot spot mutants, there was no clear correlation between the frequency of *p53* mutation and the degree of radiosensitivity in *p53* mutant cells (Figure 3). A previous report indicated that the pathological response to DNA damage does not contribute to *p53*-mediated tumour suppression (24). Therefore, the radiosensitivity of mutant *p53* cells may be independent of *p53*-mediated tumour suppression.

We have already reported that the sensitivity of transformants to anticancer drugs varies with the type of *p53* mutation (123A, 143A, 175H, 273H) (25). As the radiosensitivity or chemosensitivity of cells might depend on the position of the mutation in the *p53* gene, it seems important to determine the mutation position in assays to predict the responsiveness of individual tumours to radiotherapy and chemotherapy.

Acknowledgements

This work was partly supported by the 21st Century Center of Excellence (COE) and Global COE Program from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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Received March 20, 2008
Revised June 19, 2008
Accepted July 1, 2008