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Changes in Radiation Sensitivity of Human Osteosarcoma Cells after p53 Introduction

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Human osteosarcoma SAOS-2 cells, which have a deletion in p53 gene, were transfected with plasmid pMSVneop53 containing human p53 cDNA and neomycin-resistance gene. Three clones (SAOS-MC10, SAOS-MC11 and SAOS-MC43) among 60 clones expressed p53 mRNA. No p53 protein was observed in SAOS-MC10, while SAOS-MC11 and SAOS-MC43 produced p53 protein. The molecular weight of p53 protein in SAOS-MC43 was lower than that in SAOS-MC11. SAOS-MC11 and SAOS-MC43 were more sensitive and more resistant, respectively, to ionizing radiation than the parental SAOS-2. We suggest that exogenous p53 protein might be one of the factors determining cellular radiosensitivity.

Key words: p53 introduction — p53 expression — Radiation sensitivity — Human osteosarcoma SAOS-2 cells

In human cancer, p53 gene mutation is one of the most frequently identified of the mutations which are believed to be implicated in the multi-step processes leading to malignancy.[1,2] Cells in which p53 is mutated or absent appear to be unable to initiate the characteristic G_1 arrest following DNA damage by ionizing radiation,[3] and cells derived from null mice show enhanced resistance to ionizing radiations with little apoptosis following lethal doses of irradiation.[4,5] Similarly, Burkitt's lymphoma cells with wild-type p53 gene are radiosensitive in comparison with those having mutant p53.[6] These findings suggest that p53 gene plays an essential role in the induction of cell death by ionizing radiation.

SAOS-2 cells are derived from a human osteosarcoma and have a deletion in the coding region of p53 gene encompassing exons 2 through 11.[7,8] Therefore, the cells are deficient in p53 expression and lack functional p53. By using an electroporation unit, we transfected SAOS-2 cells with plasmid pMSVneop53, containing neomycin-resistance gene and human p53 cDNA derived from pProSp53. After the pMSVneop53 transfection and G418 treatment, 60 clones were isolated. The expression of p53 mRNA was observed in three clones, 10 (SAOS-MC10), 11 (SAOS-MC11), and 43 (SAOS-MC43) (Fig. 1A), but not in the other clones. The transcription size of p53 was different among these clones. The size (2.5 kb) observed in SAOS-MC11 cells is normal and that of about 2.1 kb in SAOS-MC10 and SAOS-MC43 cells is abnormal. Western immunoblot analysis showed that SAOS-MC11 and SAOS-MC43 cells produced p53 proteins, whereas no expression of p53 was observed in SAOS-MC10 cells (Fig. 1B). The molecular weight of p53 protein produced in SAOS-MC43 cells was lower than that in SAOS-MC11 cells.

To examine whether any alterations exist in the p53 cDNA introduced into these cells, the polymerase chain reaction (PCR) of the p53 fragments was performed. We amplified p53 cDNA as three fragments, F1 (nucleotides 165–621), F2 (572–1036) and F3 (991–1510). From the analyses of each fragment, the nucleotide sequence of p53 in SAOS-MC11 cells was shown to be consistent with that of wild-type p53 cDNA. However, no F1 PCR product was obtained in SAOS-MC10 cells and no F3 PCR product in SAOS-MC43 cells. Therefore, parts of F1 (including the initiation codon of nucleotides 215–217) and F3 (C-terminus region) might be deleted in SAOS-MC10 and SAOS-MC43 cells, respectively.

Fig. 2 shows the X-ray dose-survival curves of the parental SAOS-2, the negative control SAOS-MSV, SAOS-MC10, SAOS-MC11 and SAOS-MC43 cells. SAOS-MC11 cells were more sensitive and SAOS-MC43 cells were more resistant to radiation than the other cells. The D_0 values of SAOS-2, SAOS-MSV, SAOS-MC10, SAOS-MC11 and SAOS-MC43 cells were 1.15 Gy, 1.17 Gy, 1.17 Gy, 0.80 Gy and 1.75 Gy, respectively.

In the treatment of cancer, sensitivity or resistance of tumor cells to ionizing radiation has substantial clinical consequences. The lethal effects of ionizing radiation in the clinically relevant dose range appear to be due primarily to DNA damage. However, molecular mechanisms involved in the repair of DNA damage by ionizing radiation are not well understood in mammalian cells.

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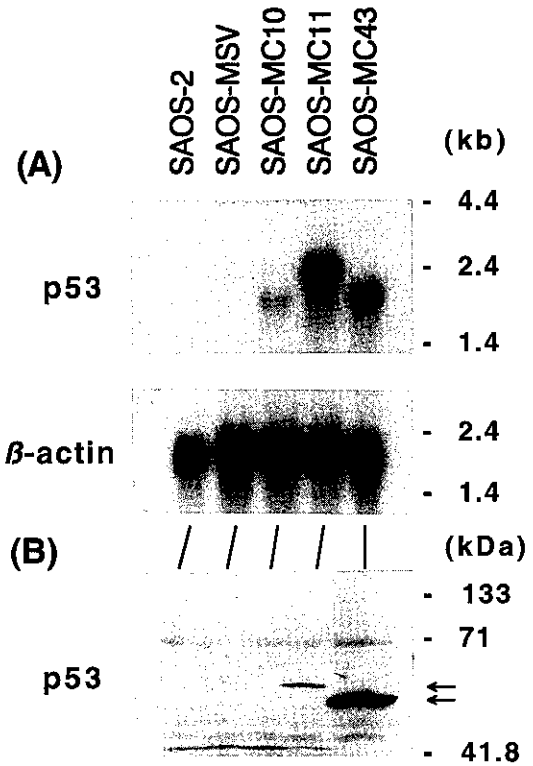

Fig. 1. Northern blot (A) and western immunoblot (B) analyses of p53 in the parental SAOS-2, the negative control SAOS-MSV, SAOS-MC10, SAOS-MC11, and SAOS-MC43 cells. For northern blots, the p53 DNA probe used was a 2.2 kb *Bam*HI fragment of php53B including human p53 cDNA (purchased from ATCC), and the β-actin probe was a 0.5 kb *Hin*fI fragment including exons 3 and 4 of the human β-actin gene. For the western blot, a monoclonal anti-p53 antibody (PAb421, Oncogene Science) was used. The blot was autoradiographed utilizing enhanced chemiluminescence according to the instructions of the manufacturer (Amersham International plc). Experiments were repeated twice and no significant difference was observed between the results.

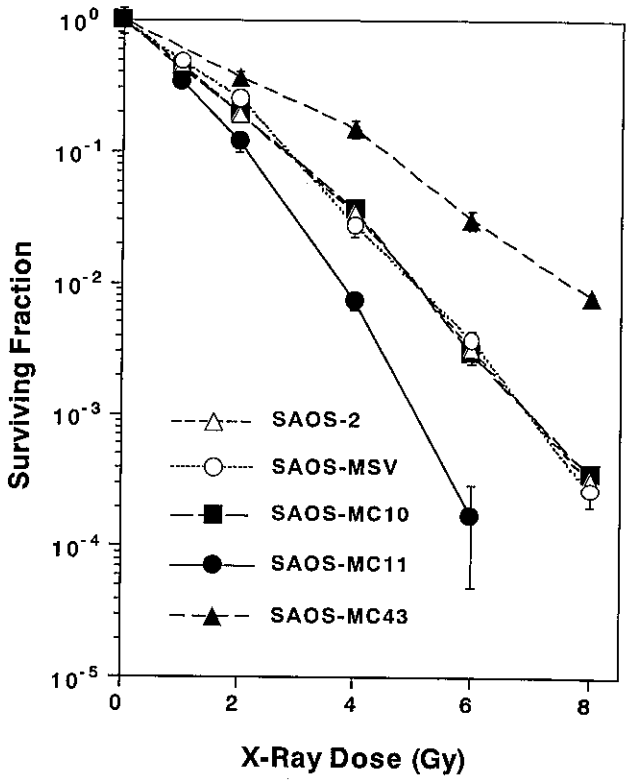

Fig. 2. X-ray dose-survival curves of the parental SAOS-2, the negative control SAOS-MSV, SAOS-MC10, SAOS-MC11 and SAOS-MC43 cells. All irradiations were performed at 37°C in 95% air and 5% CO_2. The dose rate was approximately 1 Gy/min. Five replicate plates per experiment were used for each survival point and the experiments were repeated at least three times. Error bars represent standard deviations.

Detection of the presence of wild or mutant protein in human tumors may be helpful to predict whether a tumor will respond to ionizing radiation. We observed both an increase and a decrease in the radiosensitivity of the p53-negative tumor cells following the introduction of p53 cDNA (Fig. 2). This finding suggests that exogenous p53 protein might be one of the factors determining cellular radiosensitivity. To clarify the exact role(s) of p53 in determining cellular radiosensitivity, functional analysis of the p53 proteins produced in SAOS-MC11 and SAOS-MC43 cells is required.

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