— **Minireview** —

The Role of p53 Molecule in Radiation and Hyperthermic Therapies

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In recent years, cancer-related genes have been analyzed at the molecular level as predictive indicators for cancer therapy. Among those genes, the tumor suppressor gene *p53* is worthy of notice in cancer therapy, because the p53 molecule prevents the malignant degeneration of non-cancer cells by regulating cell-cycle arrest, apoptosis, and DNA repair. An abnormality of the *p53* gene introduces a genetic instability and increases the incidence of carcinogenesis and teratogenesis. Therefore, *p53* is called a guardian of the genome. Mutations of *p53* are observed at a high frequency in human tumors, and are recognized in about half of all malignant tumors in human head and neck cancers. We previously reported that radio- and heat-sensitivities of human cultured tongue squamous cell carcinoma cells are *p53*-dependent, and are closely correlated with the induction of apoptosis. In a human cell culture system, the interactive hyperthermic enhancement of radiosensitivity was observed in wild-type p53 cells, but not in mutated p53 cells. In a transplanted tumor system, the combination therapies of radiation and hyperthermia induced efficient tumor growth depression and apoptosis in the wild-type p53 tumors. In this review, we discuss the p53 activation signaling pathways through the modification of p53 molecules, such as phosphorylation after radiation and hyperthermia treatments.

Key words —— p53, predictive indicator, cancer therapy, X-ray, hyperthermia, apoptosis

INTRODUCTION

Combinations of radiation and hyperthermia therapies have been widely adopted for interdisciplinary cancer therapy. It is well known that ionizing radiation-induced cell killing is enhanced by hyperthermia *in vitro*.[1,2] Previous studies have shown that heat treatment depresses the DNA repair of radiation-induced DNA strand breaks and thymine lesions.[3,4] In addition, it has been reported that the activities of DNA polymerases, α and β are sensitive to heat treatment at temperatures higher than 40°C.[5,6] Thus, it has been understood that the synergistic effects of hyperthermia on radiation-induced cell killing is induced mainly through the inhibition of DNA repair mechanisms. Moreover, another possible mechanism of radiosensitization by hyperthermia has been suggested to involve the hyperthermic instability of the Ku subunits of DNA-PK, which contribute to the repair of radiation-induced double-strand breaks in DNA.[7,8]

Patients with tumors that have p53 mutations often have a worse prognosis than those with tumors that have wild-type p53 (wtp53).[9] For prognosis-predictive assays of cancer therapy, the genetic status of the *p53* gene is the most important candidate among various cancer-related genes.[10] We previously reported that the radio- and heat-sensitivities of human cultured tongue squamous cell carcinoma cells are p53-dependent, and are closely correlated with the induction of apoptosis.[11,12] The interactive hyperthermic enhancement of radiosensitivity was also observed in wtp53 cells, but not in mutated p53 (mp53) cells.[13] However, it remains unclear whether the hyperthermic enhancement of tumor growth inhibition by irradiation is p53-dependent. To clarify the problem, we described in this review whether the *p53* gene products contribute to the hyperthermic enhancement of tumor growth inhibition by X-

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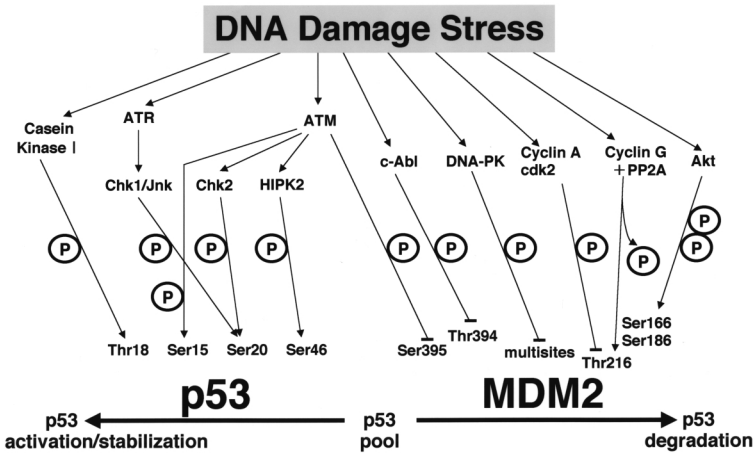

Fig. 1. p53-Centered Signal Transduction Pathway
→, accelerate; —|, suppress; Ⓟ, phosphate.

ray irradiation, using transplantable human cultured tongue squamous cell carcinoma cells with an identical genotype except for the *p53* gene status, as an *in vivo* experimental model from the view point of p53 activation and deactivation through the modification and degradation of p53 molecules.

p53 and the Signal Pathways

The ancestor of the mammalian p53 tumor suppressor protein is homologous to drosophila and nematodes.[14] The gene product prevents the malignant degeneration of a normal cell. In cancer cells bearing m*p53*, genetic stability is lost and mutations are accumulated, and therefore, malignant changes in the cancer progress at a high frequency. *p53*-mutant and *p53*-deleted cells account for about 50% of total advanced cancer cells.[15] The p53 molecules are modified for activation by many kinds of different protein kinases at different portions after cell stress (Fig. 1).[16]

p53 regulates the transcription of the phenotypic expressions of target genes by binding to a specific sequence. One of the p53 target genes, wild-type p53 activated fragment 1 (WAF1) inactivates proliferating cell nuclear antigen (PCNA) regulating DNA replication,[17] and induces *p53*-dependent G_1 arrest through the inhibition of cyclin/cyclin-dependent kinase (cyclin/CDK) activity.[18,19] During cell cycle arrest, *p53*-regulated pathways, such as growth arrest and DNA damage inducible 45 (gadd45) and p53 ribonucleotide reductase small subunit 2 (p53R2), play an important role in the repair of damaged DNA.[20,21] Otherwise, DNA damage induces apoptosis by several processes of *p53*-regulated pathways, such as *Bcl-associated X protein* (*Bax*),[22] *Fas/APO-1*,[23] and *PAG608*.[24] In contrast, *p53*-regulated murine double minute 2 (MDM 2)[25] functions in the negative feedback regulation of p53 activity.[26] It is reported that these determinations of cell cycle arrest or apoptosis are divided by the modification of p53 molecules, such as phosphorylation[25] (Fig. 1), acetylation,[27] poly (ADP-ribosyl)ation[28] and sumoylation.[29,30] When the cells are stressed, Ser15/Ser20 of p53 is phosphorylated, then MDM 2 is separated from the phosphorylated p53, and finally p53 is stabilized and activated,[31–33] Thereafter, p53 binds to the promoter of *WAF1* or the *p53R2* gene related to DNA repair, and induces their gene expression. When there are too many irreparable DNA lesions, p53 is phosphorylated at Ser46, and then binds to the promoter of the *p53-regulated apoptosis-inducing protein 1* (*p53AIP1*) gene. Then induction of p53AIP1 mediates apoptosis in the serious damaged cells.[34,35] Other p53 modifications of acetylation and sumoylation have also been reported. It is understood that acetylation is performed at the C-terminal region of p53, and the binding capacity of p53 to specific DNA is enhanced.[36] In addition, there are several reports regarding the sumoylation of lysine 386 in p53, which seems to activate a *p53* response gene.[29,30,37–39] In these modifications, it is thought that the structural change of p53 molecules, which bind to specific DNA sequences known as p53 concensus sequence, is brought about by many kinds of the up-stream genes. On the other hand, p53 molecules are deactivated and degradated by activated MDM 2 molecules, which are phosphorylated at multi-sites by other protein kinases (Fig. 1).

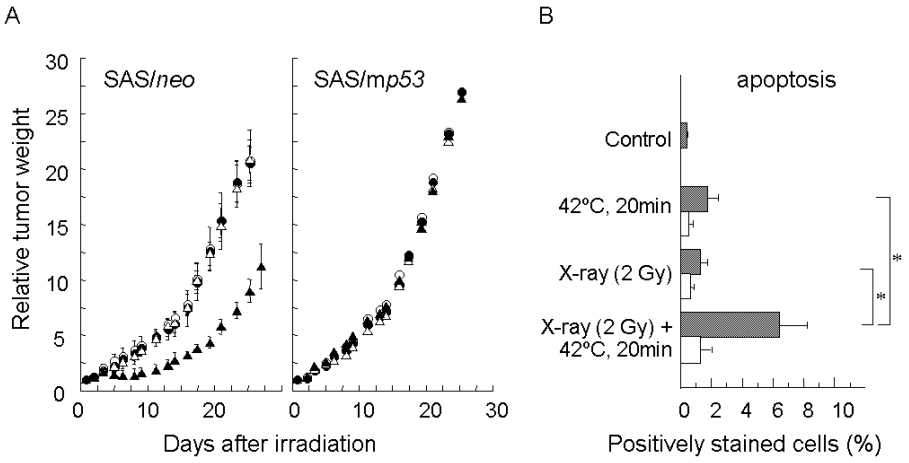

Fig. 2. The Effects of Hyperthermia and/or X-Ray Treatments on Growth (A) and Apoptosis (B) of Human Tongue Carcinoma

A, Tumor growth curves of human tongue carcinomas. ○, non-treated group; ●, hyperthermia at 42°C for 20 min alone; △, X-rays at 2 Gy alone; ▲, combined treatment with X-rays at 2 Gy and hyperthermia at 42°C for 20 min. B, The rate of cells positively stained for apoptosis between SAS/*neo* tumors (closed column) and SAS/m*p53* tumors (open column) 72 hr after treatment. In all cases, three individuals, who were blind to the source of the specimen, counted a total of 500 cells in three random fields. The error bars indicate S.D. *, a highly significant difference ($p < 0.01$) by Student's *t*-test.

Moreover, p53 is reported to bind to other proteins, such as heat shock proteins (HSPs), which are a famous stress protein.[40,41] Thus, p53 regulates the fate of the cells after stresses, such as cancer therapies.

p53-Dependent Synergism between Hyperthermia and X-Ray Treatment

We previously reported that radio- and heat-sensitivities of cultured human tongue squamous cell carcinoma cells are *p53*-dependent, and are closely correlated with the induction of apoptosis[11,12] *in vitro*. To confirm that the hyperthermic enhancement of tumor growth inhibition by X-ray irradiation is dependent on the *p53* gene status, we used two kinds of cancer cell lines carrying a different *p53* gene status, wt*p53* and m*p53*. We compared heat and X-ray induced cell killing and apoptosis frequencies in the wt*p53* cells and the m*p53* cells though Bax and Caspase-3 pathways.[11,12] We adopted mild treatments with radiation and hyperthermia for transplantation on nude mice to examine the synergistic effects on tumor growth inhibition. Tumor growth curves are shown in Fig. 2A.

Individual treatment with hyperthermia at 42°C for 20 min or X-irradiation (2 Gy) showed almost no effect on the growth curves of the both wtp53 and mp53 tumors in the non-treated control groups (Fig. 2A). When the tumors were treated with a combination of X-rays and hyperthermia, the apparent enhancement of tumor growth inhibition was observed in the wtp53 tumors alone.

We examined both the accumulation of apoptosis-related proteins and the incidence of apoptosis by immunohistochemical analysis (Fig. 2B). The apoptosis incidence was apparently higher in the wtp53 tumors 72 hr after the combined treatment, but not in the mp53 tumors. The fragmentation of poly (ADP-ribose) polymerase (PARP) and Caspase-3 as the activation of Caspase-3 showed almost the same pattern as the incidence of apoptosis in the wtp53 tumor.[42]

p53 Gene Status as a Predictive Indicator in Cancer Therapy

As previously reported, if the hyperthermic enhancement of radiosensitivity results in heat-induced denaturation of repair enzymes for DNA damage, the synergism of the combination therapy of radiation and hyperthermia should be similarly detected in both wtp53 and mp53 tumors. However, it is very interesting that the synergistic depression of tumor growth was found only in the wtp53 tumors. These findings suggest that the hyperthermic enhancement of tumor growth inhibition by irradiation may result in *p53*-dependent apoptosis due to heat-induced inactivation of the cell survival system(s), through either regulation of the cell cycle or induction of DNA repair. Thus, mp53 tumors may rarely include this function, which promotes cell killing by the heat-induced interactive inactivation of the repair enzyme(s) for certain types of sublethal damage. As participants in the *p53*-dependent apoptotic processes, the induction of apoptosis mediated by

Caspase-3 activation was examined in these tumors. In fact, activated Caspase-3 was confirmed from the proteolysis of several important molecules, such as PARP and Caspase-3 itself so called markers of apoptosis.[43,44] The induction of *p53*-dependent apoptosis showed a pattern similar to the tumor growth inhibition after combined treatments with radiation and hyperthermia (Fig. 2B). Therefore, gene analysis of the *p53* gene status of cancer cells can be used as a predictive assay for the effectiveness of combined therapy with radiation and hyperthermia.

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