The role of the p53 molecule in cancer therapies with radiation and/or hyperthermia

ABSTRACT
In recent years, cancer-related genes have been analyzed as predictive indicators for cancer therapies. Among those genes, the gene product of a tumor suppressor gene p53 plays an important role in cancer therapy, because the p53 molecule induces cell-cycle arrest, apoptosis and depression of DNA repair after cancer therapies such as radiation, hyperthermia and anti-cancer agents. An abnormality of the p53 gene might introduce low efficiency in their cancer therapies. Mutations of p53 are observed at a high frequency in human tumors, and are recognized in about half of all malignant tumors in human. In the both systems of a human cell culture and their transplanted tumor, the sensitivities to radiation, heat and anti-cancer agents were observed in wild-type p53 cells, but not in mutated or deleted p53 cells. In this review, we discuss the p53 activation signaling pathways through the modification of p53 molecules such as phosphorylation after radiation and/or 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. Previous studies have shown that heat treatment depresses the DNA repair of radiation-induced DNA strand breaks and thymine lesions. 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. 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.

Patients with tumors that have p53 mutations often have a worse prognosis than those with tumors that don’t have wild-type p53 (wtp53). For prognosis-predictive assays of cancer therapy, the genetic status of the p53 gene is the most important candidate among various cancer-related genes. We previously reported that the radio-, heat- and chemo-sensitivities of human cultured tongue squamous cell carcinoma cells are p53-dependent, and are closely correlated with the induction of apoptosis in in vitro and in vivo. The interactive hyperthermic enhancement of radiosensitivity was also observed in wtp53 cells, but not in mutated p53 (mp53) cells. 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-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. The gene product prevents the malignant degeneration of a normal cell. In cancer cells bearing mp53, 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. The p53 molecules are modified for activation by many kinds of different protein kinases at different portions after cell stress [Figure 1].

p53 regulates the transcription of the phenotypic expressions of target genes by binding to a specific sequence. One of the p53 target genes, WAF1 (wild-type p53 activated fragment 1) inactivates PCNA regulating DNA replication, and induces p53-dependent G0 arrest through the inhibition of cyclin/CDK activity. During cell cycle arrest, p53-regulated pathways, such as gadd45 (growth arrest and DNA damage inducible 45) and p53R2 (ribonucleotide reductase small subunit 2), play an important role in the repair of damaged DNA. Otherwise, DNA damage induces apoptosis by several processes of p53-regulated pathways, such as Bax (Bcl-associated X protein), Fas/APO-1, and PAG608. In contrast, p53-regulated MDM 2 (murine double minute 2) functions in the negative feedback regulation of p53 activity. It is reported that these determinations of cell cycle arrest or apoptosis are divided by the modification of p53 molecules, such as phosphorylation (Figure 1), acetylation, poly (ADP-ribosyl)ation, and sumoylation. 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. 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 DNA lesions, however, G0 arrest and DNA repair do not succeed. In this case, p53 is phosphorylated at Ser46, and then binds to the promoter of the p53AIP1 (p53-regulated Apoptosis-Inducing Protein 1) gene. Therefore, p53AIP1 is accumulated. Apoptosis happens in the damaged cells. 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. In addition, there are several reports regarding the sumoylation of lysine 386 in p53, which seems to activate a p53-downstream transcriptional factor. In these modifications, it is thought that the structural change of p53 molecules, which bind to specific DNA sequences known as p53 CON, is brought about by many kinds of the up-stream genes. On the other hand, p53 molecules are deactivated and degraded by activated MDM2 molecules, which are phosphorylated at multi-sites by other protein kinases [Figure 1].

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

**p53 Dependent Synergism after 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 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, wtp53 and mp53. We compared heat and X-ray induced cell killing and apoptosis frequencies in the wtp53 cells and the mp53 cells though Bax and Caspase-3 pathways. We adopted mild treatments with radiation and hyperthermia for transplantation on nude mice to examine the effects of hyperthermia and radiotherapy on tongue carcinoma cells.

![Figure 1: p53-centered signal transduction pathway, accelerate, suppress, phosphate](image1)

**Figure 2:** A, tumor growth curves of human tongue carcinomas. B, the rate of cells positively stained for apoptosis between SAS/neo tumors (closed column) and SAS/mp53 tumors (open column) 72 h 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 SD.*: a highly significant difference (P < 0.01) by Student’s t-test.
the synergistic effects on tumor growth inhibition. Tumor growth curves are shown in [Figure 2A].

We applied an individual treatment with hyperthermia at 42°C for 20 min or X-irradiation (2 Gy) which showed almost no effect on the growth curves of the both wt-p53 and mp53 tumors in the non-treated control groups [Figure 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 wt-p53 tumors, but not the mp53 tumors.

We examined both the accumulation of apoptosis-related proteins and the incidence of apoptosis by immunohistochemical analysis [Figure 2B]. The apoptosis incidence was apparently higher in the wt-p53 tumors 72 h after the combined treatment, but not in the mp53 tumors. The fragmentation of PARP and Caspase-3 as the activation of Caspase-3 showed almost the same pattern as the incidence of apoptosis in the wt-p53 tumor.[40]

**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 wt-p53 and mp53 tumors. However, it is very interesting that the synergistic depression of tumor growth was found only in the wt-p53 tumors.[46] 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.[47,48] The induction of p53-dependent apoptosis showed a pattern similar to the tumor growth inhibition after combined treatments with radiation and hyperthermia [Figure 3]. 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 [Figure 3].

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