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Immunohistochemical study of p53 expression in cancer tissues from patients undergoing radiation therapy

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Summary. Immunostaining using p53 monoclonal antibodies (p53(Ab-3) recognizes mutant type and p53(Ab-6) the wild type of p53 protein) was performed on frozen sections of biopsy specimens obtained before and during preoperative radiotherapy from 23 patients with head and neck squamous cell carcinoma. The positive staining rates of p53(Ab-3) before radiotherapy and at radiation doses of 4Gy, 10Gy and 20Gy were 30.0%, 38.9%, 25.0% and 6.25%, and those of p53(Ab-6) 10,5%, 11.8%, 5.0% and 0% respectively. The relationship between the immunohistochemical findings and antitumor effect at radiation dose of 20Gy was examined on the correspondent haematoxylin-eosin sections. In patients whose p53(Ab-3) stainings were positive at any doses of radiotherapy, the antitumor effect at the cumulative dose of 20Gy was either remarkable or effective. Moreover, the frequency of the expression of mutant type p53 protein tended to increase in rather radiosensitive tumors. As for wild type p53 protein, there was no remarkable relationship between the staining of p53(Ab-6) and the antitumor effect.

Key words: Immunohistochemistry, p53, Radiotherapy, Head and neck cancer

Introduction

Since its discovery, the p53 protein has been the subject of extensive study. Initially, this protein was detected as T antigen complexed to simian virus 40 (Lane and Crawford, 1979), and subsequent studies have established that the p53 protein will also bind to adenovirus E1b-58kd protein (Sarnow et al., 1982) and to mammalian heat shock protein HSP70 (Pinhasi-Kimhi et al., 1986). The p53 protein is present in minute amounts in normal cells and tissues, on the other hand, high concentrations of the protein exist in many kinds of tumors and tumor cell lines.

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Immunohistochemical studies carried out on different kinds of human cancer tissue showed elevated p53 levels in the cell nucleus in 30-60% of the sections examined (Iggo et al., 1990; Quinlan et al., 1992; Yamaguchi et al., 1994). There are still very few studies, however, concerning p53 protein expression in cancer tissues of patients undergoing radiation therapy (Frank et al., 1994; Merritt et al., 1994). Therefore, we studied the changes in the expression of p53 protein induced by irradiation in patients undergoing radiotherapy for head and neck squamous cell carcinoma. In addition, the correlation between the therapeutic effect of radiation and expression of p53 protein was examined using an immunohistochemical method.

Materials and methods

A total of 23 patients with squamous cell carcinoma of the head and neck regions were examined (Table 1). Pieces of approximately 0.5 x 0.5 x 0.5 cm were cut, on patient's approval, from obviously viable portions of the cancer tissue before and after delivery of 4, 10 and 20Gy (cumulative dose) of irradiation. All the specimens were histopathologically diagnosed as squamous cell carcinoma. Samples from these tissue specimens were immediately stored in liquid nitrogen and subsequently cut into serial sections of 6 μm in thickness in the cryostat. These sections were immunohistochemically stained by the streptavidin-biotin peroxidase method with Histofine SAB-PO kit, according to the instruction manual, using monoclonal antibodies against two kinds of p53 protein; p53(Ab-3) which recognizes mutant type of p53 protein, and p53(Ab-6) which recognizes the wild type (Table 2). The procedure has been described elsewhere (Ogawa et al., 1987, 1988, 1990; Manabe and Ohtsuki, 1992). Irradiation was administered with 4MV X-rays on an ML-15MDX linear accelerator (Mitsubishi Electric Co. Ltd., Japan). Treatments were performed five times a week, the daily fraction size being 2Gy. The grade of expression of each kind of p53 protein in the cancer tissue was evaluated according to the following

Table 1. Summarized data of patients studied.

CASE	SEX	AGE	SITE	TNM STAGE[1]	HISTOLOGY[2]	TREATMENT[3]	ANTITUMOR EFFECT[4]
1	M	52	Maxillary sinus	T4N0M0 IV	PD-SCC	52Gy + chemo + op	remarkable
2	M	63	Maxillary sinus	T3N0M0 III	SCC	42Gy + chemo + op	remarkable
3	M	73	Maxillary sinus	T4N0M0 IV	SCC	60Gy + chemo + op	poor
4	M	45	Maxillary sinus	T3N0M0 III	SCC	50Gy + chemo + op	effective
5	M	62	Tongue	T2N1M0 III	WD-SCC	20Gy + op	poor
6	M	67	Tongue	T4N3M0 IV	MD-SCC	60Gy	effective
7	F	70	Tongue	T4N1M0 IV	WD-SCC	20Gy + op	poor
8	F	58	Tongue	T3N3M0 IV	WD-SCC	56Gy	
9	M	68	Tongue	T1N0M0 I	WD-SCC	20Gy + op	effective
10	M	60	Tongue	T2N2M0 IV	WD-SCC	20Gy + op	effective
11	M	83	Tongue	T2N0M0 II	WD-SCC	26Gy + op	remarkable
12	M	60	Tongue	T4N2M0 IV	MD-SCC	60Gy	poor
13	M	68	Oral floor	T2N0M0 II	SCC	20Gy + op	poor
14	M	57	Oral floor	T2N2M0 IV	MD-SCC	20Gy + op	poor
15	M	53	Oral floor	T4N2M0 IV	MD-SCC	20Gy + op	effective
16	F	69	Gingiva	T1N0M0 I	MD-SCC	20Gy + op	effective
17	M	59	Gingiva	T4N0M0 IV	MD-SCC	20Gy + op	effective
18	F	65	Hard palate	T1N0M0 I	WD-SCC	20Gy + op	poor
19	M	67	Oropharynx	T3N2M0 IV	WD-SCC	70Gy	remarkable
20	M	72	Oropharynx	T2N2M0 IV	WD-SCC	20Gy + op	effective
21	M	59	Oropharynx	T4N3M0 IV	MD-SCC	60Gy + chemo + op	poor
22	M	65	Oropharynx	T3N0M0 III	WD-SCC	20Gy + op	poor
23	F	60	Hypopharynx	T2N2M0 IV	MD-SCC	20Gy + op	poor

[1]: UICC classification (1978). [2]: WD-SCC, well differentiated squamous cell carcinoma; MD-SCC, moderately differentiated squamous cell carcinoma; PD-SCC, poorly differentiated squamous cell carcinoma. [3]: chemo, chemotherapy; op, operation. [4]: poor, viable residual cancer cells; effective, degenerated cancer cells; remarkable, disappearance of cancer cells.

Table 2. Characteristic of the monoclonal antibodies used.

	p53(Ab-3)	p53(Ab-6)
Producer	Oncogene Science, Inc.	Oncogene Science, Inc
Origin	Clone PAb 240	Clone DO-1
Hybridoma	BALB/c mice splenocytes X SP2 mouse myeloma cells	BALB/c mice splenocytes X NS-1 mouse myeloma cells
Class	IgG1 (mouse)	IgG2a (mouse)
Specificity	Human mutant-type p53 protein and others	Human wild-type p53 protein and others

standards: 0% (-); <10% (±); 10-50% (+); >50% (++). Specimens that revealed + or ++ expression were evaluated positive. The relationship between the immunohistochemical findings and therapeutic effect of cumulative doses of 20Gy of radiotherapy was examined on correspondent haematoxylin-eosin sections.

Results

The results are summarized in Table 3. Specimens from 6 of the 20 patients evaluated showed positive staining (grade + or ++) for p53(Ab-3) before irradiation. During radiotherapy, positive staining was observed in 7 out of 18 samples, 5 out of 20 and 1 out of 16, at 4Gy, 10Gy and 20Gy, respectively.

As for p53(Ab-6), specimens from 2 out of 19 patients showed positive staining before irradiation. During radiotherapy, positive staining was observed in 2 out of 17 and 1 out of 20 samples, at 4Gy and 10Gy, respectively. At the cumulative dose of 20Gy of irradiation, none of the specimens showed positive labelling.

The correlation between p53 expression and the antitumor effect of radiotherapy was examined on H&E-stained sections at the cumulative dose of 20Gy. The antitumor effect was evaluated as poor, effective or remarkable. The number of samples with poor, effective and remarkable grades were 10, 8 and 4, respectively. Six out of 6 patients whose specimens showed p53(Ab-3)-positive staining before radiotherapy responded well to radiotherapy and the therapeutic effect was classified as remarkable or effective. In 7 out of 7 and 4 out of 5 patients with positive staining at 4Gy and 10Gy, respectively, the therapeutic effect was regarded as remarkable or effective.

The immunohistochemical staining pattern of case 19, which exhibited p53-positive expression both before and during radiotherapy and for which the antitumor effect of radiotherapy was estimated as remarkable, is shown in Figs. 1-3.

The immunohistochemical staining pattern of case 7, which was negative for p53 expression both before and during radiotherapy and for which the antitumor effect of radiotherapy was classified as poor is illustrated in Figs. 4-6.

Discussion

There are still very few reports concerning the effect

of irradiation on p53 expression (Frank et al., 1994; Merritt et al., 1994). We have studied the correlation between the grade and subsets of mononuclear cells infiltrated into cancer tissues (Ogawa et al., 1990), the significance of fractions of proliferating cancer cells (Ogawa et al., 1992) and extracellular matrix, such as

Table 3. p53 expression.

CASE	EXPRESSION OF p53 (Ab-3)				EXPRESSION OF p53 (Ab-6)			
	0Gy	4Gy	10Gy	20Gy	0Gy	4Gy	10Gy	20Gy
1	++	-						
2	+	+	++	-	±	+	+	-
3		±	±	±		-	-	-
4	+	+	±	-	-	-	-	-
5	±							
6	++		+	+	+		-	-
7	-	-	-	-	-	-	-	-
8	-	±	+	-	-	-	-	-
9	±	+	-	-	-	±	-	-
10	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-
12	-	-	-	-	-	-	-	-
13		±				-		
14			±	±	±		-	-
15	++	+	+		++	+	-	-
16	±	+	±	±	±	-	-	-
17	-	±	±	-	-	-	-	-
18	-		-	-	-		-	-
19	+	+	+		-	-	-	
20	-	+	±		±	-	-	
21	±	±	±	±	-	-	-	-
22	-	-	-	-	-	-	-	-
23	±		±		-		-	

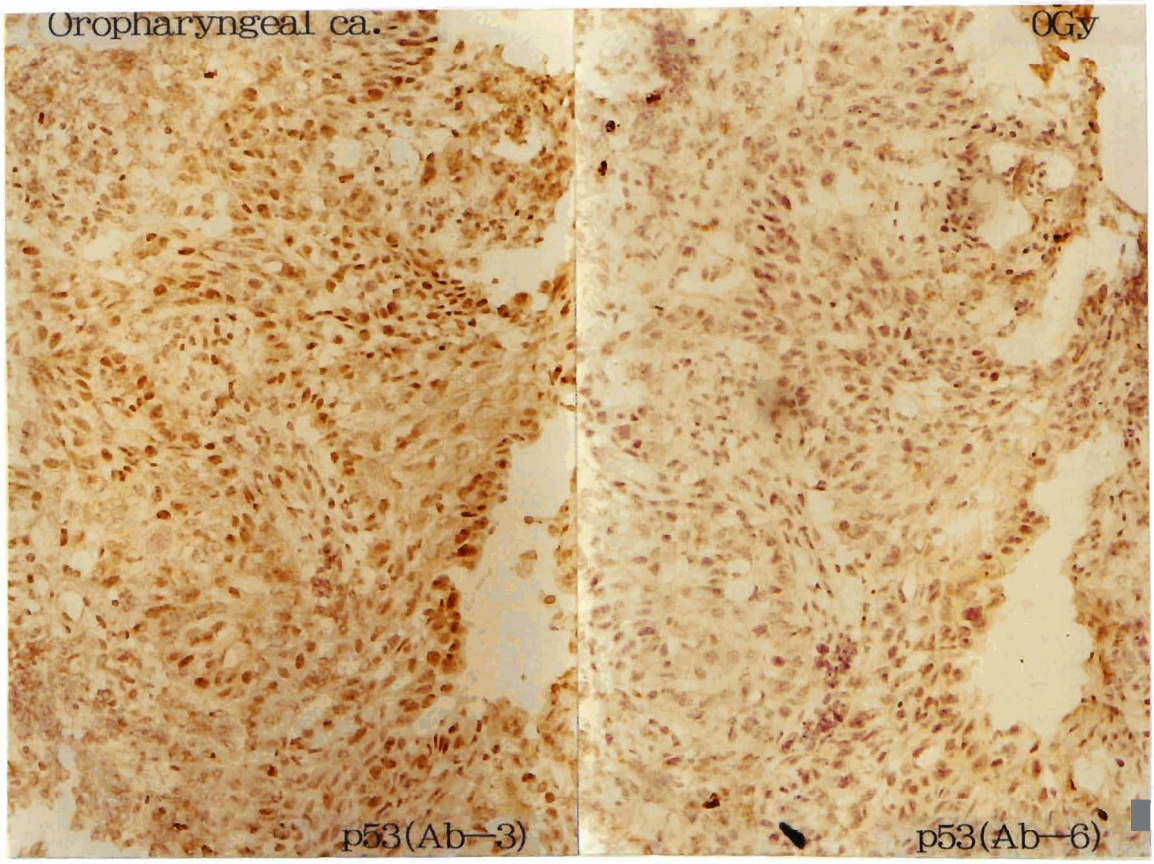

Fig. 1. Immunohistochemical stainings of an oropharyngeal cancer tissue (case 19) before irradiation. Positive stainings of p53(Ab-3) in nuclei of cancer cells. Negative stainings of p53(Ab-6) in nuclei of cancer cells. x 200

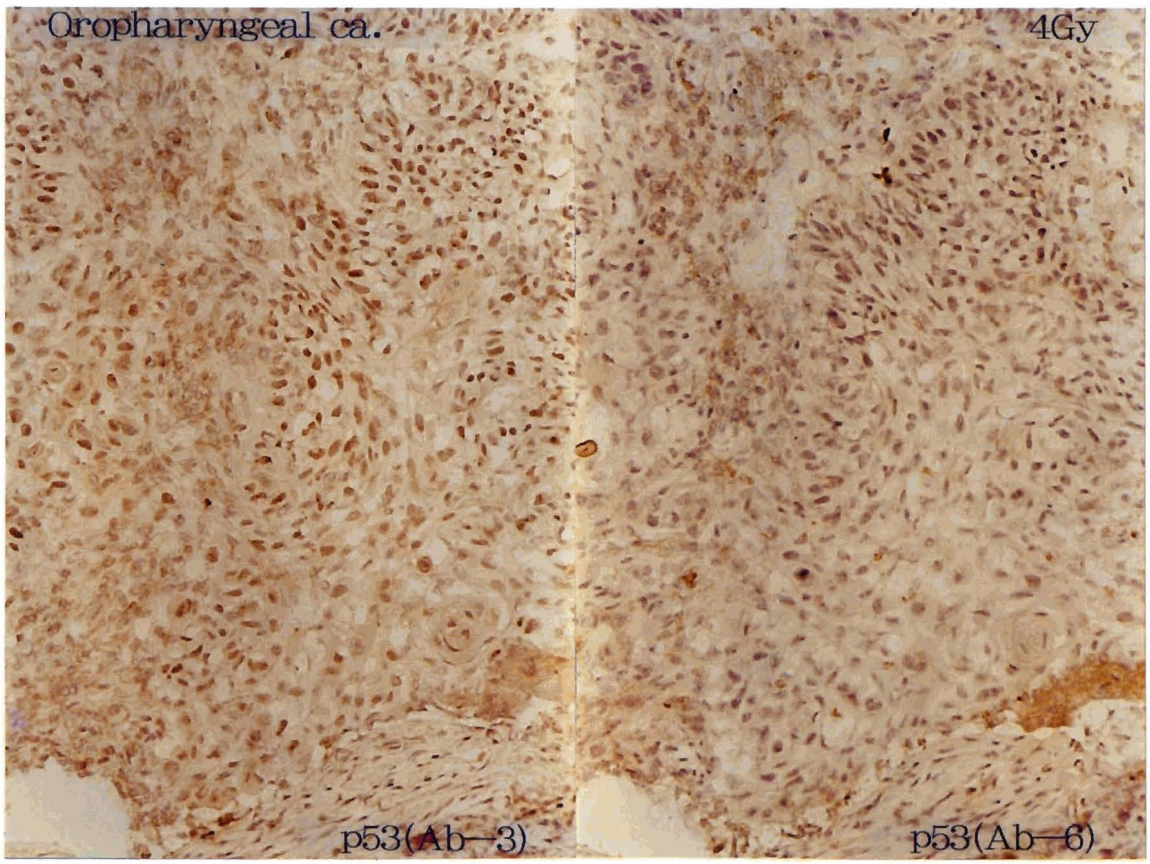

Fig. 2. Immunohistochemical stainings of an oropharyngeal cancer tissue (case 19) after 4Gy. Positive stainings of p53(Ab-3) in nuclei of cancer cells. Negative stainings of p53(Ab-6) in nuclei of cancer cells. x 200

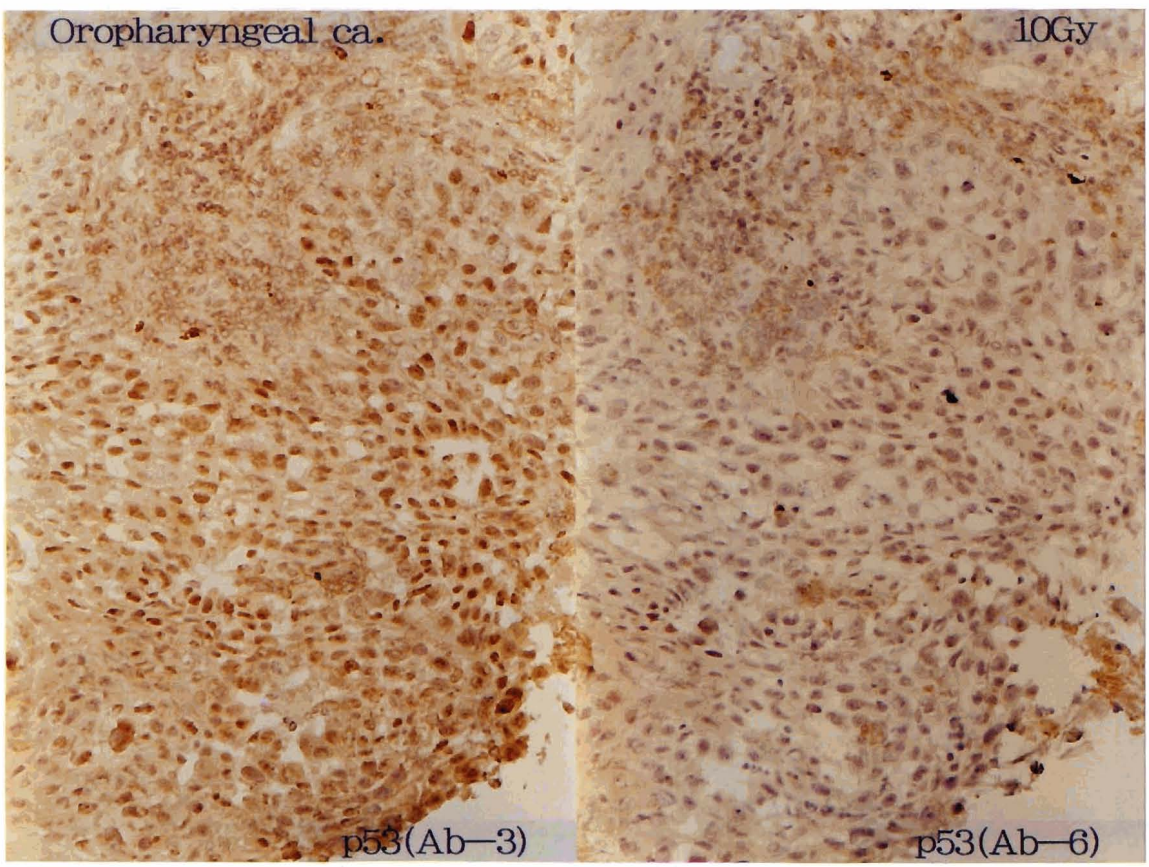

Fig. 3. Immunohistochemical stainings of an oropharyngeal cancer tissue (case 19) after 10Gy. Positive stainings of p53(Ab-3) in nuclei of cancer cells. Negative stainings of p53(Ab-6) in nuclei of cancer cells. x 200

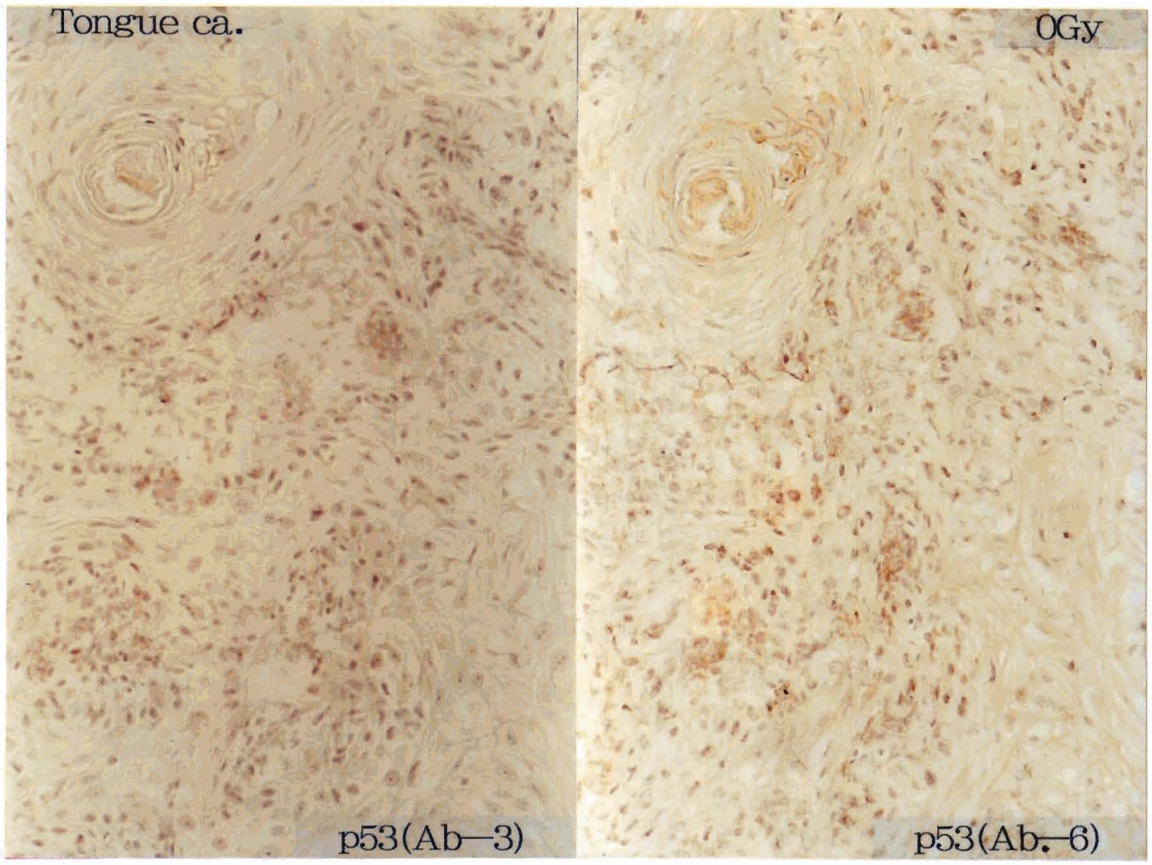

Fig. 4. Immunohistochemical stainings of a tongue cancer tissue (case 7) before irradiation. Negative stainings of both p53(Ab-3) and p53(Ab-6) in nuclei of cancer cells. x 200

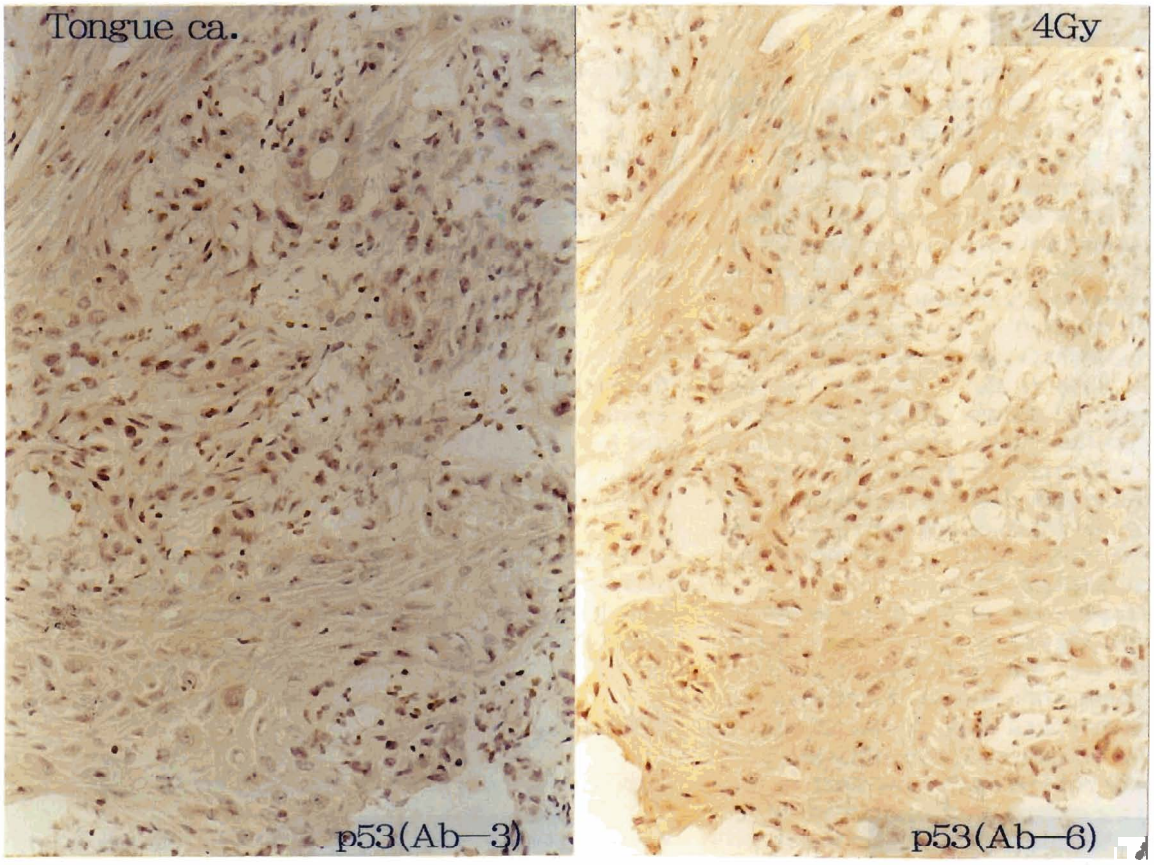

Fig. 5. Immunohistochemical stainings of a tongue cancer tissue (case 7) after 4Gy. Negative stainings of both p53(Ab-3) and p53(Ab-6) in nuclei of cancer cells. x 200

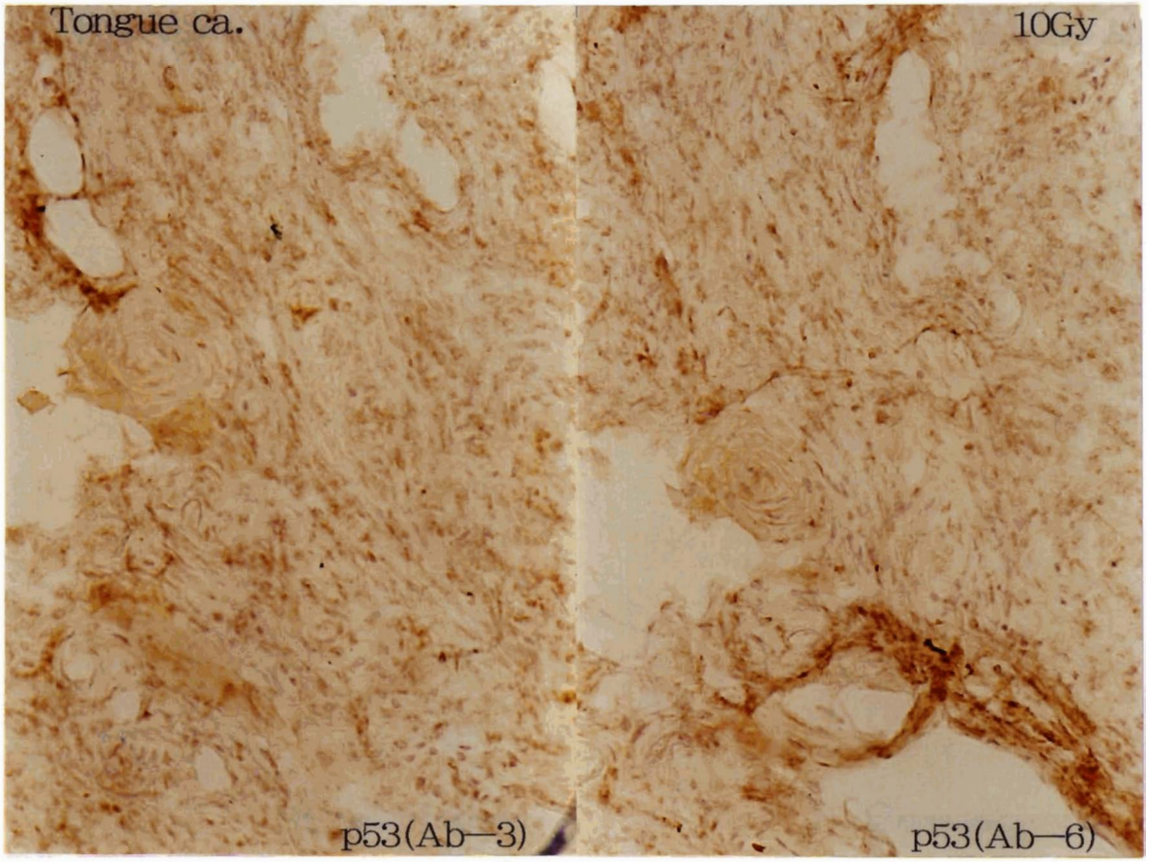

Fig. 6. Immunohistochemical stainings of a tongue cancer tissue (case 7) after 10 Gy of irradiation. Negative stainings of both p53(Ab-3) and p53(Ab-6) in nuclei of cancer cells. x 200

fibronectin (Nishioka et al., 1993), before and during irradiation. In this study, we examined the changes in the expression of two kinds of p53 proteins (mutant and wild type) before and during radiotherapy for head and neck squamous cell carcinoma and their possible use as histopathological aids in the assessment of these lesions.

Our findings regarding the rate of overexpression of mutant p53 protein (30.0%), as detected by p53(Ab-3), in samples of squamous cell carcinomas of head and neck regions before irradiation are similar to those reported by other researchers (Gusterson et al., 1991; Frank et al., 1994; Shin et al., 1994). As for the expression of wild-type p53 protein (10.5%), the results are in agreement with the current view that although overexpression the wild-type p53 protein is a normal physiological response to slow down the cell cycle as the G1 phase to allow for the repair of damaged DNA, it is short lived and, therefore, difficult to detect by histochemical means (Battifora, 1994).

Regarding the expression of the p53 protein in head and neck tissue. Ogden et al. (1992) reported that the p53 protein was not identified in normal, benign or premalignant oral mucosa. Later studies, however, have demonstrated that, in premalignant lesions adjacent to tumors in these regions, p53 expression increased continuously, not only in the incidence but also in the amount of p53 expressed, as the tissue progressed from normal, to hyperplasia, to dysplasia and to squamous cell carcinoma (Shin et al., 1994).

In this study, inspection of haematoxylin-eosin sections at the cumulative dose of 20Gy revealed that the antitumor effect of irradiation was either remarkable or effective in patients showing positive mutant type p53 protein. Moreover, the frequency of the expression of mutant type p53 protein tended to increase in rather radiosensitive tumors.

As patients with head and neck cancer commonly have rather better prognosis than other cancer patients, the evaluation of the correlation between the grade of expression of each type of p53 protein in cancer tissues and the patient's prognosis could not be reported in this study. The results will therefore, have to wait until a sufficient follow up period of these patients has elapsed.

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