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Fibronectin expression in cancer tissues from patients undergoing radiation therapy

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Summary. Fibronectin expression and distribution were examined in cancer tissues from 19 patients with cancer of the head and neck regions. Samples taken before and after irradiation of approximately 10 Gy, 20 Gy or 30 Gy were analyzed by the avidin-biotin-horseradish peroxidase method using mouse monoclonal antibodies against human fibronectin. The results were correlated with the patient's prognosis after radiation therapy. No remarkable changes in the fibronectin expression or distribution were found between tissue specimens taken before and after each dose of irradiation. The prognosis, however, varied according to the degree of expression and the distribution pattern of fibronectin. Seven patients in which the cancer tissue was encircled by a thick fibronectin network are still alive without recurrence 4.5-6 years after treatment, whereas 6 patients in which fibronectin was only faintly expressed or focally distributed died or developed recurrence soon after treatment. The present findings demonstrate that fibronectin expression and distribution in cancer tissue are intimately related to the patient's prognosis, and that the analysis of these two parameters is applicable as a predictive assay in radiotherapy of cancer of the head and neck regions.

Key words: Fibronectin, Immunohistochemistry, Radiation therapy, Head and neck cancer, Cancer prognosis

Introduction

The extracellular matrix has been the subject of extensive biochemical research during the past few years (Pierschbacher and Ruoslahti, 1984; Hynes, 1985), and its components are currently believed to play important roles in many physiological activities as well as in cancer invasion and metastasis (Yamada and Olden, 1978; Smith et al., 1979; Murray et al., 1980; Stenman and Vaheri, 1981; Vlodavsky and Gospodarowicz, 1981; Terranova et al., 1984; Humphries et al., 1986). Fibronectin, one of the most representative components of the extracellular matrix, is a high molecular weight glycoprotein which consists of a dimer of two subunits, each about 250 Kilodaltons (Hynes, 1985). This protein has been reported to be involved in a wide variety of cellular activities (Pearlstein et al., 1980; Ruoslahti et al., 1981; Yamada, 1988) such as cellular adhesion (Yamada et al., 1976; Pierschbacher and Rouslahti, 1984), migration (Duband and Thiery, 1982), differentiation, phagocytosis (Bevilacqua et al., 1981), repair of tissue damage (Gudewicz et al., 1980) and carcinogenesis (Yamada and Olden, 1978; Stenman and Vaheri, 1981). In addition, fibronectin is considered to inhibit local invasion and distant metastasis of cancer (Smith et al., 1979; Murray et al., 1980; Vlodavsky and Gospodarowicz, 1981; Terranova et al., 1984; Humphries et al., 1986). The present study was thus undertaken in order to examine the effect of irradiation on the fibronectin expression in cancers of the head and neck regions.

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Materials and methods

Nineteen patients with cancer of the head and neck regions were examined (Table 1). Pieces of approximately 0.5 x 0.5 x 0.5 cm were cut from the margins of the cancer tissue before and after delivery of 10 Gy, 20 Gy or 30 Gy 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 avidin-biotin-horseradish peroxidase method (ABC method) using a monoclonal antibody against human fibronectin (Table 2). The proceudre has been described in detail elsewhere (Ogawa et al., 1987). Irradiation was administered with 10 MV X-rays on an ML-15MIII linear accelerator (Mitsubishi Electric Co. Ltd., Japan), according to the standard method described

Table 1. Summarized data of patients studied

CASE	SEX	AGE	SITE	TNM STAGE[1]	HISTOLOGY	TREATMENT
1	M	61	Nasal cavity	T3N0M0 III*	PD-SCC	30Gy+op.
2	F	71	Maxillary sinus	T3N1M0 III	WD-SCC	50Gy+chemo.+op.
3	M	62	Maxillary sinus	T4N0M0 IV	WD-SCC	50Gy+chemo.+op.
4	M	75	Maxillary sinus	T4N0M0 IV	WD-SCC	50Gy+chemo.+op.
5	M	65	Maxillary sinus	T4N0M0 IV	WD-SCC	30Gy+chemo.+op.
6	M	40	Tongue	T2N0M0 II	WD-SCC	30Gy+op.
7	M	50	Tongue	T2N1M0 III	WD-SCC	30Gy+op.
8	M	84	Tongue	T2N0M0 II	MD-SCC	60Gy
9	F	57	Tongue	T3N2M0 IV	WD-SCC	80Gy
10	M	76	Tongue	T3N2M0 IV	WD-SCC	60Gy
11	M	38	Tongue	T2N0M0 II	WD-SCC	20Gy+op.
12	M	45	Tongue	T2N0M0 II	WD-SCC	20Gy+chemo.+op.
13	M	66	Parotid gland	T3bN0M0 III	PD-SCC	30Gy+op.
14	F	57	Epipharynx	T2N3M0 IV	PD-SCC	30Gy+chemo.
15	M	60	Larynx	T4N1M0 IV	MD-SCC	20Gy+op.
16	M	60	Larynx	T3N1M0 III	MD-SCC	20Gy+op.
17	M	87	Hypopharynx	T2N0M0 II	PD-SCC	60Gy
18	M	66	Oral floor	T1N0M0 I	WD-SCC	60Gy
19	M	75	Buccal mucosa	T4N0M0 IV	SCC	40Gy+chemo.

[1]: UICC classification (1987); WD-SCC: well differentiated squamous cell carcinoma; MD-SCC: moderately differentiated squamous cell carcinoma; PD-SCC: poorly differentiated squamous cell carcinoma; op.: operation; chemo.: chemotherapy; *: refer to UICC classification of maxillary cancer.

Table 2. Characteristic of the monoclonal anti-fibronectin antibody used.

PRODUCER	Hybritech
IMMUNOGEN	Human fibronectin (amniotic fluid)
HYBRIDOMA	Murine myeloma x Balb/C mouse spleen cells
CLASS	IgG1 (mouse)
SPECIFICITY	Human fibronectin and others

in Fletcher's textbook (Fletcher, 1980). Treatments were performed five times a week, the daily fraction size being 2 Gy. The grade of expression of fibronectin in the cancer tissue was evaluated according to the following standards: absent or faint (-); slight (+); moderate (++); and marked (+++). The distribution pattern was classified as encircled, diffuse, or focal. Finally, the relationship between the immunohistochemical findings and the patient's prognosis was examined.

Results

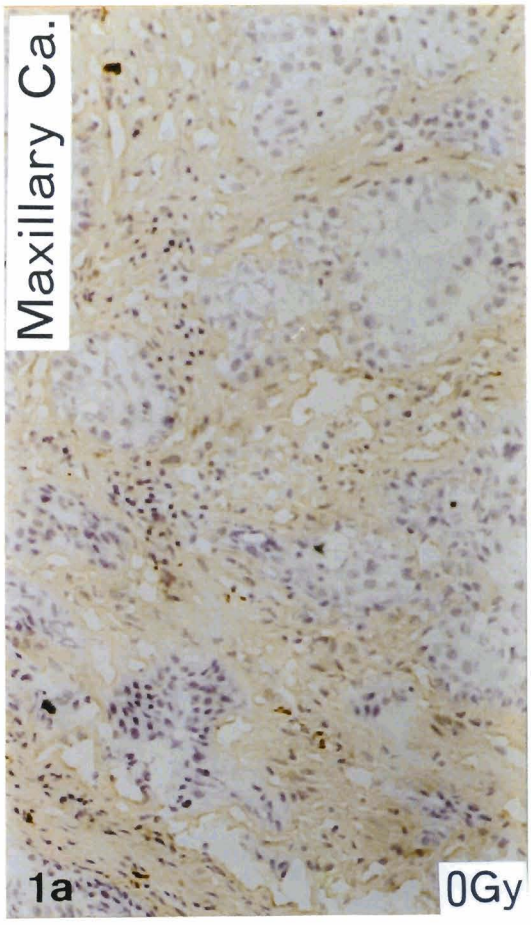
The results are summarized in Table 3. No remarkable changes were found as regards the grade of expression and the distribution pattern of fibronectin in the cancer tissue among the specimens taken before and after each dose of irradiation. Moreover, no clear correlation was found between the distribution pattern and the region, stage or malignancy of the carcinomas examined.

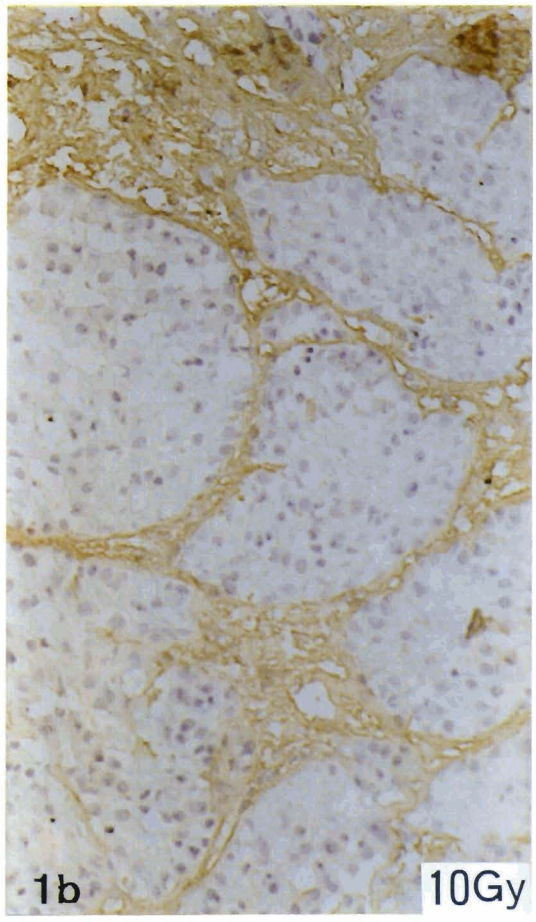
The prognosis, on the other hand, differed with each of the distribution patterns observed. Of the eight patients in which the cancer tissue was thickly encircled by fibronectin (cases 2, 4, 5, 6, 10, 11, 12 and 18) seven are still alive without recurrence after 4.5 to 6 years (Fig. 1a-c). However, 6 patients (cases 3, 7, 8, 9, 14 and 19) in which fibronectin was only faintly expressed or focally distributed (Fig. 2a-c) in the cancer tissue died or had recurrences 6-18 months after radiotherapy. The prognosis varied for the other 5 patients in which fibronectin exhibited a diffuse distribution pattern in the

Table 3. Fibronectin expression and distribution, and prognosis.

CASE	EXPRESSION AND DISTRIBUTION OF FIBRONECTIN			PROGNOSIS	
	0 Gy	10 Gy	20 or 30 Gy		
1	D++	D++	D+	Fair: dead	4 years
2	E+	E ++		Good: alive	6 years
3	F+	F +		Fair: alive	5.5 years
4	E+	E ++	E++	Good: alive	4.5 years
5	E+++	E ++	E +	Good: alive	4.5 years
6	E++	E++		Good: alive	5 years
7	-	-		Poor: dead	10 months
8	F++	F++		Poor: dead	6 months
9	F +	F++		Poor: dead	1 year
10	E ++	E++	E++	Good: alive	4.5 years
11		E++		Good: alive	5 years
12	E+			Good: alive	4.5 years
13	D+	D+		Good: alive	6 years
14	F++	F++	F+++	Poor: dead	1.5 years
15	D++	D+	D+	Fair: dead	3 years
16	D+	D+		Fair: dead	4.5 years
17	D+	D+		Good: alive	4.5 years
18	E++	E++		: dead*	2.5 years
19		-		Poor: dead	8 months

D (diffuse): fibronectin was diffusely distributed in cancer tissue. F (focal): fibronectin was focally distributed in cancer tissue. E (encircled): cancer tissue was encircled by fibronectin. Good: alive without recurrence. Fair: dead after more than 2 years or alive with recurrence. Poor: dead within 2 years. *: patient died of adenocarcinoma of the lung.





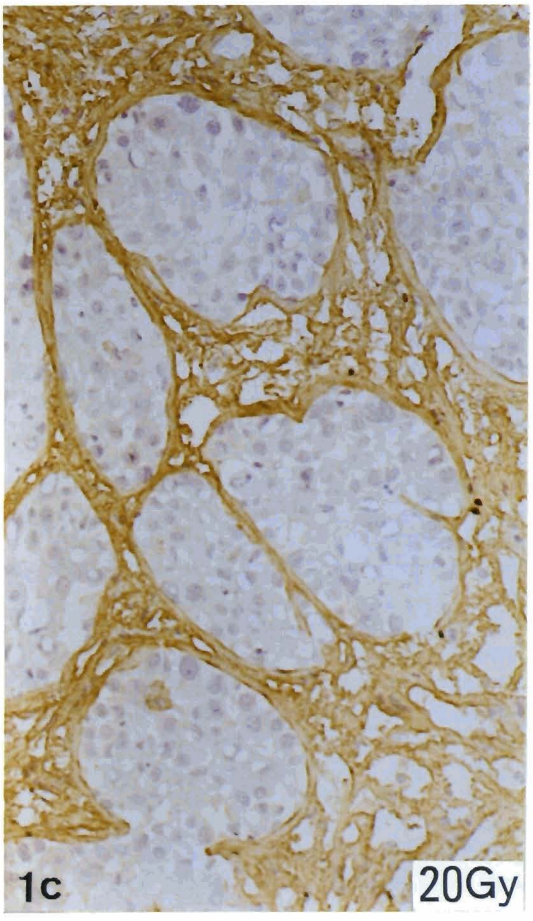


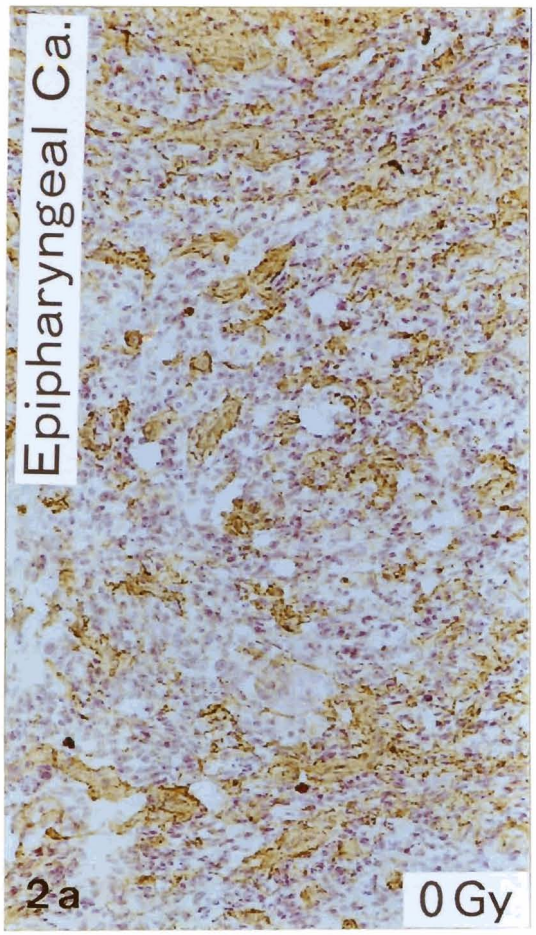
Fig. 1. Immunohistochemical staining of maxillary cancer tissue (case 4). **a:** before irradiation, **b:** after 10Gy, and **c:** after 20 Gy of irradiation. The cancer tissue is completely encircled by fibronectin. x 200

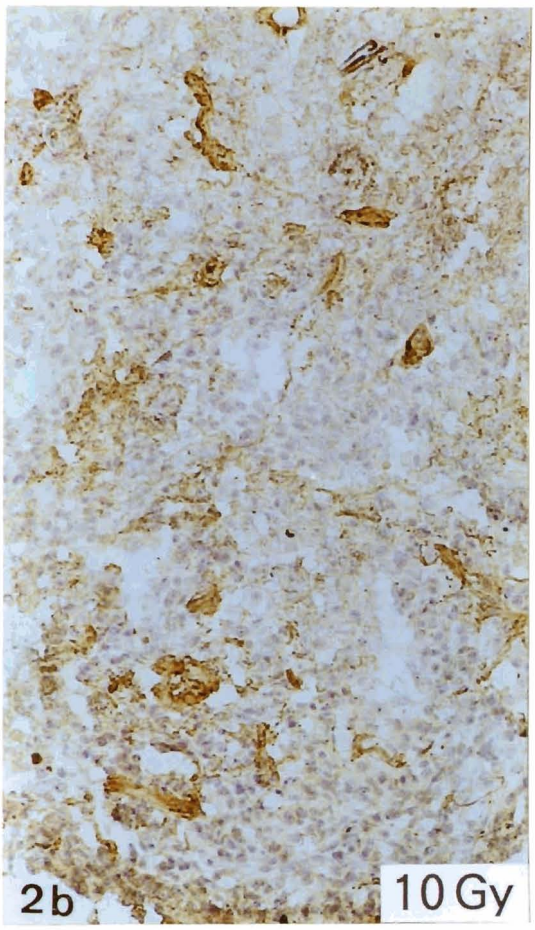
cancer tissue: cases 1, 15 and 16 had fair prognosis, and cases 13 and 17 had good prognosis (Fig. 3a-c).

Discussion

The effect of irradiation on cancer tissues is often clinically experienced to vary with each patient in spite of the similarities in their pathologic and oncogenic conditions. The results of previous studies indicated that the heterogeneity of the effect is due to the tumor immunity induced by low-dose irradiation. Hence, the importance of evaluating the tumor immune response to irradiation has been pointed out (Ogawa et al., 1983, 1986, 1988; Hirota et al., 1987). Moreover, recent biochemical studies of the extracellular matrix have indicated that the extracellular matrix, like the tumor matrix, should be considered one of the most important factors involved in carcinogenesis (Yamada and Olden, 1978; Smith et al., 1979; Murray et al., 1980; Stenman and Vaheri, 1981; Vlodavsky and Gospodarowicz, 1981; Terranova et al., 1984; Humphries et al., 1986).

The present results indicated that low-dose irradiation (less than 30 Gy) has no significant effect on the fibronectin expression and distribution in the cancer tissue. The prognosis, however, was good for the patients in which the cancer tissue was encircled by fibronectin, and poor for those showing only faint





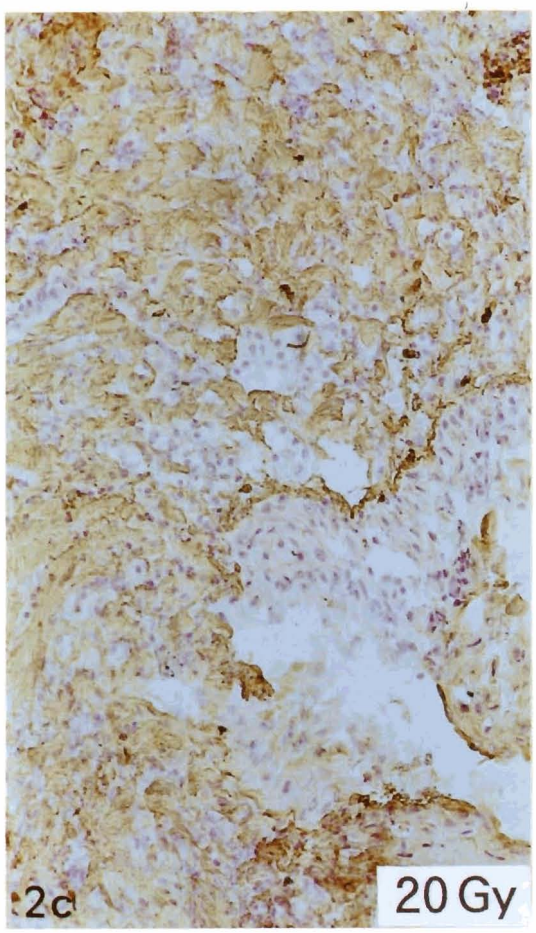
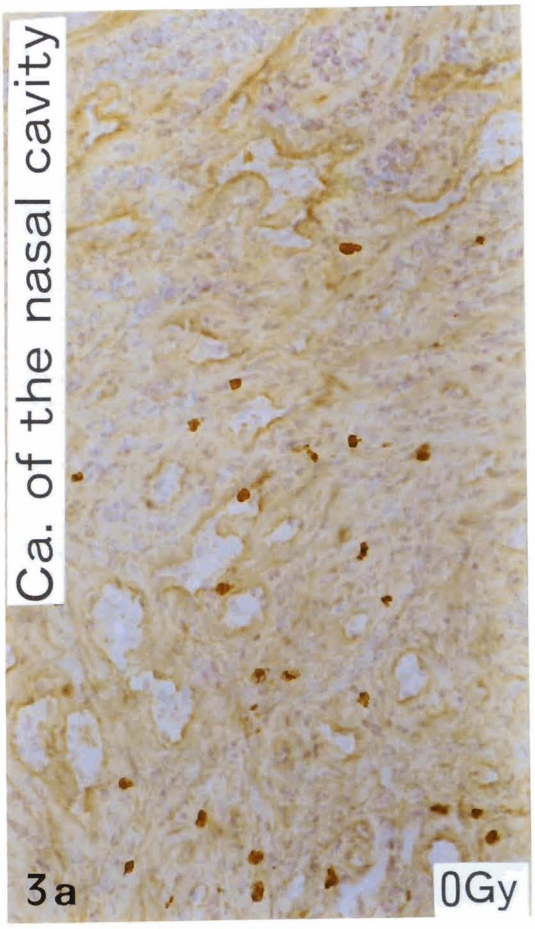


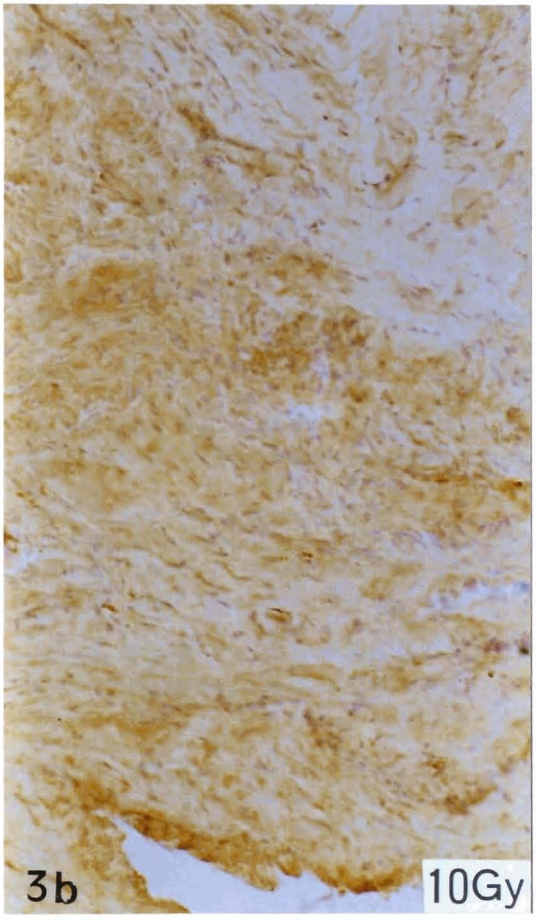
Fig. 2. Immunohistochemical staining of epipharyngeal cancer tissue (case 14). **a:** before irradiation, **b:** after 10Gy and **c:** after 20Gy of irradiation. Fibronectin is focally distributed in the cancer tissue. x 200

expression or focal distribution. As for the patients with a diffuse pattern of fibronectin distribution, the prognosis varied (Table 3). These findings seem to confirm previous reports indicating that fibronectin inhibits local invasion and distant metastasis of cancer (Smith et al., 1979; Murray et al., 1980; Terranova et al., 1984; Humphries et al., 1986). In addition, they suggest that expression alone is not sufficient for fibronectin to exert its inhibitory effect on local invasion or distant metastasis, and that its physical distribution in the cancer tissue is a factor of great importance.

It was also demonstrated that the patient's prognosis could be forecast with considerable accuracy before and after radiation therapy by analyzing the grade of expression and the distribution pattern of fibronectin in cancer tissues.

In previous papers we reported that the lymphocyte subpopulation infiltrating the cancer tissue upon delivery of low-dose irradiation (approx. 10 Gy) had an intimate relationship with the antitumor effect of radiotherapy in cancers of the head and neck regions, and that the analysis of this subpopulation was applicable as a predictive assay in radiotherapy (Nishioka et al., 1988; Ogawa et al., 1988, 1990). It is, therefore, expected that the analysis of fibronectin expression and distribution in cancer tissues will enable us to further improve the





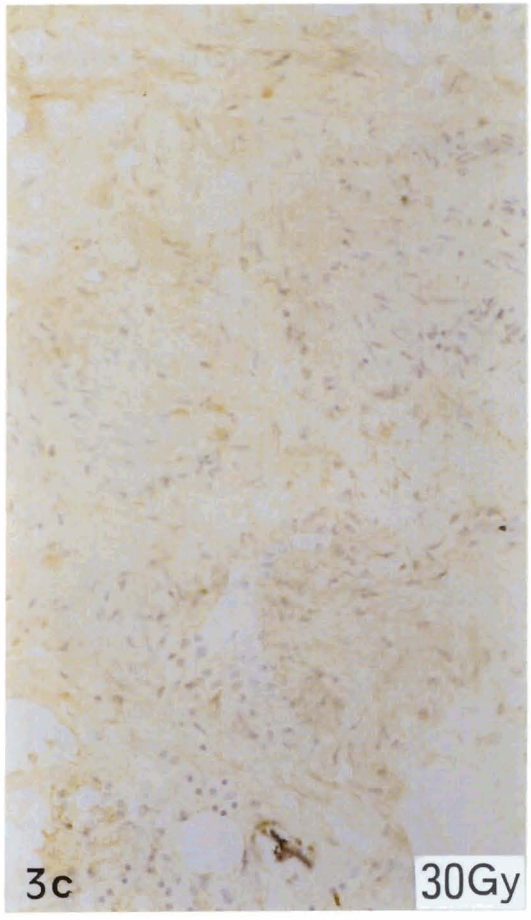


Fig. 3. Immunohistochemical staining of cancer tissue of the nasal cavity (case 1). **a:** before irradiation, **b:** after 10Gy and **c:** after 30 Gy of irradiation. Fibronectin is diffusely distributed in the cancer tissue. x 200

accuracy of the assay and the future management of patients with cancers of the head and neck regions undergoing radiation therapy.

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