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Correlated Expression of Glutathione S-Transferase-π and c-Jun or Other Oncogene Products in Human Squamous Cell Carcinomas of the Head and Neck: Relevance to Relapse after Radiation Therapy

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The expression of glutathione S-transferase (GST)-π and four oncogene products, c-Jun, c-Fos, c-H-Ras, and c-Myc, in human squamous cell carcinomas of the head and neck was investigated immunohistochemically before and after radiation therapy, to examine whether these oncogene products might be involved in GST-π expression, and also to examine the relationship between their expression and therapeutic response. Clinical response to radiation was evaluated in terms of both tumor regression and relapse over two-year follow-up periods. The overall positive rates in 83 carcinoma specimens before therapy were 60.2% for GST-π and 28.9–51.8% for the individual oncogene products, the positive rates for the oncogene products being higher in GST-π-positive than in GST-π-negative cancers. c-Jun was most highly correlated with GST-π expression. Following radiation, the expression of GST-π and the oncogene products was altered in about a half of 30 patients. Eleven of the 18 patients who exhibited prior positivity for GST-π showed negative conversion, while 4 of the 12 patients with prior negativity demonstrated positive conversion. In most cases, changes in c-Jun staining coincided with those in GST-π. Regarding clinical response to radiation therapy, the positive rates for GST-π and c-Jun before radiation were higher in the residual cancer or relapse cases than in the group showing complete response without relapse. Examination of 26 patients with laryngeal cancer revealed that relapse occurred more frequently in cases exhibiting positive reactions for GST-π, c-Jun, or c-H-Ras. These results suggest a direct link between c-Jun and GST-π in head and neck cancers before and after radiation. Although GST-π and the oncogene products can be influenced by radiation, GST-π and c-H-Ras expression may be a risk factor for relapse of laryngeal cancer.

Key words: Glutathione S-transferase — c-Jun — Oncogene — Radiation therapy — Head and neck cancer

The glutathione S-transferases (GSTs) are a family of multifunctional proteins that act as both enzymes and binding proteins in various detoxication processes.[1] One of the isoenzymes, GST-π (GST P1–1 in the new nomenclature[2]), is strongly expressed in a wide range of human malignant tumors, including cancers of the colon, uterine cervix, head and neck, and esophagus, and in many cancer cell lines resistant to alkylating agents, doxorubicin, and cisplatin.[3–6] Since the *GST-π* gene possesses a 12-*O*-tetradecanoylphorbol-13-acetate-responsive element (TRE) in its enhancer region,[7,8] GST-π expression in cultured cells has been suggested to be partly regulated by the oncogene products, c-Jun and c-Fos.[8–10] However, it remains to be clarified what factors are actually responsible for the expression of GST-π in cancer tissues.

Our previous study revealed that GST-π expression is repressed by radiation therapy and is not directly involved in determining response to radiation.[11,12] Although oncogenes such as *ras* and *myc* have been suggested as factors influencing radiosensitivity in cultured cell lines,[13–19] their significance as determining factors remains to be established in clinical cases.[13]

In the present retrospective study, the expression of GST-π and four oncogene products, c-Jun, c-Fos, c-H-Ras, and c-Myc, in human squamous cell carcinomas of the head and neck was investigated immunohistochemically before and after radiation therapy, to examine whether these oncogene products might be involved in GST-π expression *in vivo*, and also to examine the relationship between their expression and therapeutic response.

MATERIALS AND METHODS

Patients Tissues were obtained at biopsy before treatment from a total of 83 patients with squamous cell carcinomas of the head and neck in the Department of Otorhinolaryngology, Hirosaki University Hospital, from 1991 to 1994. The patients included 35 with laryn-

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geal carcinoma, 24 with pharyngeal carcinoma, 14 with maxillary carcinoma, 5 with carcinoma of the oral cavity, and 5 with tongue carcinoma, and the ages of these patients (67 men and 16 women) ranged from 37 to 83 years (mean age, 62.3 years). Staging and histologic classification of the carcinomas were performed in accordance with the general rules for TNM classification (International Union Against Cancer) and Clinical and Pathologic Studies on Head and Neck Cancer (Japan Society for Head and Neck Cancer), respectively.

Four to 6 weeks after radiation therapy, residual cancers were removed surgically from 37 patients and also used for examination of GST-π and oncogene products.

Radiation therapy Of these 83 patients, 77 received curative or preoperative radiation therapy. The radiation was administered with 10-MeV photons from a linear accelerator or cobalt 60 gamma rays. Irradiation was performed with doses of 200 cGy per fraction, and five fractions per week. The total radiation dose was in the range of 2,600–8,000 cGy. Radium 226-needles were also utilized against carcinomas of the oral cavity or tongue. Forty-four of these patients also received intravenous administration of cisplatin and pepleomycin or 5-fluorouracil during the period of radiation therapy as reported previously.[11] These chemotherapy regimens were repeated in some patients.

Antibody preparations A gene fusion vector utilizing cDNA of *Schistosoma japonicum* GST was used for the expression of oncogene products in *Escherichia coli*, essentially according to the method of Smith and Johnson.[20] The plasmid expression vector, *p*GEX-3X, was purchased from Pharmacia. Rat c-*jun* cDNA (*p*RJ101)[21] and human c-H-*ras* (*p*RG12)[22] were kindly donated by Dr. M. Sakai and Dr. E. Ohtsuka, respectively. Human c-*fos* (*p*SPT-fos)[23] and c-*myc*-exon-2 (*p*Myc6514-2)[24,25] were obtained from the Japanese Cancer Research Resources Bank. The plasmids for GST-Jun or GST-Fos fusion proteins were prepared as reported previously.[26] The plasmid for GST-Ras fusion protein was made by an in-frame insertion of the *Bam*H I-*Eco*R I fragment of human c-H-*ras* nucleotides 5–421 (corresponding to amino acids 1–139) into the *p*GEX-3X vector, while that for GST-Myc fusion protein was produced by insertion of human c-*myc*-exon 2 nucleotides 2849–3605 (amino acids 1–252) amplified by polymerase chain reaction. The expressed GST-fusion proteins were purified from the respective bacterial lysates through a GSH-agarose column as reported previously.[26] Antibodies to the respective fusion proteins were raised in rabbits as described previously.[27] Anti-fusion protein antibodies were absorbed four times with the carrier GST and confirmed to be specifically reactive to portions of oncogene products, and not reactive to GST-π or other human GST forms by immunoblotting. These absorbed antibodies were used for immunohistochemical staining. Rabbit anti-GST-π antibody was prepared as reported previously.[28] Its specificity for GST-π was confirmed by immunoblotting using human GST forms and placenta tissues as reported previously.[29]

Immunohistochemical staining methods All samples were fixed in formaldehyde and embedded in paraffin. Serial sections 6 μm thick were routinely passed through xylene and a graded alcohol series and stained for GST-π and oncogene products by the avidin-biotin-peroxidase complex (ABC) method using specific antibodies. Affinity-purified, biotin-labeled goat anti-rabbit immunoglobulin G and ABC complex (Vectastain ABC kit, PK4001) were obtained from Vector Laboratories Inc. (Burlingame, CA). The sites of peroxidase binding were determined by the diaminobenzidine method as described previously.[30] Sections were then lightly counterstained with hematoxylin for microscopic examination. Sections were also stained with hematoxylin and eosin. As negative controls, preimmune rabbit sera or antibodies absorbed with the respective antigens were used instead of the antibodies. As positive controls, sections from a laryngeal cancer that had exhibited positive reactions were used in every experiment.

Evaluation and statistical methods Clinical response to radiation therapy together with chemotherapy was evaluated in terms of tumor response at 2 to 8 weeks after the last irradiation and relapse within a two-year follow-up period. Assessment was made by reviewing the results of serial clinical examinations, including radiologic investigations, and confirmed by pathologic findings. For evaluation of tumor response, the following four categories were used, essentially according to the recommendations of the World Health Organization (WHO): complete response, partial response, no response, and progressive disease.[31] The disease-free survival time was defined as the period that elapsed between the date of disappearance of tumors and relapse. Kaplan-Meier and log-rank tests were used to compare disease-free survival curves. In immunohistochemistry, whole cancer tissues on each slide were examined and carcinomas in which 30% or more of the component cells were stained for GST-π or oncogene products were evaluated as positive (+), whereas tissues consisting of less than 30% stained cells were counted as negative (−). Evaluation of staining results for the individual oncogene products was performed without information on results for the other oncogene products. The staining data were analyzed statistically by the χ^2 method.

RESULTS

Correlated expression of GST-π and oncogene products in head and neck carcinomas The expression of four oncogene products, c-Jun, c-Fos, c-H-Ras, and c-Myc,

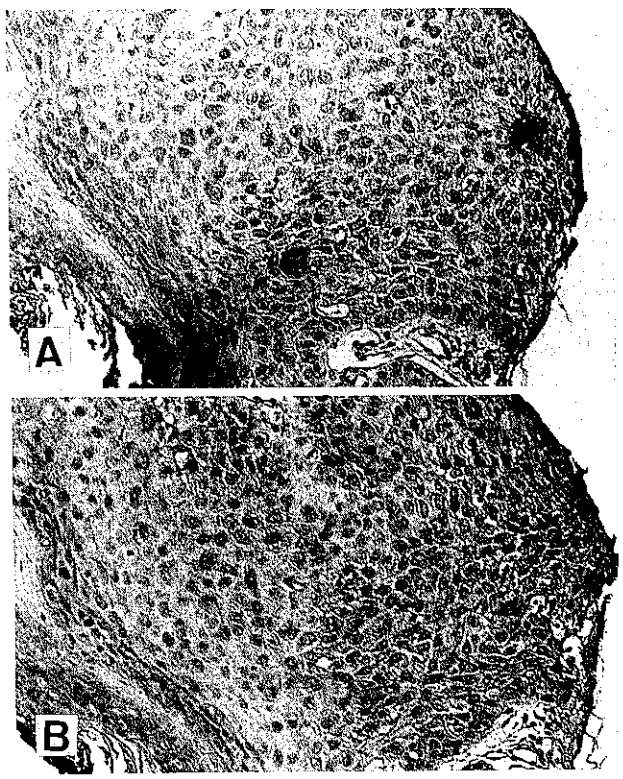

Fig. 1. Nuclear localization of c-Jun (A) and c-Myc (B) in a laryngeal carcinoma case visualized by immunohistochemical staining. Original magnification ×400.

was immunohistochemically examined in serial sections of squamous cell carcinomas of the head and neck before treatment, and compared with that of GST-π. The nuclear oncogene products, c-Jun (Fig. 1A), c-Fos and c-Myc (Fig. 1B), were only detected in nuclei of some cases, but most of the positive cases exhibited both cytoplasmic and nuclear staining. Typical staining patterns for GST-π and c-Jun are shown in Fig. 2A and 2B, respectively. GST-π and c-H-Ras were in general homogeneously stained in the cytoplasm of positive cases. Control staining using preimmune sera and antibodies absorbed with the respective antigens proved negative. Fig. 2C illustrates the result in the case of absorbed anti-c-Jun antibody. Cells exhibiting either cytoplasmic or nuclear staining for c-Jun or c-Fos were only localized within areas exhibiting a positive reaction for GST-π. In most specimens positive for c-Ras or c-Myc, positive areas were also localized within GST-π-positive areas.

The overall positive rates in 83 carcinoma specimens were 60.2% for GST-π and 28.9–51.8% for the oncogene products, as shown in Table I. Carcinoma tissues positive for c-Jun or c-Fos were all positive for GST-π and those negative for GST-π were negative for c-Jun or c-Fos.

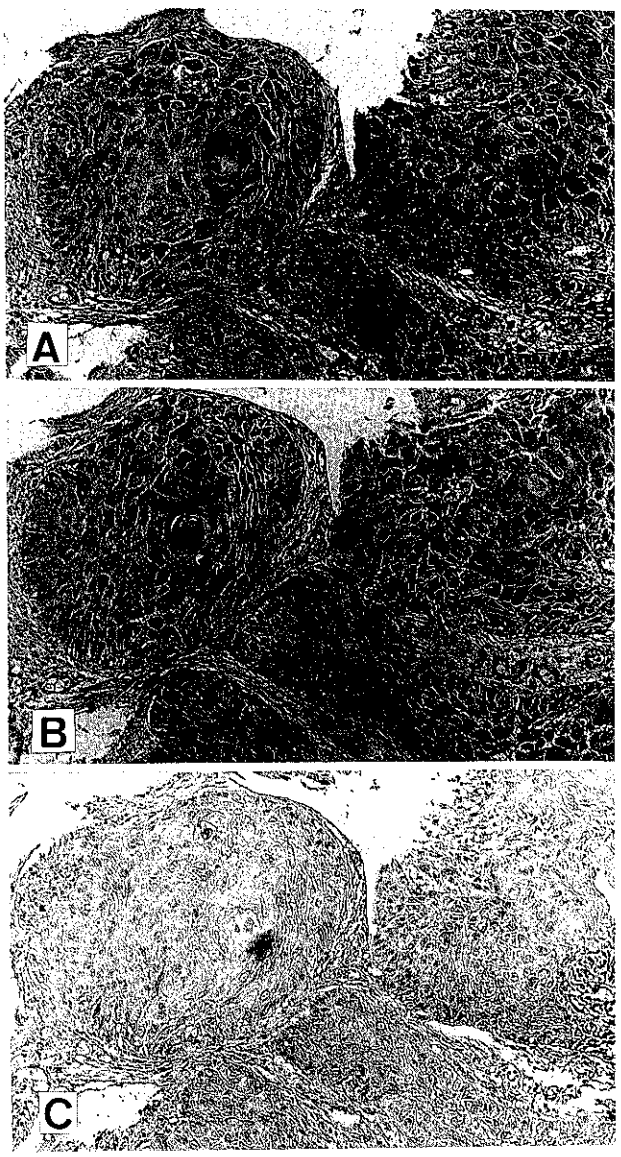

Fig. 2. Immunohistochemical staining for GST-π (A) and c-Jun (B) in a well differentiated squamous cell carcinoma of the larynx. The panel (C) shows the result using anti-c-Jun antibody absorbed with the antigen. GST-π and c-Jun are seen mainly in cytoplasm of cancer cells. Cancer tissues stained for c-Jun are also positive for GST-π. Cytoplasmic and nuclear staining for c-Jun is competed out after absorption of the antibody. Original magnification ×400.

Most specimens positive for c-H-Ras or c-Myc were also positive for GST-π. Comparison of the positive rates of the individual oncogene products revealed values to be higher for GST-π-positive cancers than for GST-π-negative cancers (Table I, $P<0.01$ for c-Jun, c-Fos, and c-H-Ras; $P<0.05$ for c-Myc), indicating correlated ex-

Table I. Staining of GST-π and Oncogene Products in Squamous Cell Carcinomas of the Head and Neck before Radiation Therapy

GST-π staining	No. of patients	No. of patients exhibiting positive reaction (%)			
		c-Jun	c-Fos	c-H-Ras	c-Myc
Positive	50	43 (86.0)[a]	24 (48.0)	26 (52.0)	24 (48.0)
Negative	33	0**	0**	4 (12.1)[b]**	7 (21.2)*
Total	83	43 (51.8)[c]	24 (28.9)	30 (36.1)	31 (37.3)

a) Positive rate in patients exhibiting positivity for GST-π.
b) Positive rate in patients exhibiting negativity for GST-π.
c) Positive rate in total patients.
* $P<0.05$ and ** $P<0.01$ compared with GST-π-positive group.

Table II. Positive Rates of GST-π and Oncogene Products before Therapy in Head and Neck Cancers or Laryngeal Cancer according to Response to Radiation

Cancer	Response to radiation	No. of patients	No. of positive patients (%)				
			GST-π	c-Jun	c-Fos	c-H-Ras	c-Myc
A. Head and neck	Residual cancer or relapse	42	29 (69.0)	25 (59.5)	13 (31.0)	17 (40.5)	15 (35.7)
	Complete response without relapse	31	13 (41.9)*	9 (29.0)*	6 (19.4)	9 (29.0)	12 (38.7)
B. Laryngeal	Residual cancer or relapse	14	13 (92.9)	10 (71.4)	6 (42.9)	7 (40.0)	5 (35.7)
	Complete response without relapse	12	4 (33.3)**	3 (25.0)*	2 (16.7)	1 (8.3)	4 (33.3)

* $P<0.05$ and ** $P<0.01$ compared with residual cancer or relapse groups in head and neck cancer and laryngeal cancer, respectively.

pression. In particular, c-Jun expression was highly correlated with GST-π expression. No significant relationships between positivity for GST-π or oncogene products and tumor stage, degree of differentiation, or lymph node metastasis were apparent for these cancers (data not shown).

Relationship between staining of GST-π or oncogene products and response to radiation therapy Relationships between clinical response to radiation therapy and the GST-π or oncogene product staining results prior to therapy were examined in 73 patients followed-up for more than two years. Some cases received one or two cycles of chemotherapy. The positive rates for GST-π or individual oncogene products were not significantly different among the four categories of tumor response evaluated at 2 to 8 weeks after the last irradiation (data not shown), confirming our previous results.[11] Clinical response was evaluated in terms of both tumor response and relapse in the follow-up period. The 73 patients were divided into two groups, one exhibiting a complete response without relapse and the other showing residual cancers or relapse. The latter group included patients demonstrating a partial response, no change, or progressive disease as well as those developing tumors after an initial disappearance. The positive rates for GST-π and the oncogene products in the two groups are summarized in Table IIA. GST-π (69.0%) and c-Jun values (59.5%) were higher in the residual cancer or relapse group ($P<0.05$) while the other oncogene products did not significantly differ between the two groups.

Since these cases included several carcinomas (29 laryngeal carcinomas, 21 pharyngeal carcinomas, 14 maxillary carcinomas, 4 carcinomas of the oral cavity, and 5 tongue carcinomas) and the distributions of stages, degree of differentiation, and radiation doses were not similar in the two groups, comparisons were also performed in 26 cases of laryngeal carcinoma that showed similar distributions in these characteristics between the two groups (Table IIB). The residual cancer or relapse group included 14 cases (5 T1, 6 T2, and 3 T3; 7 well, and 7 moderately differentiated carcinomas), of which 6 cases showed relapse. The total radiation dose of this

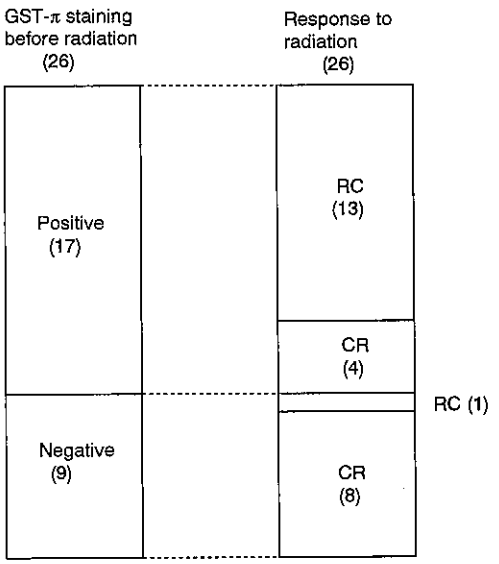

Fig. 3. Relationship between GST-π staining before radiation and response to therapy. Numbers in parentheses indicate numbers of cases. CR, complete response without relapse; RC, relapse or residual cancers, including partial response, no change, and progressive disease categories.

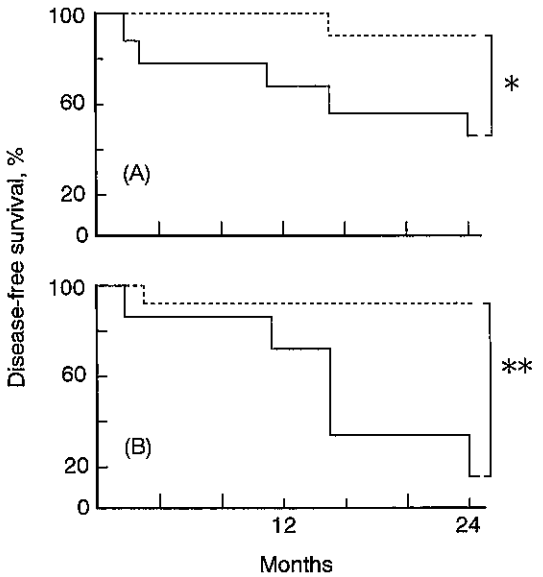

Fig. 4. Disease-free survival in relation to GST-π staining (A) or c-H-Ras staining (B) before radiation therapy. In panel A, solid line, GST-π-positive group (9 cases); dashed line, GST-π-negative group (9 cases). In panel B, solid line, c-H-Ras-positive group (6 cases); dashed line, c-H-Ras-negative group (12 cases). * $P<0.05$; ** $P<0.01$.

group was in the range of 3,000 to 7,000 cGy (average 5,800 cGy). The other group of complete response without relapse included 12 cases (5 T1, 6 T2, and 1 T3; 4 well, 7 moderately, and 1 poorly differentiated carcinomas). The total radiation dose was in the range of 6,600 to 7,000 cGy (average 6,700 cGy). The positive rates for GST-π and c-Jun were significantly higher in the residual cancer or relapse group ($P<0.01$ for GST-π; $P<0.05$ for c-Jun), while those for the other oncogene products did not significantly differ between the two groups (Table IIB). Of 6 cases with relapse, 5 each showed positive reactions for GST-π, c-Jun, and c-H-Ras; 3 and 4 were positive for c-Fos and c-Myc, respectively.

To exclude the possible involvement of chemotherapy, positive rates were also compared in 17 cases that received radiation therapy alone. The rate of GST-π positivity was also higher in the residual cancer or relapse group ($P<0.05$, data not shown). Fig. 3 illustrates the relationship between response to radiation therapy and GST-π staining results in the 26 laryngeal carcinoma cases. Thirteen of 17 GST-π-positive cases developed residual cancers or relapse while 8 of 9 GST-π-negative cases showed complete response without relapse.

Of 26 cases with laryngeal carcinoma, 8 featured residual cancers while the remaining 18 cases exhibited disappearance of tumors up to 8 weeks after the last irradiation. These 18 cases were further divided into two groups depending on staining results for GST-π or the oncogene products before therapy to compare their disease-free survival times. The GST-π-positive group included 9 cases (5 T1 and 4 T2), of which 5 demonstrated relapse during the follow-up period, as compared to 1 of 9 in the GST-π-negative group (4 T1, 4 T2, and 1 T3). Life-table plots of disease-free survival are shown in Fig. 4, with 2-year disease-free survival rates of 88.9 and 44.4% in the GST-π-negative and positive groups, respectively. The difference in survival distribution between the two groups was significant by the log-rank test ($P<0.05$, Fig. 4A). Significant differences were also observed for c-Jun and c-H-Ras ($P<0.05$ for c-Jun; $P<0.01$ for c-H-Ras, Fig. 4B), with higher disease-free survival rates in the oncogene product-negative groups. No significant differences were apparent for the other oncogene products (data not shown).

Altered expression of GST-π and oncogene products after radiation therapy Four to 6 weeks after radiation therapy, 37 patients exhibiting residual cancers underwent surgery, and the tissues obtained from 30 of them were examined for expression of GST-π and the oncogene products to allow comparison with data before radiation. The results are summarized in Table III. The overall positive rate for GST-π in the 30 specimens after radiation was rather low as compared with the value before radiation but the difference was not significant

Table III. Comparison of Positive Rates of GST-π and Oncogene Products in Head and Neck Cancers between before and after Radiation Therapy

	GST-π staining	No. of patients	No. of positive patients (%)			
			c-Jun	c-Fos	c-H-Ras	c-Myc
Before radiation	Positive	18	16 (88.9)[a)]	8 (44.4)	9 (50.0)	8 (44.4)
	Negative	12	0**	0**	1 (8.3)[b)]**	3 (25.0)*
	Total	30	16 (53.3)	8 (26.7)	10 (33.3)	11 (36.7)
After radiation	Positive	11	11 (100)	2 (18.2)	8 (72.7)	10 (90.9)§
	Negative	19	1 (5.2)**	1 (5.2)**	1 (5.2)**	2 (10.5)**
	Total	30	12 (40.0)	3 (10.0)	9 (30.0)	12 (40.0)

a) Positive rate in patients exhibiting positivity for GST-π.
b) Positive rate in patients exhibiting negativity for GST-π.
* $P<0.05$ and ** $P<0.01$ compared with GST-π-positive group before and after radiation, respectively.
§ $P<0.05$ compared with the corresponding value of GST-π-positive group before radiation.

Table IV. Alterations in Staining of GST-π and Oncogene Products in Individual Head and Neck Cancers after Radiation Therapy

Alteration in staining		No. of patients				
Before radiation	After radiation	GST-π	c-Jun	c-Fos	c-H-Ras	c-Myc
Positive	Negative	11	10 (10)[a)]	7 (6)	7 (5)	8 (5)
Positive	Positive	7	6 (6)	1 (1)	3 (3)	3 (3)
Negative	Positive	4	6 (4)	2 (0)	6 (3)	9 (3)
Negative	Negative	8	8 (7)	20 (7)	14 (7)	10 (4)

a) Numbers in parentheses indicate numbers of patients who shared common alterations in the staining of both GST-π and individual oncogene products.

(36.7 vs. 60.0%, $P=0.07$), while the values for the individual oncogene products were essentially similar between before and after radiation. As with the results before radiation, the positive rates of the respective oncogene products were higher in GST-π-positive residual cancers than in GST-π-negative ones ($P<0.01$ for all oncogene products), indicating that the great majority of GST-π-positive residual cancers coexpressed oncogene products other than c-Fos. Of 11 cases exhibiting GST-π-positive residual cancers, 10 also showed positive reactions for c-Myc, the rate being more frequent than the value before radiation ($P<0.05$, Table III). At the tissue level, areas positive for the oncogene products were localized within or coincided with GST-π-positive areas except for a few cases.

Alterations in staining results in individual cases after radiation therapy are summarized in Table IV. In the case of GST-π, 11 of 18 patients who exhibited prior positivity showed negative conversion, while 4 of 12 patients with prior negativity demonstrated positive conversion. Of the 11 exhibiting negative conversion, 10 also showed the same conversion for c-Jun. Four patients that showed positive conversion for GST-π all exhibited the same alteration in c-Jun. Of 15 patients retaining prior GST-π-positive or negative reactions, 13 also retained the same reactions for c-Jun. Thus, in the great majority of patients, changes in c-Jun staining coincided with those in GST-π staining, indicating that c-Jun was more closely correlated with GST-π expression than the other oncogene products. c-Myc staining revealed that 8 of 11 patients having a positive reaction before radiation showed a negative reaction after radiation (Table IV), while 9 of 19 patients having prior negativity showed positive conversion. Thus, in a total of 12 patients that showed positive reactions for c-Myc after radiation, only 3 retained this prior positivity. A similar tendency was also observed for c-H-Ras. Of 9 patients exhibiting a positive reaction, only 3 retained staining.

DISCUSSION

The present immunohistochemical investigation revealed appreciable expression of GST-π and c-Jun in 60.2 and 51.8%, respectively, of squamous cell carcinomas of the head and neck before treatment. The positive rates for the other oncogene products, c-Fos, c-H-Ras, and

c-Myc, were 28.9–37.3% (Table I), these values being essentially similar to those reported by other investigators.[32–35] Since positive areas for these oncogene products were generally not as broad as that for GST-π, carcinomas were evaluated as positive when 30% or more of the component cells were stained. About a half to two-thirds of positive cases were judged as negative, if cut-off values were set as 50%, as used for GST-π in our previous study.[11] When staining results were divided into three or more categories depending on percent of positive areas, the relationships between the individual staining results could not be analyzed owing to their complexity. Thus, the two categories of positivity and negativity were employed, cut-off values being set as 30%. The findings that c-Jun, c-Fos, and c-Myc were generally stained in both cytoplasm and nuclei (Fig. 2), were consistent with their localization in colorectal cancers reported by Magrisso *et al.*[36] and Melhem *et al.*[37] and our previous results in rat tissues.[26] Nuclear translocation of c-Jun synthesized in the cytoplasm is suggested to be modulated by phosphorylation or other modification.[38, 39]

The positive rates for the four oncogene products examined were over 2-fold higher in GST-π-positive cancers than in their GST-π-negative counterparts (Table I), suggesting that these oncogene products might be involved in the expression of GST-π. In particular, c-Jun expression was highly correlated with GST-π expression. The distribution of areas expressing c-Jun also coincided with that for GST-π in most positive cases. As compared to the positive rate for c-Jun, the value for c-Fos was rather low. This may reflect the shorter half-life of c-Fos than that of c-Jun.[40] However, not only c-Jun/c-Fos heterodimers (AP-1) but also c-Jun/c-Jun homodimers are known to be able to activate the transcription of genes possessing TRE.[41, 42] Ras proteins are suggested to enhance the trans-activating potential of c-Jun via activation of the protein kinase C pathway and post-translational phosphorylation of c-Jun.[43, 44] Thus, the three oncogene products, c-Jun, c-Fos, and c-H-Ras, may be linked in enhancement of GST-π expression in squamous cell carcinoma, with c-Jun being the most directly involved.

The current data on 26 patients with laryngeal carcinoma suggest that GST-π expression might be partly responsible for determining sensitivity to radiation therapy (Fig. 3 and Table IIB). This finding differs from our previous indication of no clear relationship between GST-π and radiation response.[11] In this study, response to radiation was evaluated in terms of both immediate tumor response and relapse within a two-year follow-up period. Inclusion of relapse seems to be relevant to the difference, because tumor response evaluation by itself did not give a significant difference between GST-π-positive and negative cases (data not shown). Furthermore, among the 18 cases of complete response after radiation therapy, relapse occurred more frequently in GST-π-positive cases than in negative ones (Fig. 4A). Similar tendencies were also observed for c-Jun and c-H-Ras (Fig. 4B). These results suggest that the expression of GST-π and these oncogene products may be risk factors for relapse of laryngeal cancer. GST-π expression has been suggested as a predictor of chemotherapeutic results for acute nonlymphoblastic leukemia.[45] The expression of *ras* and *myc* oncogenes has been suggested to affect radiosensitivity in several cancer cell lines.[13–17] Our present results support the possible involvement of c-H-Ras, but not c-Myc, expression in radioresistance of laryngeal cancers (Table III). Most relapse cases also exhibited correlated expression of GST-π and the oncogene products before radiation, as observed in residual cancers. However, it seems unlikely that such prior properties in relapse cases were directly linked to those in residual cancers, since prior positive reactions for c-Myc or c-H-Ras were altered in most individuals after radiation.

The present study also revealed that the expression of GST-π and the oncogene products can be influenced by radiation therapy (Table IV). Alterations in the expression of all but c-Fos occurred as negative or positive conversions in about half the cases. Following radiation, negative conversion of GST-π occurred more often than positive conversion. In the residual cancers, the expression of c-Myc was more closely correlated with GST-π expression, as compared with before radiation (Table III). The high correlation between GST-π and c-Jun was retained after radiation therapy and changes in c-Jun staining almost completely coincided with those in GST-π (Table IV).

In conclusion, the present results indicate a relationship between GST-π expression and oncogene products in head and neck cancers. In particular, c-Jun is most highly correlated with GST-π expression. Although influenced by radiation, GST-π and c-H-Ras expression is suggested to be a risk factor for relapse in cases of laryngeal cancers.

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