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Glutathione Peroxidases in Human Head and Neck Cancer

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Glutathione peroxidases (GPX), enzymes that catalyze the reduction of reactive intermediates have been implicated in the action of several cytostatic drugs. Two major types of GPX have been found: a selenium-dependent form (SeGPX) which is active with both hydrogen peroxide and organic hydroperoxides, and a selenium-independent GPX which is only active with organic hydroperoxides. SeGPX and total GPX (tGPX) activity were assayed in cytosolic fractions from malignant and adjacent normal tissue in 13 patients with oral/oropharyngeal, and 10 patients with laryngeal squamous cell carcinoma. Neck lymph node metastases were available from 2 and 5 of these patient respectively. Tumors from the oral/oropharyngeal region contained significantly less SeGPX and tGPX activity than laryngeal tumors. Primary oral/oropharyngeal and laryngeal tumors had lower SeGPX activities than the matched normal mucosa. tGPX activities were similar in normal and tumor tissue. Metastases contained slightly more SeGPX and tGPX activity than the matched tumor tissue. We conclude that the inherent anti-tumor drug resistance of human head and neck squamous cell carcinoma is not mediated by increased glutathione peroxidase enzyme activity in the tumor tissue. *Key words*: *glutathione peroxidase, head and neck cancer, drug resistance.*

INTRODUCTION

Oral/oropharyngeal and laryngeal cancer account for 2.1 and 1.3 cancer deaths respectively per 100,000 inhabitants per year in The Netherlands (1). Early involvement of regional lymph nodes tends to occur in patients with these types of cancers. Chemotherapy, usually added for the treatment of extensive disease, is of limited use due to either intrinsic resistance or development of (multidrug) resistance during treatment with anti-cancer drugs (2, 3).

The mechanisms involved in drug resistance are poorly understood. *In vitro* studies with tumor cell lines selected for resistance to commonly used chemotherapeutic agents showed that several interrelated mechanisms may be involved in the acquisition of the drug resistant phenotype. Decreased intracellular drug accumulation due to increased expression of P170 glycoprotein, a drug efflux pump (4), increased repair of or tolerance to drug-induced damage due to increased expression of DNA topoisomerases (5), and increased metabolic drug inactivation by glutathione-consuming reactions (6, 7) may all contribute to resistance.

Glutathione peroxidases (GPX) are enzymes that catalyze the reduction of organic hydroperoxides (lipid hydroperoxides, DNA hydroperoxides) and hydrogen peroxide, intermediates implicated in the action of cytostatic drugs such as bleomycin or adriamycin (8, 9). Two major types of GPX have been found. One type contains selenium; this enzyme is active with both organic hydroperoxides and hydrogen peroxide. The second type of GPX consists of proteins that do not depend on selenium, and have negligible activity with hydrogen peroxide. This class comprises mainly glutathione S-transferases (10). All GPX-catalyzed reactions use reduced glutathione as a cofactor (10). In sarcoma (11) and other human cell lines (12) resistant to the anti-cancer drugs adriamycin or cisplatin, increased GPX activity has been implicated as a mechanism of resistance. High GPX levels are also present in several refractory solid tumors, where they may be a barrier to effective treatment with chemotherapeutic agents (13–15). In this study we examined the GPX activity with hydrogen peroxide (selenium-dependent activity, SeGPX) and t-butylhydroperoxide (total activity, tGPX) as substrates, in tumor tissue and corresponding normal epithelium in patients with head and neck squamous cell carcinoma.

MATERIAL AND METHODS

Tissue samples

Twenty-three patients (22 men and 1 woman, median age 63 years, range 47–74 years) with head and neck squamous cell carcinoma were operated on at the Department of Otorhinolaryngology and Maxillofacial Surgery of the St Radboud University Hospital. All patients received primary surgical treatment. Tumor localizations were oral cavity ($n = 3$), tongue ($n = 6$) oropharynx ($n = 4$) and larynx ($n = 10$). One tumor was well differentiated, 19 tumors were moder-

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ately and 3 poorly differentiated. Representative parts of tumor and adjacent normal mucosa were excised by a pathologist. From 7 patients, samples of neck lymph node metastases were obtained at primary resection. All tissue samples obtained were frozen within 20 min after resection of the tumor, and were stored at −70°C. Tissue fragments (10–100 mg) were thawed and homogenized on ice in 5 volumes of homogenizing buffer (250 mM sucrose, 20 mM Tris-HCl, 1 mM dithiothreitol, pH 7.4) using small glass-glass homogenizers. Homogenates were centrifuged at 150,000 × g at 4°C for 1 h. Supernatants (cytosolic fraction) were divided into portions and stored at −70°C.

Assays

Protein was assayed by the method of Lowry et al. (16). Glutathione peroxidase enzyme activity was measured with hydrogen peroxide and t-butylhydroperoxide (Sigma Chemical Company, St Louis, MO, USA) as substrates, essentially as described by Howie et al. (14).

Statistical analysis

Data were calculated per mg total cytosolic protein and expressed as the mean ± standard error of the mean. Results for the different groups were compared with Student's *t*-test. The paired Student's *t*-test was used to evaluate differences between pairs of normal and malignant or normal and metastatic tissue. Correlations between the parameters were tested with Pearson's linear correlation procedure. Differences were considered significant if the *p* value was below 0.05.

RESULTS

Cytosolic fractions of squamous cell carcinomas from the larynx contained significantly more SeGPX activity and tGPX activity than fractions from comparable tumors of the oral cavity, tongue and oropharynx (Table I). In comparison with normal oral/oropharyngeal mucosa, normal laryngeal mucosa had higher SeGPX and tGPX activities, but the difference was statistically significant only for tGPX activity.

Tumors at both localizations contained less SeGPX activity than the corresponding normal tissues, and when data from the two localizations were combined the difference was significant ($p < 0.05$). tGPX activities were similar in normal mucosae and primary tumor tissues, both in the oral cavity and larynx.

Metastases had SeGPX activities similar to those in matched normal mucosae. tGPX activity in the metastases was slightly higher in comparison with both primary tumors and normal mucosae, but the difference with respect to normal tissues was significant only for metastases of oral/oropharyngeal tumors (Table I).

When SeGPX activity was plotted against tGPX activity for all 53 times samples analyzed, a regression line with a correlation coefficient of 0.90 ($p < 0.001$) was obtained.

DISCUSSION

tGPX activity was highly correlated with SeGPX activity, indicating that GPX activity in normal and malignant tissue from human head and neck mucosa is mediated mainly by SeGPX. Howie et al. (14) reported very similar results for normal and malignant human lung, colon and breast tissue, in which tGPX activity also closely paralleled SeGPX activity.

Parise et al. (17) reported higher concentrations of glutathione and glutathione S-transferases in human head and neck squamous cell carcinomas in comparison with normal tissue. Glutathione S-transferases constitute a major part of selenium-independent GPX activity (10), therefore higher tGPX activities might have been expected in these tumors. Significantly higher SeGPX and tGPX activities in tumor tissues

Table I. *Selenium–dependent glutathione peroxidase (SeGPX) and total glutathione peroxidase (tGPX) activity in cytosols of primary tumors, corresponding normal mucosae and metastases from human head and neck squamous cell carcinoma*

Tumor localization		SeGPX activity (nmol/min/mg protein)	tGPX activity (nmol/min/mg protein)
Oral cavity, tongue and oropharynx	Normal mucosa (n = 13)	211 ± 28	130 ± 18
	Tumor (n = 13)	156 ± 12	116 ± 9
	Metastasis (n = 2)	202 ± 45	172 ± 27[c]
Larynx	Normal mucosa (n = 10)	273 ± 40	200 ± 29[a]
	Tumor (n = 10)	230 ± 12[b]	198 ± 9[b]
	Metastasis (*n* = 5)	293 ± 32	244 ± 37

[a] $p < 0.05$ vs. normal oral and oropharyngeal mucosa.
[b] $p < 0.001$ vs. oral and oropharyngeal tumor tissue.
[c] $p < 0.05$ vs. normal oral and oropharyngeal mucosa.

than in adjacent normal tissues have been reported in malignancies from breast (14), lung (13, 15), colon, stomach and kidney (14). The results of the present study contrast with reports of other solid tumors, as lower SeGPX activities and similar tGPX activities were found in human head and neck squamous cell carcinomas in comparison with adjacent normal tissue. However, the combination of similar tGPX activities and lower SeGPX activites indicates that Se–independent GPX activities (i.e. glutathione S-transferases) are increased in the primary tumors.

Metastases had higher SeGPX and tGPX activities than the corresponding primary tumors, and mean values were very similar (SeGPX) or slightly above (tGPX) activities detected in the corresponding normal tissues. Janot et al. (18) assayed several principal drug- and carcinogen-metabolizing enzymes in pairs of tumor and adjacent normal tissue in the pyriform sinus of the larynx. Except for a decrease in epoxide-hydrolase and an increase in glutathione concentration in the tumors, these authors found no significant difference between normal and tumor tissues.

Our results also show that GPX activity in human head and neck squamous cell carcinoma varies with the localization of the tumor. Malignancies in the oral region contained significantly less SeGPX and tGPX activity than laryngeal tumors.

In conclusion, human head and neck squamous cell carcinomas contain less SeGPX activity and similar amounts of tGPX activity in comparison with adjacent normal tissues. Tumors in the oral cavity had significantly lower SeGPX and tGPX activities than laryngeal tumors. These results do not support the hypothesis that GPX plays a role in the intrinsic resistance of these tumors to anti-cancer drugs. Because both glutathione S-transferase enzyme activities (17) and Se–independent GPX activities (this study) are increased in human head and neck squamous cell carcinomas, further studies are indicated to examine the composition of glutathione S-transferase subclasses in these tumors.

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