Glutathion-S-Transferase-π Activity in Benign and Malignant Human Oral Epithelial Lesions

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SUMMARY

Histological sections of 14 squamous cell carcinomas, 3 carcinomas in situ, 6 papillomas, 17 dysplasias, and 5 hyperkeratoses from patients were examined for expression of the placental form of glutathion-S-transferase (GST-π) using a monoclonal antibody with an immunoperoxidase technique. As controls, 5 oral mucosal epithelia from healthy individuals and 6 histologically normal oral mucosal epithelia adjacent to cancerous lesions were also investigated. No GST-π activity was detected in the normal oral mucosal epithelia from healthy individuals, while histologically normal oral mucosal epithelia adjacent to cancerous lesions exhibited expression of GST-π with patchy distribution of staining in the epithelial components. In benign and precancerous lesions, GST-π was present in only 1 out of 5 hyperkeratoses, 3 out of 6 mild dysplasias, 10 out of 11 moderate to severe dysplasias and 2 out of 6 papillomas. On the other hand, GST-π was detected in all 17 cases of carcinoma in situ and squamous cell carcinoma with an intense and homogeneous pattern of staining of GST-π. Our study strongly suggests that GST-π may be useful as a immunohistochemical marker for the analysis of tumor development in human oral mucosal epithelium.

Key words: GST-π, Immunohistochemistry, Oral cancer

INTRODUCTION

In patients with oral mucosal cancer, squamous cell carcinoma antigen (SCC) has been utilized as a relatively useful tumor marker for serum diagnosis (1), and γ-glutamyl transpeptidase (GGT) as an immunohistochemical marker

for analysis of cancer development(2). However, these markers are not always reliable because of the difficulty of early detection of cancer based on the serum levels of SCC(3) and the suggestive evaluation of GGT as a marker for differentiation rather than atypical proliferation of cancer cells(4). Therefore, the establishment of a more useful and reliable marker for serum diagnosis and for morphological analysis of oral mucosal cancer is desirable.

Glutathion-S-transferases (GSTs) are a family of multifunctional enzymes which are involved in the metabolism, that is the detoxification, of a broad range of xenobiotics(5). In a recent study, the placental form (GST-π) of GST has been considered to be the most reliable for the identification of precancerous and cancerous lesions on liver carcinogenesis of rat(6). Furthermore, prominent GST-π activity has been observed biochemically and immunohistochemically in various malignant tumors in human, indicating that GST-π may be a ubiquitous tumor marker(7, 8, 9, 10, 11, 12, 13, 14). These findings led us to investigate the usefulness of GST-π as a potentialy new marker for human oral mucosal cancers. In this context, we have previously reported significantly elevated plasma levels of GST-π in oral cancer patients(15). In the present study, we immunohistochemically examined precancerous and cancerous lesions of human oral mucosal epithelium using anti-GST-π antibody to investigate the possible application of GST-π as a marker for the analysis of cancer development.

MATERIALS AND METHODS

Antiserum

GST-π was purified from human placenta, and 5 anti-GST-π monoclonal antibodies recognizing 4 independent epitopes were prepared as reported previously(16, 17). An antibody designated 5F was used for immunohistochemical staining since it gave the most discrete staining in a preliminary study(17).

Tissue sections

A total of 45 biopsy specimens of oral epithelial lesions, consisting of 22 clinical leukoplakias, 3 erythroplasias, 6 papillomas, and 14 invasive carcinomas were examined for the expression of GST-π activity. As controls, 5 normal oral mucosal tissues from healthy individuals and 6 histologically normal oral mucosal tissues adjacent to cancerous lesions were also examined for GST-π expression. All these tissue samples were quickly frozen in liquid nitrogen-isopentan, and stored at -80℃. Tissue sections were cut into 6-μm size in a cryostat and placed on glass slides. Tissue sections were also cut from buffered formalin -fixed, paraffin-embedded blocks and dried on glass slides. The tissues were rehydrated in xylene and alcohol for the immunoperioxidase assay.

Histology and Immunohistochemistry

Frozen sections fixed with cold acetone and paraffin sections were stained with hematoxilin-eosin. Histological diagnosis was made on the basis of the classification by Pindborg(18).

The remaining serial sections were examined for GST-π activity with an avidin-biotin complex immunoperoxidase technique using a Vecstain Kit. In brief, after blocking the nonspecific binding with normal goat serum for 10 minutes, the sections were treated with 1% H_2O_2 in phosphate-buffered saline (PBS) for 20 minutes to eliminate endogenous peroxidase activity. Tissue sections were covered with a diluted solution(1 : 600) of anti-GST-π antibody for 30 minutes at room temperature. The sections were then reacted with biotinylated goat anti -mouse Ig serum for 30 minutes and further reacted with avidin biotin peroxidase complex for 30 minutes. The enzyme reaction was promoted by using diaminobenzidine as the substrate with an incubation time of 5 to 10 minutes. Each step was followed by a brief washing with PBS. Sections were then counterstained with hematoxilin. Negative control sections were prepared by the same procedure mentioned above except for substitution of the GST-π immune serum with nonimmune serum.

RESULTS

Histological diagnosis

Of 22 clinical leukoplakias, 5 were simple hyperkeratosis, 6 mild dysplasia, 6 moderate dysplasia, and 5 severe dysplasia. The 3 clinical erythroplasias were all carcinoma in situ. Fourteen invasive squamous cell carcinomas consisted of 5 well differentiated, 6 moderately differentiated, and 3 poorly differentiated types (Table 1).

Evidence for GST-π activity

GST-π activity was immunohistochemically visualized in sections by the presence of a brown precipitate(Figs. 2, 3, 4, 5, 6). Controls incubated in the preimmune serum at the first step of staining showed no colored deposit. The intensity of GST-π expression in tissue sections were defined as negative or positive, and the positivity was further categorized into two groups of strong or weak staining (Table 1).

GST-π activity in normal oral mucosal tissues

In normal oral epithelia from healthy individuals, essentially no GST-π activity was found (Fig. 1).

Table 1 *GST-π activity in normal tissue, benign and precancerous lesions, and malignant lesions in human oral mucosal epithelium*

Histological diagnosis	Total	GST-π positive		GST-π negative
		strong	weak	
Normal				
oral epithelium from healthy individual	5			5
oral epithelium adjacent to cancerous lesion	6	1	5	
Benign and precancerous lesion				
hyperkeratosis	5		1	4
mild dysplasia	6	1	2	3
moderate dysplasia	6	3	2	1
severe dysplasia	5	4	1	
papilloma	6	1	1	4
Malignant				
carcinoma in situ	3	3		
squamous cell carcinoma				
well differentiated	5	5		
moderately differentiated	6	6		
poorly differentiated	3	3		

However, in all tissue sections (100%) of histologically normal oral epithelia adjacent to cancerous lesions, GST-π activity was detected, although the staining was generally weak as compared to those of cancerous lesions (Fig. 2). Five out of 6 specimens revealed focal and weak reaction for GST-π activity in both the nuclei and cytoplasm of the cells in the parabasal or intermediate layers of the epithelium, whereas one specimen showed patchy but relatively intense GST-π activity in the epithelium other than the superficial layers (Table 1).

GST-π activity in benign and precancerous lesions of oral mucosal epithelium

In only 1 out of 5 hyperkeratoses (20%) and in 3 out of 6 mild dysplasias (50%), GST-π activity was noted with the same distribution pattern of GST-π activity as seen in histologically normal oral mucosal epithelia adjacent to cancerous lesions (Table 1, Fig. 3).

Five out of 6 moderate (83%) and all 5 severe (100%) dysplasias showed relatively intense GST-π activity in the basal and intermediate layers of the epithelium (Table 1, Fig. 4).

Two out of 6 papillomas (33%) had focally weak GST-π activity localized in the epithelial components (Table 1, Fig. 5).

GST-π activity in carcinomas in situ and squamous cell carcinomas

All 3 carcinomas in situ (100%) and all 14 invasive squamous cell carcinomas (100%) showed intense GST-π activity which was localized in the cytoplasm of cancer cells with a homogeneous distribution pattern in the epithelial foci, but not in stromal connective tissues (Table 1, Fig. 6). There was no direct relationship beween the grade of tumor differentiation and the intensity of GST -π activity (Table 1).

DISCUSSION

In the present study, normal human oral mucosal epithelia from healthy individuals showed no reaction of staining for GST-π activity. However, GST-π immunoreactivity was identified in all cases of carcinomas in situ and invasive squamous cell carcinomas with intense reaction of staining, while benign and precancerous lesions revealed lower levels of occurrence and intensity of the expression of GST-π activity. Our results are consistent with previous findings of the presence of GST-π in tissues of other cancers, including tumors of colon (7, 13, 14), stomach(9, 13), esophagus(14), pancreas(14), lung(11, 12), breast(14), uterine cervix(8) and skin(10). Elevated serum levels of GST-π have also been described in patients with colorectal cancer(13, 14), gastric cancer(13), hepatocellular carcinoma(13, 14), breast cancer(14), and cancer of the uterine cervix(14). The high immunohistochemical expression of GST-π in oral mucosal cancers further emphasizes its potential as a tumor marker as did in our previous report (15). Moreover, the finding that precancerous lesions of oral mucosal epithelium contain GST-π activity suggests that GST-π could be useful not only as a marker for the morphological analysis of cancer development but also in screening tests for detection of patients with a high risk of cancer, in view of the fact that normal oral mucosal epithelia from healthy individuals are immunohistochemically negative for GST-π.

Of special interest was the finding that the more the precancerous lesions progress, the more intense the GST-π expression. The GST-π-positive areas in precancerous lesions of oral mucosal epithelium may express phenotypically altered changes that will promote them to cancer and be associated with the evolution of the cancer. From such speculation, it is not surprising that the histologically normal oral mucosal epithelium adjacent to cancer contained GST-π activity, because phenotypically it might already have acquired the processes of both initiation and promotion to cancer development through a long latent period, which

may associate it with tumor reccurrence when not excised at surgery.

Thus, GST-π is of potent diagnostic use as a marker in human oral mucosal cancers. The markedly increased expression of GST-π in cancer cells suggests an association of GST-π expression with the property of cancer cell. With regard to the relationship to differentiation, there was no relationship observed between the degree of tumor differentiation and the occurrence of GST-π immunoreactivity. Essentially, GST-π is one of the multifunctional detoxifying enzyme families that plays a role in drug resistance of cells. From the present study, however, it was impossible to determine whether GST-π was expressed as the result of the metabolic changes due to drug resistance during malignant transformation. Its role in the multivariate analysis of atypical proliferative lesions, its relationship to drug resistance, and its potential prognostic value in evaluation of human oral cancers all deserve further study.

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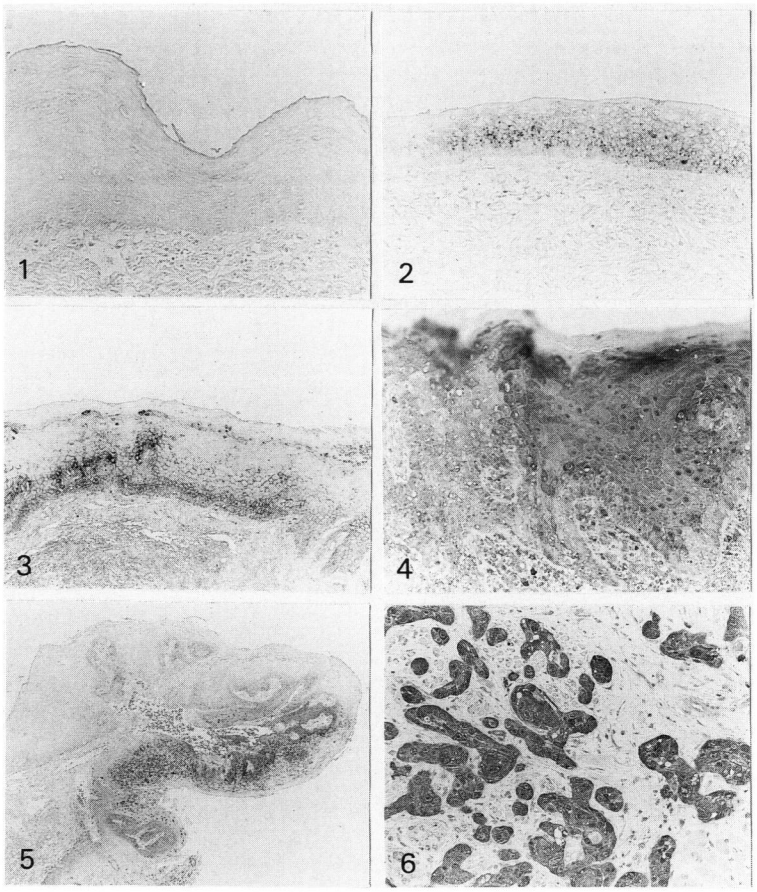

Fig. 1 A normal oral mucosal tissue from a healthy individual. No GST-π activity was seen. Frozen section. ×109.

Fig. 2 A histologically normal oral mucosal tissue adjacent to cancerous lesion. GST-π activity was focally distributed in both nuclei and cytoplasm of the squamous epithelial cells. Paraffin section. ×54.

Fig. 3 A mild dysplasia with marked keratosis. GST-π activity was mainly observed in the basal and intermediate layers of the epithelium. Paraffin section. ×54.

Fig. 4 A severe dysplasia with a diffuse but relatively intense activity of GST-π. Paraffin section. ×109.

Fig. 5 A papilloma with a focal distribution of GST-π activity in the epithelial portion. Paraffin section. ×22.

Fig. 6 A poorly differentiated squamous cell carcinoma. Intense GST-π activity was homogeneously distributed in the cytoplasm of the cancer cells. Paraffin section. × 109.