HISTOCHEMICAL DEMONSTRATION OF GAMMA-GLUTAMYL TRANSPEPTIDASE (GGT) ACTIVITY IN HUMAN THYROID TISSUES

SHINICHI MORIYAMA, NORIO HIROTA* AND TAKESHI YOKOYAMA*

The 2nd Department of Pathology, Yamanashi Medical School, Yamanashi 409-38 and Department of Pathology*, Jichi Medical School, Tochigi 329-04

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A histochemical analysis of gamma-glutamyl transpeptidase (GGT) activity was performed on 42 cases of various human thyroid diseases. In contrast to the negative reaction for GGT in the normal thyroid tissues, a rather widespread distribution of activity was demonstrated in diseased thyroids. Among thyroid neoplasms, papillary carcinoma showed the strongest and most stable activity, while a more variable activity occurred in follicular carcinoma. Anaplastic carcinoma and medullary carcinoma were negligible in the activity. In non-neoplastic thyroid diseases, the activity was localized in distribution and usually less pronounced, as compared with the neoplastic lesions.

This study suggests that the appearance of GGT activity in thyroid tissues reflects not only proliferative or regenerative changes of the follicular epithelium but also functional aspects of the lesions.

Many studies have been performed on the histochemical localization in various organs of gamma-glutamyl transpeptidase (GGT, E.C. 2.3.2.2.), a membrane-bound enzyme which facilitates extra-intracellular amino acid transport, and a widespread distribution of GGT activity has been demonstrated (1, 11, 20). Furthermore, the oncofetal nature of this enzyme has been emphasized in the histochemical studies of experimental and human neoplasms (6, 8, 12, 15).

Glenner et al. (11) first reported that with the exception of the epithelium of immature follicles which were markedly positive, the GGT activity of the normal thyroid tissues in human was negligible. In an attempt to clarify the significance of GGT staining in the thyroid tissues, the present study was directed toward determining whether GGT activity was increased in various thyroid diseases and what the histochemical localization was.

MATERIALS AND METHODS

Materials: Human thyroid tissues removed surgically in the treatment of various thyroid diseases were used for histochemical analysis. Normal thyroid tissues obtained at autopsy within 3 hr after death served as control material. The thyroid tissues studied constituted of 30 cases of neoplasms and 12 cases of non-neoplastic lesions (Tables 1 and 2). Eight control thyroids were obtained from individuals between the ages of 17 weeks of gestation and 82 years.
Histochemical methods: Most fresh thyroid tissues were immediately prepared for frozen sections with cryostat, about 6 μm thick, and fixed in cold acetone (4°C) for 5 min. The other materials were fixed in cold acetone and embedded in paraffin. Paraffin sections of about 5 μm in thickness were deparaffinized in benzene and used for staining. GGT staining was performed following Rutenburg’s method (20). Gamma-glutamyl-4-methoxy-2-naphthylamide (Bachem Inc., Torrance, Cal. 90505) was used as a substrate, being fast blue BBN as coupling agents. The tissue sections were incubated in the reacting solution at room temperature for 20 to 40 min. The nuclei were counterstained with hematoxylin. Cupric sulfate (0.1 M) was used for an inhibition test (20).

RESULTS

Control study: No positive staining for GGT was found in the control thyroid tissues (Fig. 1) with the exception of a slight increase in the activity focally present in tiny immature thyroid follicles. The colloid substance occasionally showed a faint stainability, although the positivity was only focal. Connective tissues, blood vessels and inflammatory cells showed no activity. Significant age differences in GGT staining were not demonstrated, and no increased activity was present in the two fetal thyroid tissues.

Inhibition test: The tissue GGT activity was completely inhibited in 0.1 M cupric sulfate solution for 5 min.

Carcinoma: The strongest and most diffuse GGT activity was demonstrated in papillary carcinoma of the thyroid (Fig. 2). Reddish-orange and finely granular stainability was mainly observed in the apical portion of the epithelium. There was a tendency toward further increased activity in the portion with follicular pattern and infiltrative growth within the stroma. Follicular carcinoma (Fig. 3) showed a more variable stainability for GGT, although the intracellular localization of GGT activity was similar to that of papillary carcinoma. The colloid substance as well as desquamated epithelia were highly reactive in the follicular carcinoma with squamous metaplasia. There was no GGT activity in an anaplastic carcinoma of the medullary type, while focal activity was barely discernible in a carcinoma of the large cell type. A medullary carcinoma with amyloid stroma showed no positivity for GGT staining, while a small number of thyroid follicles at the periphery within the tumor showed increased activity. A weak stainability for GGT was present in the portion of squamous cell carcinoma in the rare case of so-called carcinosarcoma of the thyroid, consisting of a fibrosarcomatous component and a well-differentiated squamous cell carcinoma.

Adenoma: The analysis was limited to follicular adenoma with cystic changes in the present study. Relatively high stainability was found in the apical portion of the epithelium lining the cystic lumen.

Non-neoplastic lesions: Some regenerative and tiny thyroid follicles in chronic thyroiditis were highly reactive for GGT along with their colloid substance. A similar appearance of GGT activity of the regenerative follicles was noted in a case of primary malignant lymphoma of the thyroid (not included in Tables). In diffuse hyperplasia of Basedow’s disease, the activity was mainly confined to small follicles, although preoperative drug therapy was performed. Adenomatous goiter also-
showed a rather localized activity in the tiny follicles. However, a strong and diffuse stainability was found in a case of congenital goiter (Fig. 4), in which a deficiency in iodine transport was demonstrated.

The histochemical analysis of GGT activity in the thyroid tissues was summarized in Tables 1 and 2. The activity was found to be more preserved in frozen sections than in paraffin embedded tissues.

**DISCUSSION**

Despite several reports of histochemical investigations of GGT activity related to neoplastic diseases in humans (2, 8, 12, 14), there has been no systematic analysis on the activity in varied conditions of human thyroid tissues since Glenner et al. (11) first described a staining property for GGT in the normal human thyroid tissues in 1961. In accordance with their demonstration, the control human thyroid tissues in our present study were usually negative for GGT staining except for immature tiny follicles. Additionally, a faintly positive reaction for GGT in the colloid substance was only focal and incidental. We report herein that the histochemical distribution of GGT activity was extraordinarily widespread in human thyroid diseases, either neoplastic or non-neoplastic.

Among neoplastic human thyroid diseases, papillary carcinoma showed the strongest and most stable activity, while follicular carcinoma displayed rather variable stainability. Anaplastic carcinoma and medullary carcinoma had negligi-
Fig. 1. No GGT activity in the control thyroid tissue. Paraffin section. ×150

Fig. 2. Strong and diffuse stainability for GGT in papillary carcinoma of the thyroid. Note higher activity in the apical portion of the epithelium. Frozen section. ×150
Fig. 3.  GGT staining of follicular carcinoma of the thyroid. Positive staining of colloid substance as well as apical portion of the epithelium. Frozen section. ×300

Fig. 4.  Strong and diffuse GGT activity chiefly in the apical portion of the follicular epithelium in congenital goiter. Paraffin section. ×300
ble activity for GGT. In cystic follicular adenomas, a relatively strong activity was noted. These findings on the neoplastic lesions of the thyroid suggested that GGT activity was closely related to the proliferation rate of cell of the tumors (22). Moreover, such GGT activity appeared to be influenced by the morphological and functional differentiation of the tumors. With respect to a functional aspect in the human thyroid tissues, thyroxine and triiodothyronine were recently demonstrated in pioneering study using the immunoperoxidase method by Kawaoi et al. (16). A better understanding of the significance of GGT activity in the thyroid would be provided if our present data were compared with the immunohistochemical localization of the thyroid hormones. Study of this aspect is now under way. Concerning morphological differentiation, it was reported that GGT activity was decreased in the poorly differentiated types of human hepatoma (14) and epidermoid carcinoma of the lung (12), in contrast to a high activity of the well-differentiated tumors of the same organs. These findings seem to correspond to our results in terms of the relationship between GGT activity and cell differentiation in thyroid carcinomas.

In non-neoplastic human thyroid diseases, GGT activity was usually weak and localized, compared with that in neoplastic lesions. GGT activity was mainly confined to small thyroid follicles in chronic thyroiditis, Basedow's disease after receiving drug therapy, and adenomatous goiter. These activities were conceivably dependent upon the regenerative processes or reactive proliferation of the follicular epithelium. In the case of congenital goiter, which showed a pseudomalignant proliferation of the thyroid follicles probably caused by a prolonged TSH stimulation, a strong and diffuse GGT activity was demonstrated.

Unlike the widespread GGT activity induced by proliferative or regenerative changes of the follicular epithelium, negative reaction for GGT in medullary carcinoma was exhibited possibly due to its parafollicular cell (C-cell) derivation.

Regarding the intracellular localization of GGT activity, the apical portion of the epithelium was mainly stained, suggesting the close relation of GGT activity to the surface alterations of the plasma membrane including microvilli. Further examination by ultrastructural histochemistry is required to confirm this conjecture, as previously done by Seligman et al. (21).

It is well known that both precancerous lesions and hepatocellular carcinomas acquire a strongly positive reaction for GGT during the experimental hepatocarcinogenesis (5, 7, 13, 15). This phenomenon was interpreted as reacquisition of fetal antigens by some investigators (6, 15), because diffusely positive GGT activity exists in the rat hepatocytes of the fetal and neonatal period (3), contrasted with almost total disappearance in the normal adult liver. It is noteworthy from the point of view of oncofetal development that GGT activity appeared in the thyroid lesions, especially in the neoplastic growth. Additional detailed study of GGT activity of the thyroid tissues during fetal development is required, since such an investigation has not been reported in the literature (9). Of the non-neoplastic lesions, thyroid hyperplasia, which has been suggested to be a possible precursor lesion for malignant tumors (18, 19), revealed a significant increase in GGT activity in the present study. Further, it may be necessary to take the presence of different isozymes of GGT in the thyroid tissues into consideration in the study of this enzyme, because several GGT isozymes have been detected in the human sera and bile (10, 23).

The follicular cells of the thyroid possess diverse organ-specific functions, which
HISTOCHEMISTRY OF GGT IN HUMAN THYROIDS

are endocrine, exocrine-like and reabsorptive in nature (4). These functions are possibly responsible, to some extent, for the varied staining pattern for GGT in thyroid tissues, if GGT is involved in the various phases of synthesis of thyroid hormones (17). Another possibility is that the stainability for GGT may also indicate functional aspects of the thyroid lesions. It seems to be imperative that the role of this enzyme in the thyroid tissues is elucidated.

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