Immunohistochemical analysis of amylase isoenzymes in thyroid cancer

M Higashiyama, S Doi, N Tomita, T Monden, M Murotani, Y Kawasaki, T Kobayashi, T Shimano, M Ogawa, S Takai, T Mori

Abstract

The expression of amylase in various histological types of thyroid cancer was studied by an immunohistochemical technique, using a polyclonal anti-amylase antiserum and two monoclonal antibodies specific for salivary and pancreatic-type amylases, respectively. Amylase was expressed in 21 of 24 (88%) thyroid cancers by polyclonal antiserum analysis. Analysis by monoclonal antibodies, however, showed that only 13 (54%) cases and three (13%) cases contained salivary-type and pancreatic-type amylases, respectively. Moreover, immunoreactivity for pancreatic-type amylase was detected only in medullary carcinoma; other histological types were positive for salivary-type amylase. These results show that thyroid cancer frequently expresses amylase, and suggest that the differences between amylase isoenzymes in thyroid cancer may correlate with those found between cellular origin of tumour.

Amylase, which hydrolyses 1,4 glucosidic bonds of starch, is expressed by several types of malignant cells and tissues, including gastric,¹ gynaecological,²⁻⁵ lung,⁶⁻⁸ and mammary cancer,⁹ and multiple myeloma.¹⁰ It was shown recently that thyroid cancer also

expresses amylase.^{11 12} Although most of these tumours express the same amylase isoenzyme as that produced in the salivary gland (salivary-type), some tumours express isoenzymes of pancreatic origin (pancreatic-type) or unknown origin.

The normal thyroid gland and various histological types of thyroid adenoma have also been shown by biochemical analyses to express salivary-type amylase and several unknown types, but pancreatic-type amylase has not been detected in these tissues.^{11 12} It is not yet clear, however, what type of amylase isoenzyme is expressed by thyroid cancer. We therefore analysed amylase isoenzymes in various thyroid cancers, using an immunohistochemical method with three different anti-amylase antibodies.

Methods

Tissue sections were prepared from formalin fixed, paraffin wax blocks of 24 specimens of thyroid cancer—14 well differentiated papillary or follicular carcinomas, two poorly differentiated carcinomas, three anaplastic carcinomas and five medullary carcinomas according to Rosai's classification and that of the WHO.^{13 14}

For the immunohistochemical analysis of amylase isoenzymes, we used three types of anti-amylase antibodies. A rabbit polyclonal antiserum, which reacts to both salivary- and pancreatic-type amylase, was produced as described previously.¹⁵ Monoclonal antibody, 88E8, specific for salivary-type amylase, was supplied by Boehringer Mannheim Yamanouchi Co Ltd, Tokyo, Japan.¹⁶ Monoclonal antibody, 3B7, specific for pancreatic-type amylase was kindly provided by Kohjin Co Ltd, Ohita, Japan.¹⁷ We confirmed the specificity of each monoclonal antibody for isoenzymes by immunostaining sections prepared from normal human pancreas and salivary gland (fig 1).

Sections were stained with a biotin-streptavidin immunoperoxidase technique (BioGenex Laboratories Biotin-Streptavidin Immunostaining Kit; Dublin, California). Briefly, after endogenous peroxidase was blocked by immersion in 0.3% hydrogen peroxide, sections were exposed to 3% normal goat serum, followed by incubation with primary antibody (rabbit polyclonal $\times 500$; 88E8 $\times 10$; 3B7 $\times 10$) for one hour at room temperature. Sections were then incubated with a biotinylated goat anti-rabbit IgG or a

Second Department of Surgery, Osaka University School of Medicine, Osaka, Japan

M Higashiyama
S Doi
N Tomita
T Monden
M Murotani
Y Kawasaki
T Kobayashi
T Shimano
M Ogawa
S Takai
T Mori

Correspondence to: M Higashiyama, Department of Surgery, The Center for Adult Diseases, Osaka. Address: Nakamichi 1-3-3, Higashinari-ku, Osaka, Japan, 537

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Figure 1 Normal human pancreas and salivary gland showing immunoreactivity with monoclonal antibodies 88E8 and 3B7. (A) Pancreatic tissues showing no reactivity with 88E8. (B) Pancreatic tissues showing positive reactivity with 3B7. (C) Salivary tissues showing no reactivity with 3B7. (D) Salivary tissues showing positive reactivity with 88E8.

Immunoreactivity of anti-amylase antibodies

Thyroid cancer histological type	Polyclonal	Immunoreactivity	
		88E8 (salivary type)	3B7 (pancreatic type)
Well differentiated papillary or follicular carcinoma	14/14	12/14	0/14
Poorly differentiated carcinoma	2/2	1/2	0/2
Anaplastic carcinoma	1/3	0/3	0/3
Medullary carcinoma	4/5	0/5	3/5

biotinylated goat anti-mouse IgG, followed by treatment with a streptavidin-horseradish peroxidase conjugate. Peroxidase activity was made visible using the diaminobenzidine/H₂O₂ reaction. Sections were counterstained with haematoxylin.

Results

Results are shown in the table. Twenty one (88%) cases of thyroid cancer expressed varying degrees of immunoreactivity for

polyclonal anti-amylase antiserum. Immunoreactive intensity for amylase was strong in some cases of well differentiated carcinomas, while expression was not detectable in two anaplastic carcinomas and one medullary carcinoma. According to immunohistochemical analyses using monoclonal antibodies, 13 (54%) cases were positive for salivary-type and three (13%) for pancreatic-type amylase. Only medullary carcinomas showed immunoreactivity for pancreatic-type amylase while the other histological types were positive for salivary-type amylase (figs 2 and 3).

Figure 2 Papillary carcinoma showing immunoreactivity for amylase. Positive immunostainings were obtained with polyclonal antiserum (A) and 88E8 (B), but no immunostaining was observed with 3B7 (C).

Figure 3 Medullary carcinoma showing immunoreactivity for amylase. Positive immunostainings were obtained with polyclonal antiserum (A) and 3B7 (C), but no immunostaining was observed with 88E8 (B).

Discussion

It has been shown that papillary carcinoma of the thyroid gland expressed various degrees of amylase activity.^{11 12} In this study we showed that other histological types also frequently produced amylase. Two anaplastic and one medullary carcinoma, however, contained no amylase, whereas almost all well differentiated papillary or follicular carcinomas expressed amylase, some showing particularly strong immunoreactive intensity. It is suspected that amylase expression closely correlates with the degree of tumour differentiation.

Discrepancies between the degree of amylase expression by each antibody, such as 14/14 (100%) by polyclonal antibody compared with 12/14 (86%) by 88E8 monoclonal antibody in well differentiated carcinoma, are explained by the following: monoclonal antibody specific for salivary-type amylase may possess lower sensitivity than polyclonal antiserum which detects both types. In fact, all cases judged positive for salivary-type amylase by polyclonal antibody but negative by monoclonal antibody were faintly stained. The same is equally true of discrepancies in medullary carcinoma: the sensitivity of monoclonal antibody specific for pancreatic-type amylase may also be lower than that of polyclonal antibody.

A few malignant cells in some cases of papillary or follicular carcinoma also showed very weak staining with the antibody 3B7 (data not shown). This may be associated with the specificity or the sensitivity of the antibody. The numbers of these cells were also too small and the intensity too faint to draw definitive conclusions about their true pancreatic-type amylase positivity. The clinical importance of these observations in the cases of papillary or follicular carcinoma is unclear.

Although not all cases of staining in papillary or follicular carcinoma were clear cut, the properties of isoenzymes in thyroid cancer are

of special interest. Only medullary carcinoma showed pancreatic-type immunoreactivity; other types of thyroid cancer showed salivary-type amylase. Medullary carcinoma is now considered to originate from C-cells, while other types are of follicular cell origin.¹⁸ Accordingly, the type of isoenzyme seems to correlate with the cells of origin.

Recently, Tomita *et al.*, using molecular biological analysis, detected a novel type of human amylase in a lung carcinoid, which was encoded by the amy3 gene.¹⁹ Interestingly, both medullary carcinoma of the thyroid gland and lung carcinoid tumour are regarded as neuroendocrine tumours histologically. Medullary carcinoma may therefore express this new amylase rather than pancreatic-type amylase. Unfortunately, immunohistochemical analysis of the expression of amylase isoenzymes in lung carcinoid, using these specific monoclonal antibodies, has not been done. The monoclonal antibody 3B7 against pancreatic-type amylase cross-reacted with this novel amylase isoenzyme while the monoclonal antibody 88E8 against salivary-type amylase did not.^{19,20} An antibody to differentiate the novel amylase isoenzyme encoded by the amy3 gene from pancreatic-type amylase has not been made as yet because the two are so similar.^{19,20} On the other hand, the two different types of amylase-encoding genes, AMY2A and AMY2B, were expressed in normal human pancreas.^{21,22} While the former encodes "original" pancreatic-type amylase, the latter is identical with the amy3 gene.²³ Accordingly, although histological localisation of this novel type of amylase isoenzyme has not been possible, it may be distributed not only in neuroendocrine tumours but also in normal tissues, including neuroendocrine cells or epithelial cells. In fact, Samuelson *et al.* provided evidence that normal liver tissues also contain mRNA of the amy3 gene.²⁴ The distribution of the amy3 gene product needs to be elucidated.

In conclusion, we have shown that most thyroid cancers produced amylase. Moreover, medullary carcinomas were immunoreactive for pancreatic-type amylase whereas other histological types were immunoreactive for salivary-type amylase. To elucidate the type of amylase isoenzyme in thyroid cancer molecular biological analyses are needed in addition to biochemical or immunohistochemical studies, or both. More discriminatory and sensitive

antibodies to amylase isoenzymes, including the amy3 gene product, must also be found.

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