Significance of Thyroglobulin in Thyroid Diseases Detected by the Immunoperoxidase Method

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Abstract : **Thyroglobulin (TG) is a protein that synthesized from the thyroid follicular epithelium and can reflect the thyroid gland in origin together with the cellular activity. The aim of this study is to detect TG qualitatively and semiquantitatively by immunoperoxidase method in various thyroid lesions. The result shows that all of the nodular goitre (43 cases), primary diffuse hyperplasia (18 cases), adenoma 60 cases, papillary carcinoma (43 cases) and follicular carcinoma (22 cases) show cytoplasmic staining of TG in varying pattern and intensity, while only 3 out of the 7 anaplastic carcinoma show irregular cytoplasmic staining.**

These findings can be used in identifying the thyroid gland in origin in the metastatic lesion and the functional status of the lesion.

เรื่องย่อ : **การศึกษารูปแบบของผลการย้อม Thyroglobulin ด้วยวิธี Immunoperoxidase ในโรคต่อมไทรอยด์ชนิดต่าง ๆ**

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Thyroglobulin (TG) เป็นโปรตีนที่สร้างขึ้นโดย follicular cell ของต่อมไทรอยด์ คณะผู้ศึกษาได้ศึกษารูปแบบของการตรวจหา TG ด้วยวิธี immunoperoxidase ในภาวะต่าง ๆ ของต่อมไทรอยด์โดยเฉพาะอย่างยิ่งในเนื้องอกพบว่า Nodular goitre 43 ราย, Primary diffuse hyperplasia 18 ราย, Adenoma 60 ราย, Papillary

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carcinoma 43 ราย และ Follicular carcinoma 22 ราย สามารถตรวจพบ TG ได้ทุกราย ด้วยรูปแบบและความเข้มที่แตกต่างกันในแต่ละโรค ส่วนใน Anaplastic carcinoma 7 ราย สามารถตรวจพบ TG ได้ 3 รายเท่านั้น ผลที่ได้จากการศึกษาสามารถนำไปใช้เพื่อช่วยหาชนิดของมะเร็งที่แพร่กระจายไปอวัยวะอื่นซึ่งไม่ทราบต้นกำเนิดว่ามาจากต่อมไทรอยด์หรือไม่ รวมทั้ง activity ของรอยโรค

INTRODUCTION

Thyroglobulin (TG) is synthesized from the rough endoplasmic reticulum in the cytoplasm of the cuboidal follicular cell of the thyroid gland, then processed and transferred to the apical cell surface and exocytosed to store in the follicular lumen. It is an important protein used in the synthesis of thyroid hormone.[1]

Since most of the thyroid tumours derived from the follicular epithelium still show some organ specific functions,[2] the most important is the ability to trap and organified iodides, and also with the potential to elaborate TG,[3,4,5] the presence and amount of TG detected will reflect the site of formation and functional activity of the thyroid gland respectively. Elevated serum TG levels were found in both untreated differentiated thyroid carcinoma and treated cases with metastases.[4,6] There is also a good correlation between serum TG level and quantity of TG in tissue sections detected by immunohistochemical method.[7,8] Furthermore a high degree of correlation between the radioiodine uptake and TG content of thyroid carcinoma has already been described.[9] The aim of the present study is to apply the immunohistochemical technique to demonstrate TG in paraffin embedded tissue sections in our department and study the content and distribution of TG in various thyroid lesions especially carcinoma of the thyroid gland. Two invaluable diagnostic adjunct to light microscopy that we wish to gain are: 1) as an approach to functional determination of thyroid lesion and 2) as an aid in the differential diagnosis of thyroid carcinomas of follicular cell type from tumours of other origin.

MATERIALS AND METHODS

From the files of the Department of Pathology, Siriraj Hospital, 206 randomly selected formalin fixed, paraffin-embedded thyroid surgical specimens were studied. These comprised of 18 cases of primary diffuse hyperplasia, 43 cases of nodular goitre , 5 cases of chronic thyroiditis, 4 cases of thyroglossal duct, 60 cases of adenoma, 43 cases of papillary carcinoma (14 of which also show lymph node metastases), 22 cases of follicular carcinoma (5 of which also show lymph node metastases), 7 cases of anaplastic carcinoma and 4 cases of medullary carcinoma.

Two micron sections cut from the blocks were deparaffinized, rehydrated and rinsed in methanol. Sections were then treated as followed:

1. Inhibition of endogenous peroxidase activity by 0.5% hydrogen peroxide in methanol.
2. Trypsin treatment by incubation in 0.1% trytpsin (Sigma Chemical Co., U.K.) with 0.1% calcium chloride (adjusted pH to 7.8 with 0.5 N sodium hydroxide) at 37°c. Each case had 5 sections for various durations of trypsin treatment : 5, 15, 30, 45 and 60 minutes. Another section is not subjected to trypsin treatment
3. Indirect immunoperoxidase method. Sections were then incubated with normal swine serum diluted 1:5 with Tris bufferred saline (TBS) for 10 minutes, follwed by rabbit antithyroglobulin (Dako, Denmark) 1:50 dilution in TBS for 30 minutes, washed in TBS followed by peroxidase-conjugated swine antirabbit immunoglobulins (Dako, Denmark) diluted 1:20 with TBS for 30 minutes. After washing, sections were incubated with a freshly prepared solution of 0.08% 3,3 diaminobenzidine tetrahydrochloride (Sigma Chemical Co., U.K.) in 0.02% hydrogen peroxide in TBS for 5-10 minutes, lightly counterstained with Mayer's hematoxylin, dehydrated, cleared and mounted in DPX.

Control of the specificity of antithyroglobulin staining

Control experiments performed by sequential omission of the different reagents showed that peroxidase-positivity was specific for TG.

Sections from cases of non-thyroid lesion namely : fibroadenoma of the breast (1 case), nodular hyperplasia of the prostate gland (1 case), intradermal nevus (1 case) and pleomorphic adenoma of the parotid salivary gland (2 cases) and carcinoma of other organs including squamous cell carcinoma of the skin (1 case), adenocarcinoma of the colon (2 cases) and hepatocellular carcinoma of the liver (3 cases) also immunohistochemically stained as mentioned above for TG.

RESULTS

Before details of the immunohistochemical findings of various thyroid diseases are discussed, general findings of the technique used and a brief mention of the immunoreactivity observed in the normal thyroid and other non-thyroid tissues studied will be given first.

Effect of trypsin treatment

In general trypsin digestion is necessary for the demonstration of TG in this study. From the result of trypsin treatment in each case, we conclude that to demonstrate TG in follicular epithelium need longer trypsin treatment than in the colloid.

The colloid usually shows intense positivity for TG in the sections without trypsin treatment or in the sections with 5 minutes digestion. The intensity gradually decreases and disappears with longer duration of digestion.

In the cytoplasm of the follicular epithelium, TG can be demonstrated in the sections without trypsin treatment but the intensity is low and the distribution is irregular. Thirty minutes of trypsin digestion usually gives intense and regular positivity. With longer duration of digestion, the positivity again decreases and evidence of tissue damage appears.

Normal thyroid tissue

Most of the follicles are composed of cuboidal cells which show intense positive staining for TG. Colloid also give lesser degree of positvity. The nuclei, interstitium and blood vessels are consistently negative.

Other non-thyroid tissue

TG has not been demonstrated in any cases of tissue from the breast, prostate gland, skin, parotid salivary gland, colon and liver.

Various thyroid diseases

The results obtained with the antithyroglobulin serum using the indirect immuno-peroxidase method on formalin-fixed, paraffin embedded various thyroid diseases are summarized in the table.

Microscopic diagnosis	Total cases	Positive for TG					
		Cases	%	Intensity			
				4+	3+	2+	1+
Nodular goitre	43	43	100	14	21	8	
Primary diffuse hyperplasia	18	18	100	6	9	3	
Chronic thyroiditis	5	5	100	Inconsistent			
Thyroglossal duct cyst	4	-	0				
Adenoma	60	60	100	34	16	10	
Papillary carcinoma	43	43	100	4	22	17	
Follicular carcinoma	22	22	100	7	14	1	
Anaplastic carcinoma	7	3	43		3		
Medullary carcinoma	4	-	0				

Nodular goitre :

Positivity of TG was noted within the cytoplasm of the follicular epithelial cells and also in the colloid. Although the positivity was noted in most of the follicular epithelium, only about 32% of the cases showed very strong positivity. The distribution of the positive cell was quite irregular, the tall columnar cell usually showed more intensity than the low cuboidal or flattened cells (Figure 1A, B).

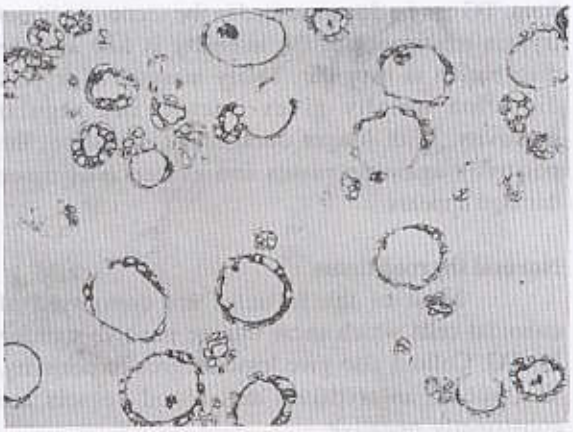


Figure 1A. Showing diffuse cytoplasmic staining of antithyroglobulin antibody (TG) with varying intensity in a case of nodular goitre. TGx100

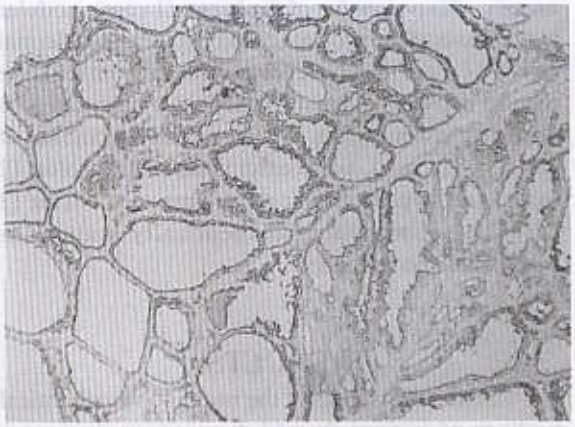


Figure 1B. Higher power showing more intensity in the cuboidal cells than in the flattened cells. TGx200

Primary diffuse hyperplasia :

TG was found in the cytoplasm of the follicular epithelium and in the colloid (Figure 2A). Distribution of the TG positive cell is rather homogenous and most prominent in the tall columnar follicular cells (Figure 2B). About 33% showed very strong positivity while most of the rest showed only slightly less strong positivity.

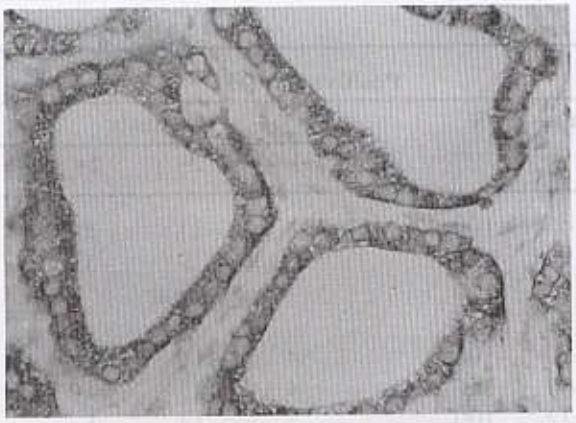


Figure 2A. Showing diffuse strong cytoplasmic staining in a case of primary diffuse hyperplasia. TGx200

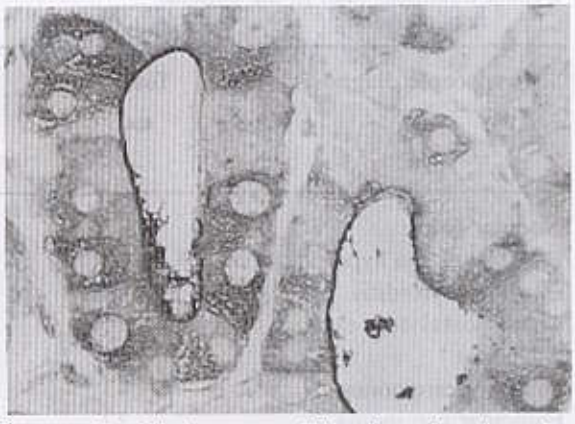


Figure 2B. Higher magnification showing the staining pattern. TGx450

Chronic Thyroiditis :

TG was demonstrated in the undamaged follicular epithelial cytoplasm and colloid but the distribution was irregular and the intensity varied.

Thyroglossal duct cyst :

The squamous and columnar epithelium found in this lesion were negative for TG while the surrounding thyroid follicles show normal pattern of positivity for TG within the follicular epithelial cytoplasm and in the colloid.

Adenoma :

TG was demonstrated in both follicular epithelium and in the colloid. (Figure 3A) The distribution of the positive cells were quite homogeneous (Figure 3B) and 57% showed intense positivity. When normal surrounding thyroid tissue was included in the section, about half of the cases show comparable intensity of TG staining while the remaining showed slightly weaker intensity than in the adenoma.

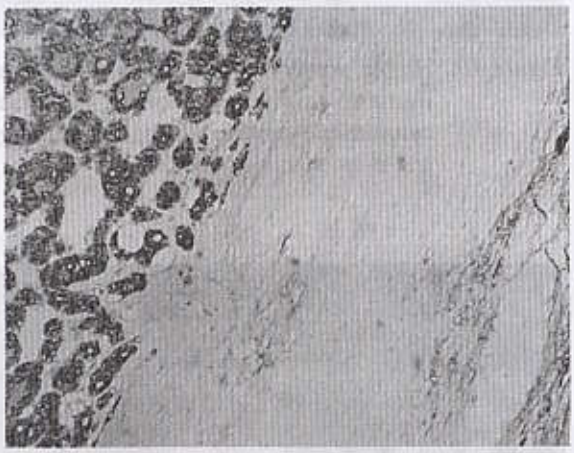


Figure 3A. Showing diffuse cytoplasmic staining in a case of adenoma (left side). TGx100

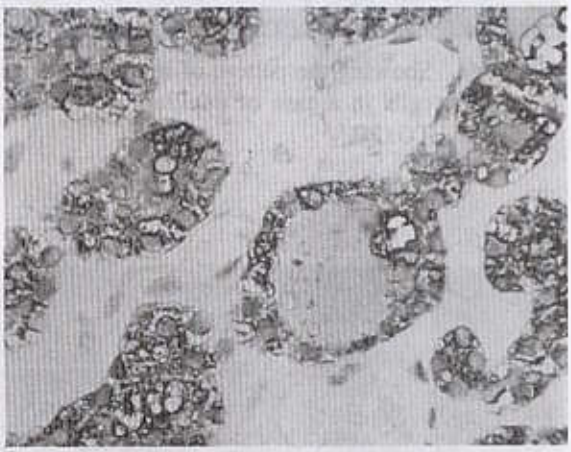


Figure 3B. Higher power, showing intense cytoplasmic staining. TGx450

Papillary carcinoma :

Three immunohistochemically different cell types were detected, often intermingled with each other (Figure 4A and B).

a. tumour cells devoid of TG

b. tumour cells with focal TG synthesis, often in the apical cytoplasm.

c. tumour cells with an intense TG staining throughout the cytoplasm.

The interpapillary spaces usually showed no staining of TG.

The follicular structures of papillary carcinomas showed identical TG content to those will be described for the follicular carcinomas. The metastatic lesion in 14 lymph nodes also showed staining of TG identical to the primary sites. The well formed papillary structure showed more positivity than the poorly formed papillary structure or solid sheet of the tumour cells. Overall, about 9.0% showed very strong positivity.

Follicular carcinoma :

TG could be demonstrated in the cytoplasm of the tumour cells in quite homogenous fashion with rather strong intensity. (Figure 5B) However, small islands of TG negative cells could be seen patchily in the immediate neighbourhood of the larger area of the TG positive cells. (Figure 5A) Overall, about 32% showed very strong positivity. There was usually no staining of TG in the follicular lumina. The metastatic lesion in 5 lymph nodes also showed staining of TG identical to the primary sites.

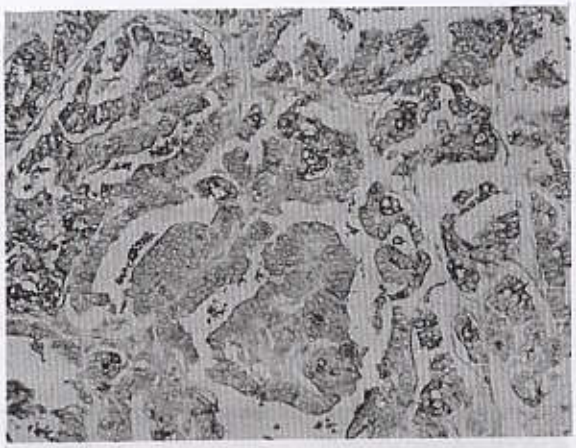


Figure 4A. Showing the pattern of immunoreactive cells in a case of papillary carcinoma. TGx200

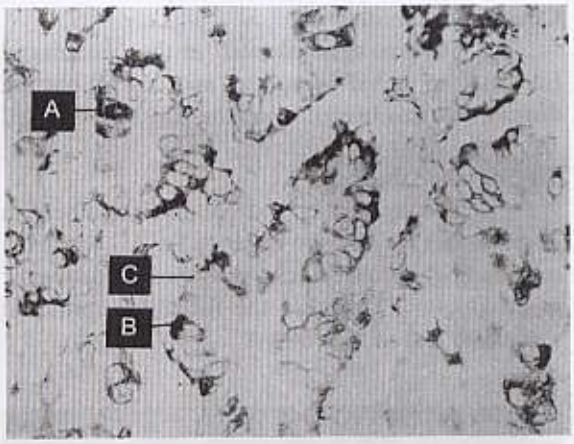


Figure 4B. Higher power showing three distinctive immunostaining pattern : a- diffuse cytoplasmic staining, b- apical staining and c- no staining. TGx450

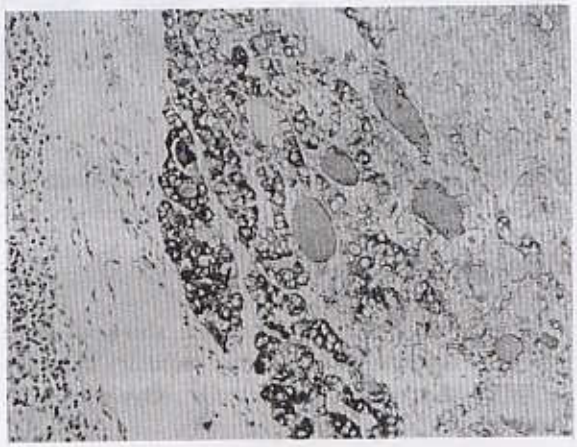


Figure 5A. Showing group of immunoreactive cells nearby an area of TG negative cells in a lymph node with metastatic follicular carcinoma from the thyroid gland. TG x35

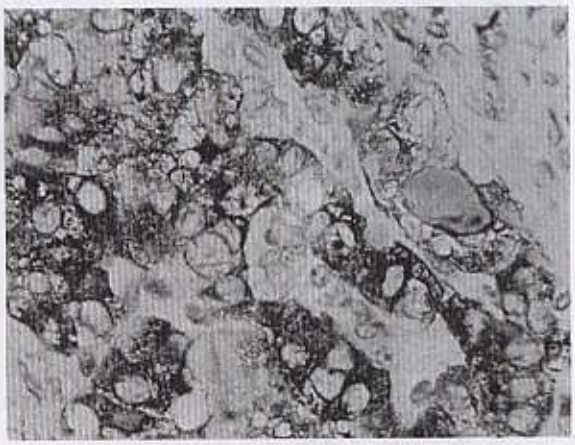


Figure 5B. Higher power showing diffuse cytoplasmic staining with strong intensity. TGx450

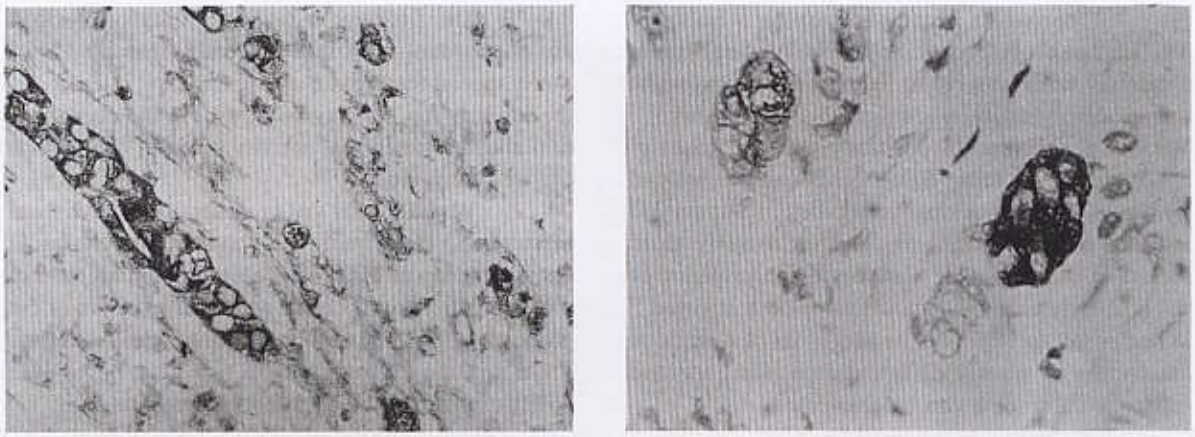


Figure 6A.,B. Showing variable intensity of immunoreactive cells in a case of anaplastic carcinoma. Immunoreactive cells can be detected in single cell or poorly-formed thyroid follicle. TGx450

Anaplastic carcinoma :

TG could be demonstrated in only a few individual tumour cells or in some poorly formed thyroid follicle. Only three out of seven cases showed TG positive cells and the intensity varied (Figure 6).

Medullary carcinoma :

All showed no staining for TG, but intense positivity of calcitonin could be demonstrated in the cytoplasm of the tumour cells.

DISCUSSION

Immunocytochemistry, a technique of identifying a tissue constituent in situ by means of a specific antigen-antidody reaction tagged by a visible label, has been applied in many fields of pathology. The applications are expanding. Several cellular and tumour markers are developed[10] and many are now commercially available. Immunoperoxidase is one of the basic method in this field which has many advantages.[10,11] It can be applied on formalin-fixed, paraffin-embedded sections. This makes it possible to examine stored materials or when fresh tissue are not available. The end result is a permanent colour product that can be visualized by an ordinary light microscope.

The immunocytochemical localization of immunoreactive TG in our study can be considered specific, as the immunoreaction was invariably negative in technical and negative controls and positive in normal thyroid parenchyma.

Detection of TG in tumour tissues indicates that they retain at least a partial function in hormone biosynthesis.[3] We can detect TG immunohistochemically in all cases of papillary and follicular carcinoma. These findings confirm the work of others whose also show very high percentage of positivity in these two carcinoma.[5] In general, the tumours with follicular differentiation contained more immunoreactive TG than papillary carcinomas, and the former showed a more uniform staining pattern than the latter. Like Leche AR et al,[7] we also found a high degree of correlation between the amount and the intensity of TG immunoreactivity and the grade of tumour differentiation. Some special pattern of staining in papillary carcinoma i.e. the apical staining and the non-homogeneous positivity may be helpful for diagnosis in the poorly differentiated cases. Furthermore, the pattern of immunoreactivity of TG in metastases of thyroid tumour is very similar to the pattern of the primary site, determination of primary tumour from thyroid gland in the metastatic lesion can be done with certainty.

The results obtained from the staining of TG in anaplastic carcinoma is inconclusive. Negative result was found in some[5,12,13,14] and some degree of positivity was reported by the other.[15,16] We can demonstrate 3 positivity out of 7 cases of anaplastic carcinoma. Better result can be obtained by the use of monoclonal antibodies of human TG instead of polyclonal antibodies usually used including in our work.[17] Caution in interpretation of the positivity in these tumours have been stressed.[12] When correctly examine the diagnosis of anaplastic carcinoma or poorly differentiated papillary and follicular carcinoma can be elucidated.

The findings in all benign thyroid lesions are concordant with those already described.[5,15-17] Some special features i.e. the pattern and intensity of the staining for TG may be helpful in the classification and the functional activity basis of the lesions can be determined.

We concluded that in addition to conventional histologic examination, immunocytochemical demonstration of TG is a reliable and valuable aid in the diagnosis, classification and determination of the grade of differentiation of malignant thyroid tumours. From this, the pathologists can provide a pathologic basis for postoperative patient management.

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