Papillary Thyroid Neoplasms in the 4th Edition WHO Classification of Tumors of Endocrine Organs: Neoplasms with Papillary Thyroid Carcinoma-type Nuclear Features

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Abstract

The introduction of borderline tumors into thyroid tumor classification significantly impacts pathology practice. As encapsulated noninvasive follicular pattern tumors with papillary thyroid carcinoma (PTC)-type nuclear features (PTC-N) were downgraded from carcinoma (noninvasive encapsulated follicular variant PTC) to borderline tumors (well-differentiated tumor of uncertain malignant potential (WDT-UMP) or noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP)), and PTC-N no longer indicates malignancy. They are now included in borderline tumors in the recent 4th edition WHO classification. Risk stratification of thyroid carcinomas for both PTCs and follicular thyroid carcinomas is one of the most important elements in the diagnosis of thyroid tumors, which aims to reduce overdiagnosis and overtreatment of thyroid carcinomas. Identification of aggressive variants, the mutation status and proliferation index helps to identify high-risk cancers. Methods to identify aggressive variants of PTC and high-risk thyroid carcinoma are highlighted in this review.

Key Words: Thyroid neoplasms, papillary carcinoma, borderline tumors, thyroid gland, risk classification, pathology, FNA cytology

Papillary thyroid carcinoma (PTC)

PTC is the predominant histological type of thyroid malignancy worldwide regardless of iodine sufficiency. PTC comprises 80% to 90% of thyroid malignancies in China and Taiwan, and more than 90% in Japan and Korea (1-3). The majority of PTC is indolent carcinoma, and the relative survival rate is almost 100% at 5 years after curative surgery according to several reports from the USA (4-5). Furthermore, the cancer recurrence rate in Japanese T1N0M0 PTC patients was approximately 3% at 10 years (6). In a review of reports published from Taiwan, the 5-year survival rate was 98.2% by Lin *et al*. (7) and cancer death occurred in only 0.8% (9/1123) of patients with low-risk PTC according to Huang *et al*. (8). Even in advanced stage patients with PTC, Lin *et al*. reported the cancer mortality rate to be 19.0% (9).

Epidemiologic studies have demonstrated an increasing incidence of thyroid cancer worldwide over the last three decades, mainly due to the increased incidence of PTC (1, 10). Taiwan is no exception. Liu *et al*. found that the incidence of thyroid cancer increased 2.2-fold from 1997 to 2012 (7). Improvements in the detection of thyroid cancer, such as cancer screening and introduction of ultrasound techniques, are considered to be the main reasons for this. However, the increase in incidence of thyroid cancer significantly varies among countries from a sharp rise in Korea, a significant increase in Australia, USA, Italy and France, to relatively modest increases in Nordic countries, the United Kingdom and Japan (1). Rapid increases in PTC incidence have been reported globally, but the rates vary among countries. Therefore, there is no one common factor for the increase in incidence.

Definition of PTC

The definition of PTC in the new WHO edition was modified significantly. It is "a malignant epithelial tumor exhibiting evidence of follicular cell differentiation and a set of distinctive nuclear features. It requires papillae, invasion or cytological features of PTC" (2). In cases without papillae or invasion, PTC-type nuclear features (PTC-N) are required for cancer diagnosis. The PTC-N are divided into 3 morphological features according to Nikiforov *et al*. (2, 11). They are: 1) changes in nuclear size and shape, 2) irregularity of the nuclear membrane, and 3) chromatin characteristics. Nuclear irregularity, overlapping, grooves and inclusions in conventional PTC are shown in Figure 1. I consider these nuclear features to be fully developed nuclear features of conventional PTC or nuclear features of BRAF-like tumors. However, PTC-N as defined in the WHO Classification is somewhat different from that in Figure 1. Please refer to the illustrations in the WHO classification (Figure 2.23) (2) or nuclear score guide provided in Nikiforov's seminal paper on NIFTP
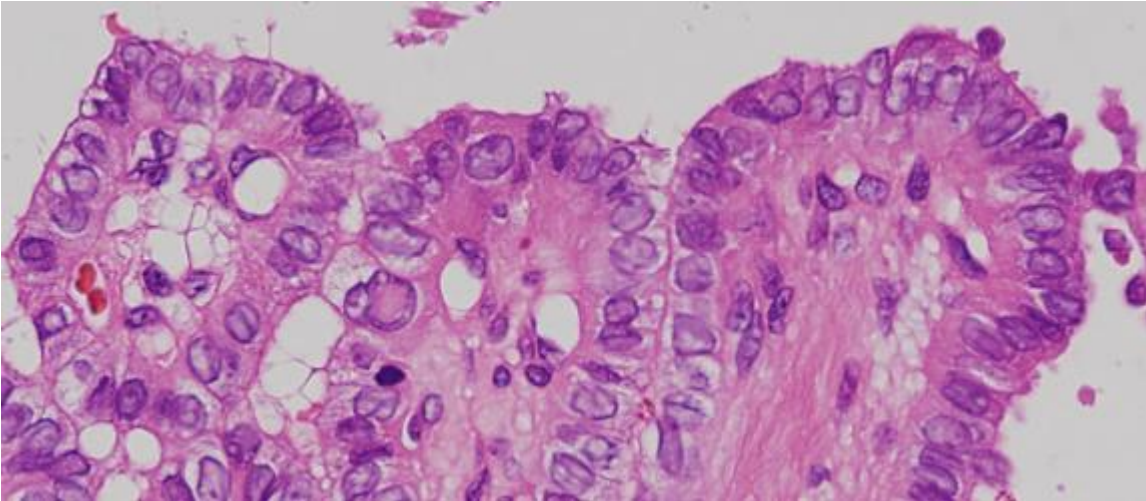
Fig. 1. Papillary thyroid carcinoma-type nuclear features in a conventional-type PTC. Nuclear size enlargement, irregular nuclear contour, including elongation, grooves and inclusion, and pale chromatin pattern are clearly observed. (HE stain, x40)
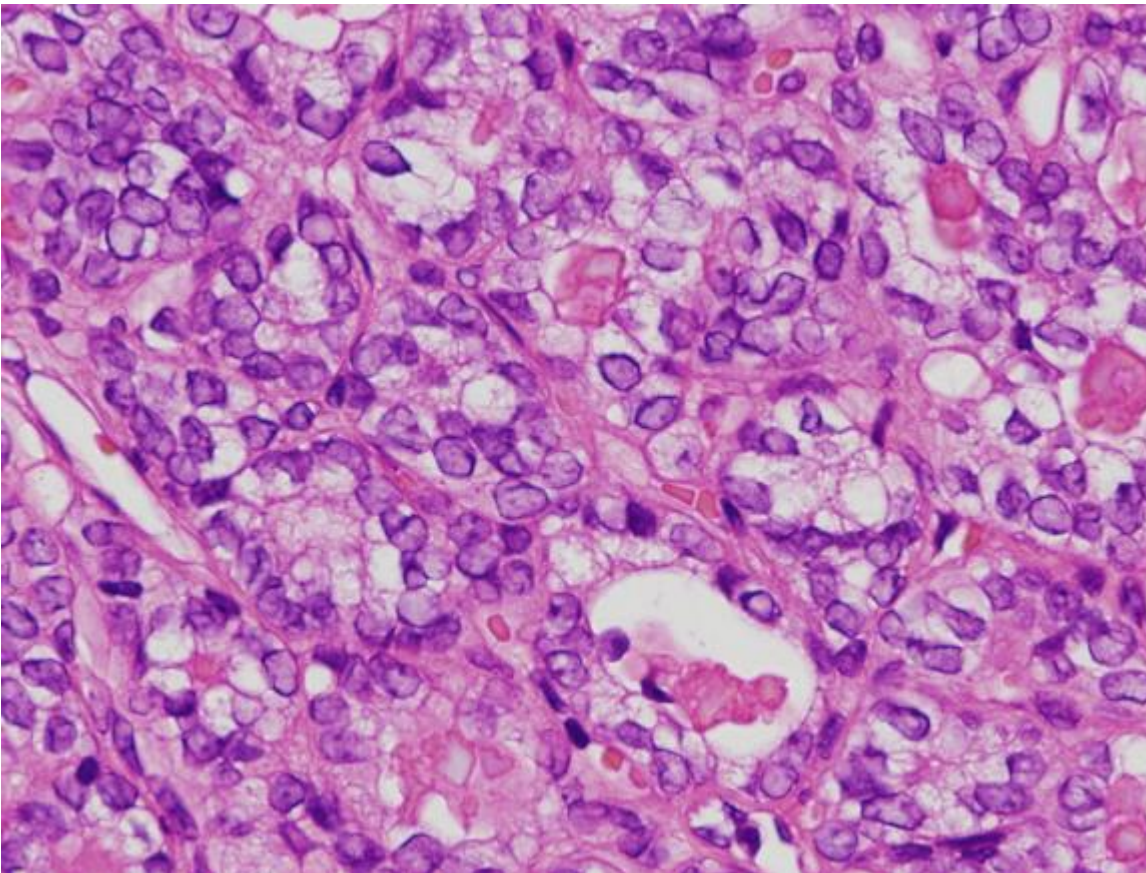

Fig. 2. Nuclear features in noninvasive encapsulated follicular thyroid neoplasm with papillary-like nuclear features (NIFTP). Mild nuclear enlargement, moderate nuclear irregularity and pale chromatin are seen in follicular pattern tumors. Only a few delicate grooves and no nuclear cytoplasmic inclusion were found. (HE stain, x40)

(http://jamanetwork.com/journals/jamaoncology/fullarticle/251325 0) (11). An example of nuclear score 3 in NIFTP (non-invasive encapsulated follicular variant PTC (FVPTC)) is shown in Figure 2, which includes mild nuclear enlargement, nuclear contour irregularity, delicate grooves and chromatin clearing (Figure 2), different from the fully developed nuclear changes in classic PTC shown in Figure 1. However, both are called PTC-N in Western practice, whereas most Asian pathologists accept only fully developed (well-developed) nuclear features as PTC-N (12-16). The diagnostic criteria for nuclear features of malignant PTC were found to be different among pathologists despite being described in the WHO classification. This is one of the possible contributing factors for the recent worldwide increase in thyroid cancer incidence and the varied rates among countries. The diagnostic threshold for PTC-type malignancy is lower in Western practice in particular. This has been reported by many pathologists (12, 13, 17-25) since 2000, but was not addressed until non-invasive encapsulated FVPTC became reclassified from the malignant category to borderline tumor (NIFTP) by Nikiforov *et al*. (11). This change has yet to be appreciated by pathologists. This NIFTP reclassification from cancer to borderline tumor means that so-called PTC-N in Western practice has been divided into 2 separate lineages, nuclear features of conventional PTC (malignant) and dysplastic nuclear features seen in NIFTP or WDT-UMP (not malignant). In a study by the Cancer Genome Atlas Research Network, PTC was divided into BRAF-like tumors and RAS-like tumors, and PTCs with RAS mutations were mostly FVPTCs (26) as confirmed in many other studies (27-33). As a result, these nuclear changes of thyroid tumors parallel each genetic change, with well-developed nuclear features in BRAF-mutated conventional PTC, and delicate nuclear changes in RAS-mutated FVPTCs, FA and borderline tumors.

Trend of follicular cell-derived thyroid neoplasms in the USA (Figure 3)

Due to the lax criteria for PTC-N, which included nuclear change in RAS-like tumors, in the USA, significant numbers of follicular adenomas (FAs) were classified as non-invasive encapsulated FVPTC in recent American pathology practice (Figure 3). This may have been due to the popularity of fine needle aspiration in thyroid practice, and PTC-N was accepted as diagnostic for PTC in the early 1970s. This trend was further enhanced by the introduction of FVPTC by Chen and Rosai in 1977 (34). Ohori and Mehrzad *et al*. suggested that some tumors previously diagnosed as follicular adenomas are often classified as FVPTC in the USA, particularly as noninvasive encapsulated FVPTC (20, 21) (Figure 3). The introduction of borderline tumors was intended to revise them from carcinoma (non-invasive encapsulated FVPTC) to the original benign category (NIFTP, WDT-UMP or FA). Although there is a significant number of cases that cannot be confidently classified as either benign or malignant, NIFTP comprised less than 5% of malignancies in most Asian practices (14, 15, 35). When a higher rate (more than 10%) of borderline tumors on histology is noted, the criteria for risk stratification of indeterminate thyroid nodules for surgery may be lax and should be improved to minimize it to less than 5%. Consequently, the ratio of borderline tumors among all thyroid malignancies or all PTC can be used as a quality measure. The average rate of NIFTP (noninvasive encapsulated FVPTC) in the USA was reported to be 17% by Faquin and Wells, and 18.6% based on data from 4 Italian and American institutions by Nikiforov *et al*. in 2016 (11, 36). The author of this review

believes that it can be reduced to less than 5% when proper risk stratification for surgery is applied to the indeterminate nodules and diagnostic surgery is limited to patients with suspicious ultrasound features or molecular profiles.

The threshold of PTC-N is key to establish diagnostic criteria for PTC

The diagnostic threshold for PTC-N varies significantly among pathologists (12, 13, 17-23, 37). The distinction between PTC and FTC using PTC-N may be not clinically important because both are malignant tumors. However, it is paramount when no other evidence of malignancy is found. When few follicles are found in the peripheral sinus of the cervical lymph node, what is your interpretation? Whether it is metastasis (malignant) or ectopic thyroid tissue (benign) relies solely on the subjective judgement of nuclear features. Figure 4 is one such example. This patient had multiple PMCs in the thyroid and multiple lymph node metastases in the lymph nodes. As there was mild nuclear enlargement, the nuclear shape irregularity and glassy chromatin in a few nuclei indicated that these follicles were metastatic PTC in a follicular growth pattern (Figure 4). This is an essential step to establish diagnostic criteria for PTC-N for malignancy, which is important for diagnosing PTC properly and downgrading NIFTP to the borderline category. Please note that the diagnostic threshold for PTC-N varies significantly among pathologists, and it is stricter in Asian practice (only BRAF-like tumors) and wider (both BRAF- and RAS-like tumors) in Western practice.

Growth pattern of PTC

In the diagnostic criteria for PTC, papillae, invasion or cytological features of PTC are required according to the recent WHO classification. The presence of papillae usually helps in making a confident diagnosis of PTC. Both patterns, follicular and papillary, are often seen in PTC in varying proportions. The papillae are composed of a central fibro-vascular stalk covered by a neoplastic epithelial lining (Figure 5A). True papillae diagnostic for PTC should be covered with neoplastic follicular cells with PTC-N (Figure 5A). When PTC-N is absent or questionable, it is likely a hyperplastic nodule with papillary hyperplasia (Figure 5B). Please compare the nuclear features between Figure 5A (true papillae diagnostic for malignancy) and Figure 5B (hyperplastic papillary growth in benign adenomatous nodule). Again, establishment of diagnostic criteria for PTC-N for malignancy is essential for good pathology practice.

Invasion as an indication of malignancy

The new WHO classification defined PTC as a malignant epithelial tumor that is usually established by identification of the invasive nature. Invasion is classified into 1) capsular invasion, 2) lymphatic invasion, 3) vascular invasion and 4) extra-thyroid invasion (Figure 6). Invasion is a prognostic factor of all histological types of thyroid carcinoma that indicates a high probability of recurrence and poor prognosis (2, 38). Figure 6 is an example of extrathyroid invasion. A locally invasive PTC exhibited invasive growth into the muscle tissue (Figure 6). Lymphatic invasion (Figure 7) and vascular invasion (please refer to Figure 9 in reference 39) are definitive for malignancy.

Aggressive variants of PTC

The majority of PTC follows an indolent clinical course and is associated with very low mortality. Risk stratification using
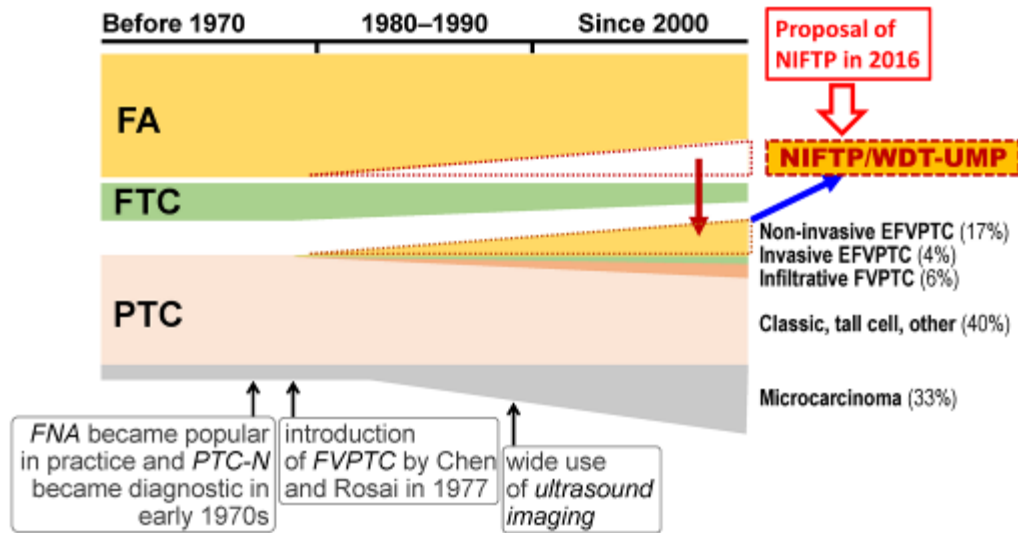
Fig. 3. Trend of follicular cell-derived thyroid neoplasms in the USA. Epidemiologic studies have reported an increasing incidence of thyroid cancer worldwide over the last three decades, mainly due to the increased incidence of PTC. Some tumors previously diagnosed as follicular adenomas are increasingly classified as FVPTC, particularly as noninvasive EFVPTC in the USA (red arrow). This trend was likely enhanced by the popularity of fine needle aspiration cytology in the 1970s, the introduction of FVPTC by Chen and Rosai in 1977 and the development of ultrasound image diagnosis. Information on the incidence of papillary thyroid carcinoma variants in the USA was adopted from Faquin and Wells (ref. 36). The introduction of borderline tumors (NIFTP) was intended to reverse this trend (blue arrow) from carcinoma (EFVPTC) to the original benign (non-malignant or biologically indolent) category (NIFTP or WDT-UMP), and to reduce overdiagnosis and overtreatment of indolent thyroid tumors. PTC: papillary thyroid carcinoma; FVPTC: follicular variant PTC; EFVPTC: encapsulated FVPTC; NIFTP: noninvasive follicular thyroid neoplasm with papillary-like nuclear features; WDT-UMP: well differentiated tumor of uncertain malignant potential.
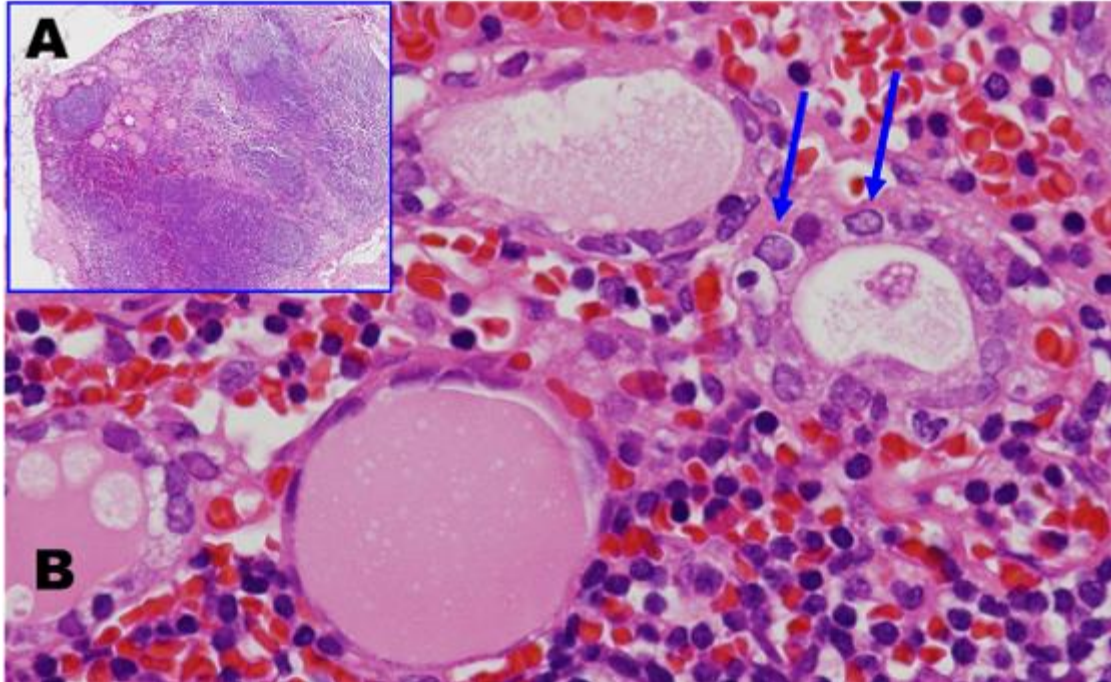

Fig. 4. Lymph node metastasis of a follicular pattern PTC. Follicles are observed in the peripheral sinus of a cervical lymph node (A: HE stain, x4). These follicles are composed of low cuboidal epithelial cells with colloid production (B: HE stain, x40). These nuclei are slightly enlarged and have irregular nuclear contours with few grooves. Nuclear chromatin is rather densely stained with hematoxylin and few nuclei (two blue arrows) have a clear chromatin pattern (B). No true papillae, psammoma bodies or nuclear cytoplasmic inclusions were found.
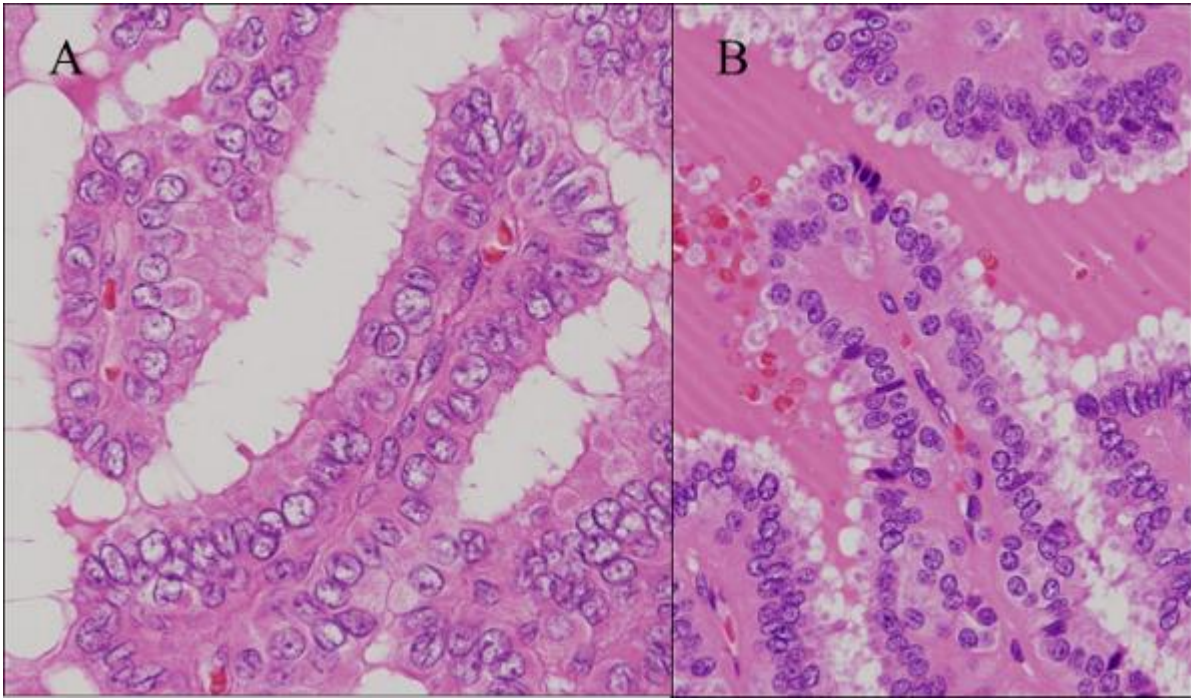
Fig. 5. True papillae (A) seen in PTC and papillary structures in a hyperplastic adenomatous nodule (B). Note true papillae are composed of a central fibro-vascular stalk covered by a neoplastic epithelial lining (A). Note the absence of PTC-N in B. (HE stain, x40)
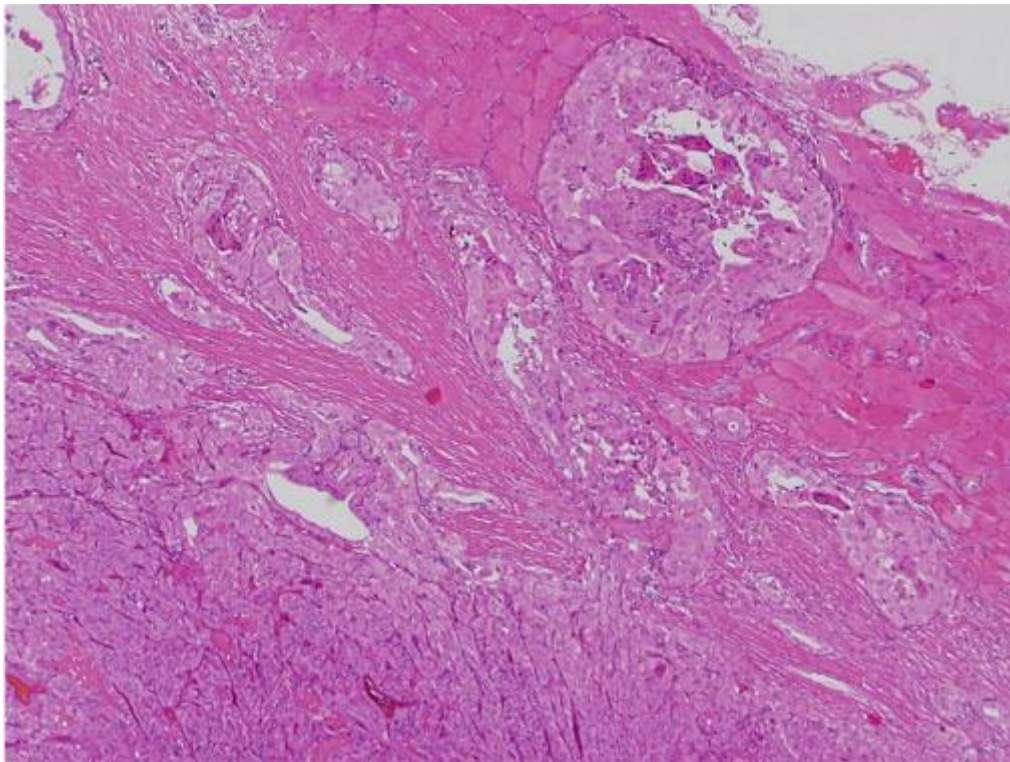

Fig. 6. Extrathyroid invasion into muscle tissue by PTC. (HE stain, x4)

Table 1. Histological types of papillary thyroid carcinoma in the 4th edition WHO classification (ref. 2)

1.	Papillary Microcarcinoma (less than 1 cm)
2.	Encapsulated variant
3.	Follicular variant
4.	Diffuse sclerosing variant
5.	Tall cell variant
6.	Columnar cell variant
7.	Cribriform-morular variant
8.	Hobnail variant (new variant in the 4th edition)
9.	PTC with fibromatosis/fasciitis-like stroma
10.	Solid/trabecular variant
11.	Oncocytic variant
12.	Spindle cell variant
13.	Clear cell variant
14.	Warthin-like variant

Note that variant numbers 4, 5, 6, 8 and 10 are so-called aggressive variants.

multiple clinical and pathological characteristics has become the standard of care for appropriate patient management. In the 2015 ATA clinical guidelines for management of thyroid nodules, thyroid follicular cell carcinomas are risk stratified for structural disease recurrence (38). Some histological parameters, such as aggressive histology, extrathyroid extension, lymph node metastasis and incomplete resection, are indicative for high risk of structural disease recurrence, and among them, aggressive histology can only be judged by pathologists. There are 14 variants of PTC and the hobnail variant was newly incorporated in the 4th edition WHO classification (Table 1). Variants 4 (diffuse scleroses), 5 (tall cell), 6 (columnar cell), 8 (hobnail) and 10 (solid/trabecular) in the Table 1 belong to the so-called aggressive variants and were reported to be biologically aggressive (2). Therefore, identification of these variants is clinically important for multidisciplinary discussion to decide further postoperative clinical management of the patient.

Diffuse sclerosing variant

The diffuse sclerosing variant of PTC is characterized by extensive lymphatic invasion (Figure 7). It occurs more commonly in young women in the second or third decade of life (2, 40, 41). Histologically, it is characterized by dense sclerosis, numerous psammoma bodies and background of chronic Hashimoto-type thyroiditis (Figure 7). The diffuse sclerosing variant is associated with a higher incidence of extrathyroid invasion, lymph node metastasis and distant metastasis. The 10-year disease-specific survival was reported to be 93% (41).

Hobnail variant

The hobnail variant is defined as >30% of cells having hobnail features (Figure 8A). Recurrence and metastasis to lymph nodes and distant organs are frequent. Histologically, complex papillary and micropapillary structures, which are covered with follicular cells containing apically located nuclei that have lost cellular cohesion, are observed (2, 42). Our group reported the loss of cellular cohesion, loss of cellular polarity (apically located nuclei) and micropapillary/hobnail histological features in PTCs as loss of cellular polarity/loss of cellular cohesion (LOP/LCC) (43-47). Minor LOP/LCC is often found at the invasive front of PTCs and we first reported that these features were associated with a less

favorable prognosis (43-47). As >30% of hobnail features are required by Asioli *et al*., it was reported as a rare variant (42). However, the minor component of LOP/LCC (hobnail) is not rare in PTCs and is often observed at the invasive front (>10% of invasive PTC) (46, 47) and in most diffuse sclerosing variant PTC. Figure 8 is an example of hobnail variant PTC (Figure 8A). It is characterized by varied nuclear positioning (so-called pseudo-stratification of nuclei) in tumor cells, but it is different from the columnar cell variant due to the loss of cellular cohesion (Please refer to columnar cell variant section below). As it is often found at the invasive front, we speculate that this morphological change can be an indicator for high risk of local invasion and local recurrence, which may be based on the epithelial-mesenchymal transition (46, 47). The Ki67 labeling index is usually high in hobnail variant PTCs (Figure 8B). The WHO classification recommended to classify it as an aggressive variant of PTC from a prognostic point of view.

Tall cell variant

The tall cell variant of PTC is composed of cells that are two-to three-times as tall as they are wide (Figure 9) (48). The tall cell variant is a common histological feature seen in conventional PTC, and must comprise more than 30% of all tumor cells to be an aggressive variant (2, 48). Some cells have oxyphilic cytoplasm (Figure 9A) and the others have non-oxyphilic cytoplasm (Figure 9B). Ito *et al*. from Kuma Hospital in Japan compared conventional PTCs and tall cell variant PTCs for cancer recurrence and cancer death in more than 1000 patients. The tall cell variant was independently correlated with a poorer disease-free survival rate and disease-specific survival rate by multivariate analysis (49).

Columnar cell variant

The columnar cell variant of PTC is a kind of tall cell variant. This variant is characterized by prominent nuclear pseudo-stratification and nuclear features that are usually not PTC-type (Figure 10A). It is a rare variant of PTC and is positive for CDX2, a putative feature of intestinal-type differentiation (50). Therefore, metastatic carcinoma from other sites must be ruled out, particularly from the alimentary tract. Columnar cell variant PTCs are usually aggressive when invasive (2, 50). The columnar cell variants usually have a high proliferation index (Figure 11B).

Solid/trabecular variant (Sakamoto-type poorly differentiated carcinoma)

Poorly differentiated carcinoma (PDC) was defined as follicular-cell neoplasms exhibiting limited structural follicular cell differentiation occupying an intermediate position morphologically and behaviorally between differentiated (follicular and papillary carcinomas) and undifferentiated carcinoma (2). The histopathological diagnostic criteria for PDC were newly listed in the Turin consensus proposal (51). In the Turin proposal, solid/trabecular carcinoma with PTC-N was excluded from PDC; therefore, solid/trabecular carcinoma with PTC features (Sakamoto-type PDC) was converted from PDC to an aggressive variant PTC in the 4th edition WHO classification. Figure 11 is an example of solid/trabecular variant PTC (Figure 11). In the previous edition WHO blue book (52), it was classified as PDC, Sakamoto-type (53). It was excluded from PDC, and was classified as solid/trabecular (aggressive) variant PTC in the 4th edition WHO classification because its prognosis in adult patients was better than that of PDC, with a 10-year disease-specific survival rate of approximately 90% (2, 54).
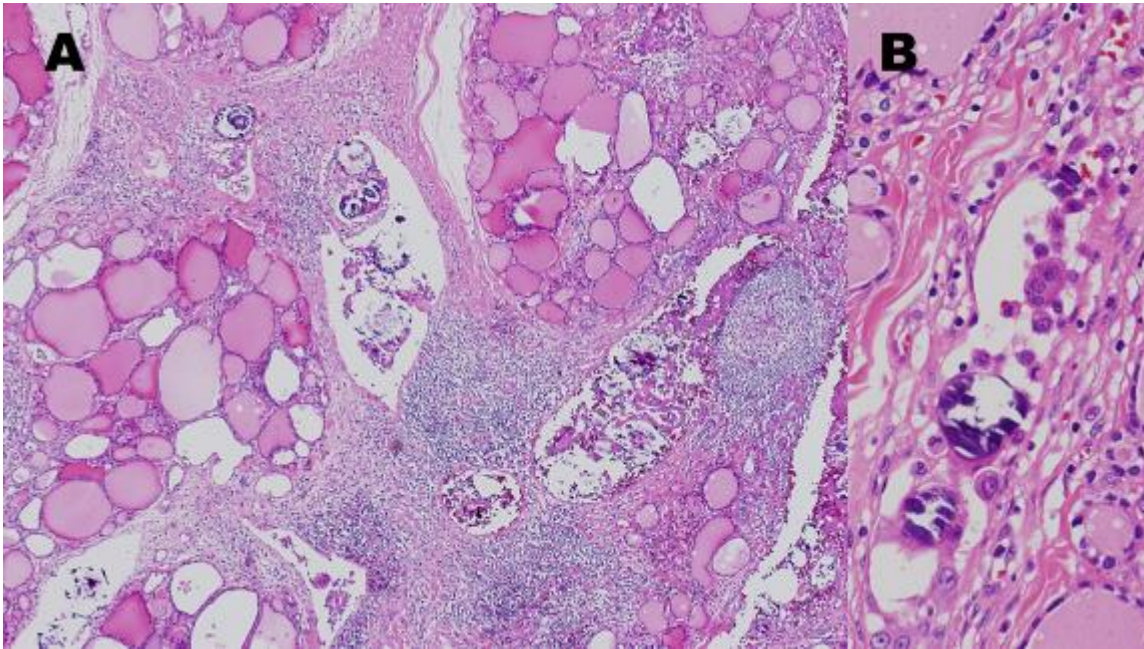
Fig. 7. Lymphatic invasion in diffuse sclerosing variant PTC. Multiple nests of tumor cells with psammoma bodies are seen in the background of chronic thyroiditis (A: HE stain, x4). Invasive growth in a micropapillary/hobnail pattern and psammoma bodies are found within a lymphatic channel (B: HE stain, x20).
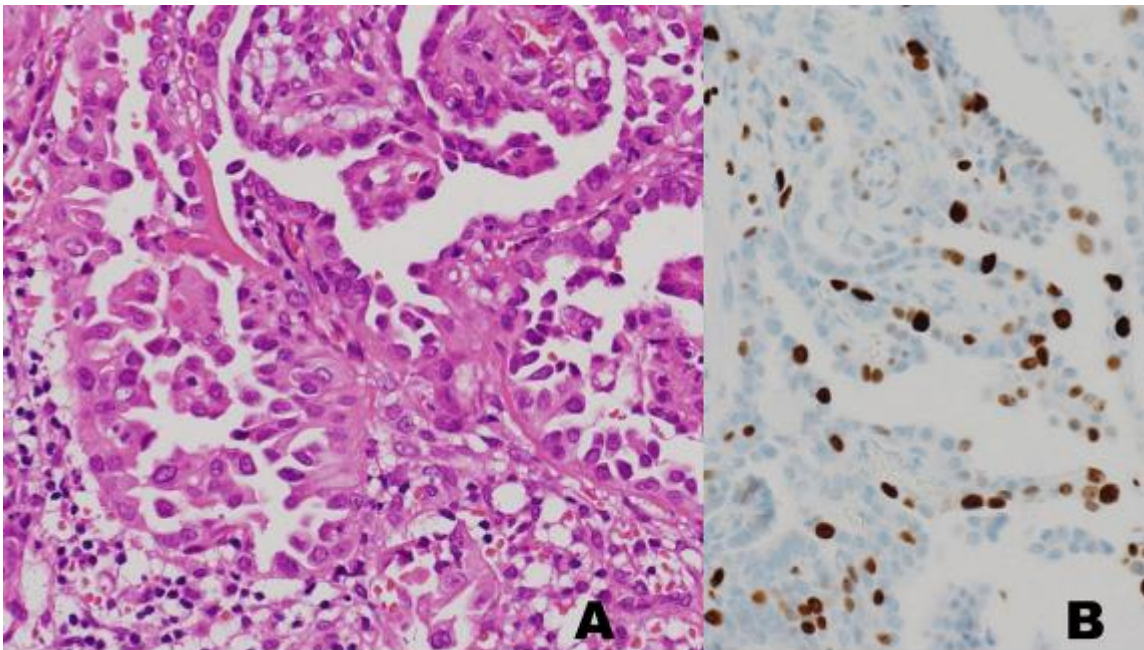

Fig. 8. Hobnail (loss of cellular polarity/loss of cellular cohesion) variant of PTC. Piling or pseudostratification of nuclei (loss of cellular polarity) in PTC tumor cells is found at the invasive front (A: HE stain, x20). Some cells are detached from the tumor cell mass (loss of cellular cohesion) floating in spaces or forming a micropapillary growth pattern (A). This is an aggressive variant and has a high Ki67 proliferation index (B: Ki67 immuno-peroxidase stain, x20).
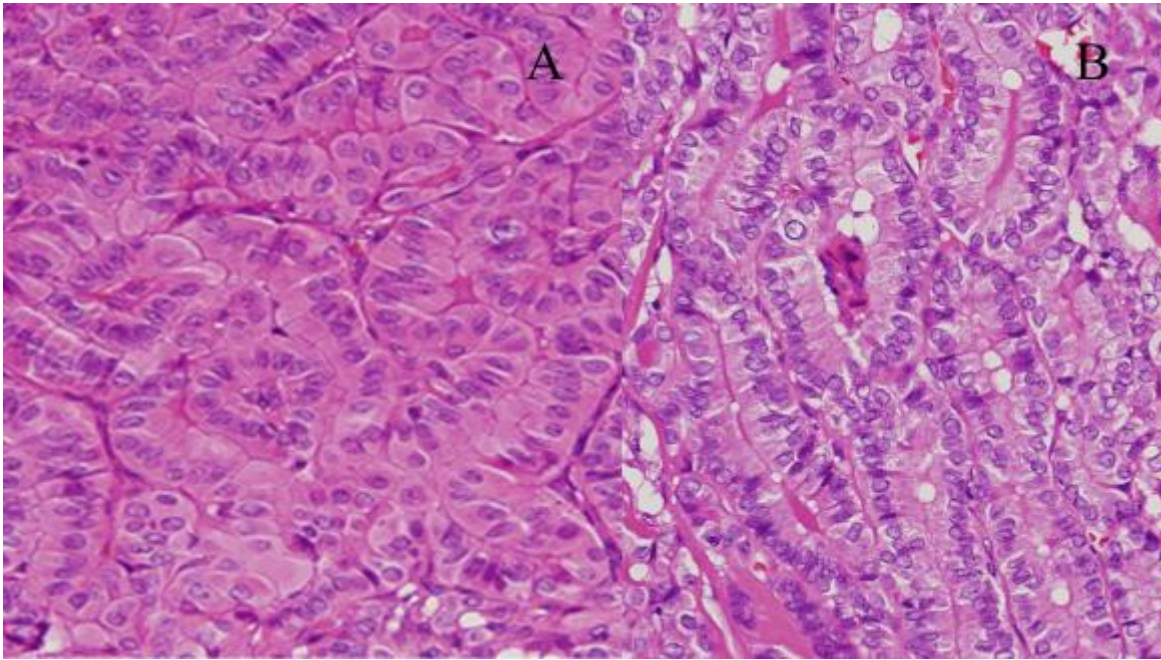
Fig. 9. Tall cell variant PTC: oxyphilic type (A) and non-oxyphilic type (B). The tall cell variant of PTC is composed of cells that are two- to three-times as tall as they are wide. The tall cell variant is a common histological feature observed in conventional PTC, and must comprise more than 30% of all tumor cells to be an aggressive variant. (HE stain, x40)
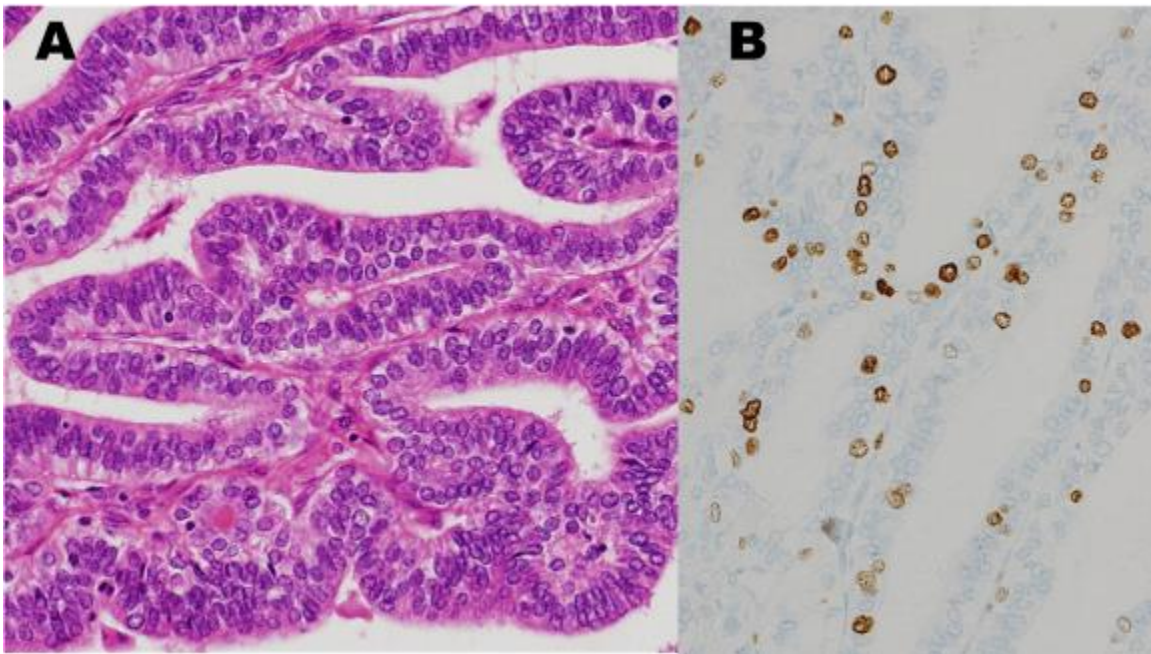

Fig. 10. Columnar cell variant PTC. The columnar cell variant is characterized by prominent nuclear pseudo-stratification and nuclear features that are usually not PTC-type (A: HE stain, x20). Although both hobnail and columnar cell variants share nuclear pseudo-stratification, loss of cellular cohesiveness is a characteristic features in the hobnail variant (Figure 8A), whereas it is not found in the columnar cell variant (A). This is an aggressive variant with a high Ki67 proliferation index (B: Ki67 immuno-peroxidase stain, x20).
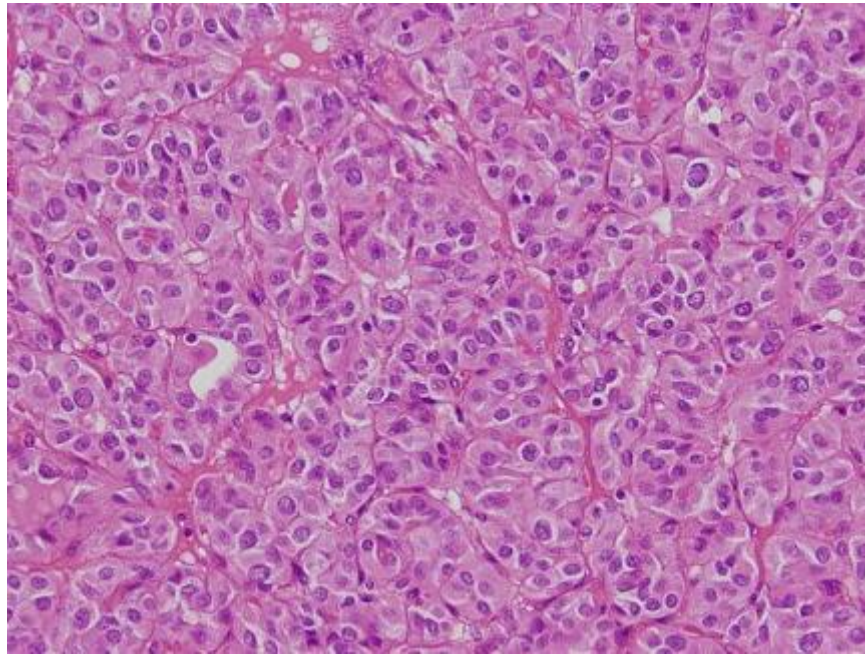
Fig. 11. Solid/trabecular variant (termed Sakamoto-type PDC). Solid/trabecular growth PTC and FTC were classified into PDC in the previous 2004 edition of the WHO classification of thyroid tumors (ref. 52, 53). Cases with PTC-N in this illustration were excluded from PDC in the 4th edition 2017 WHO classification (ref. 2). (HE stain, x20). PTC: papillary thyroid carcinoma; FTC: follicular thyroid carcinoma; PDC: poorly differentiated carcinoma.
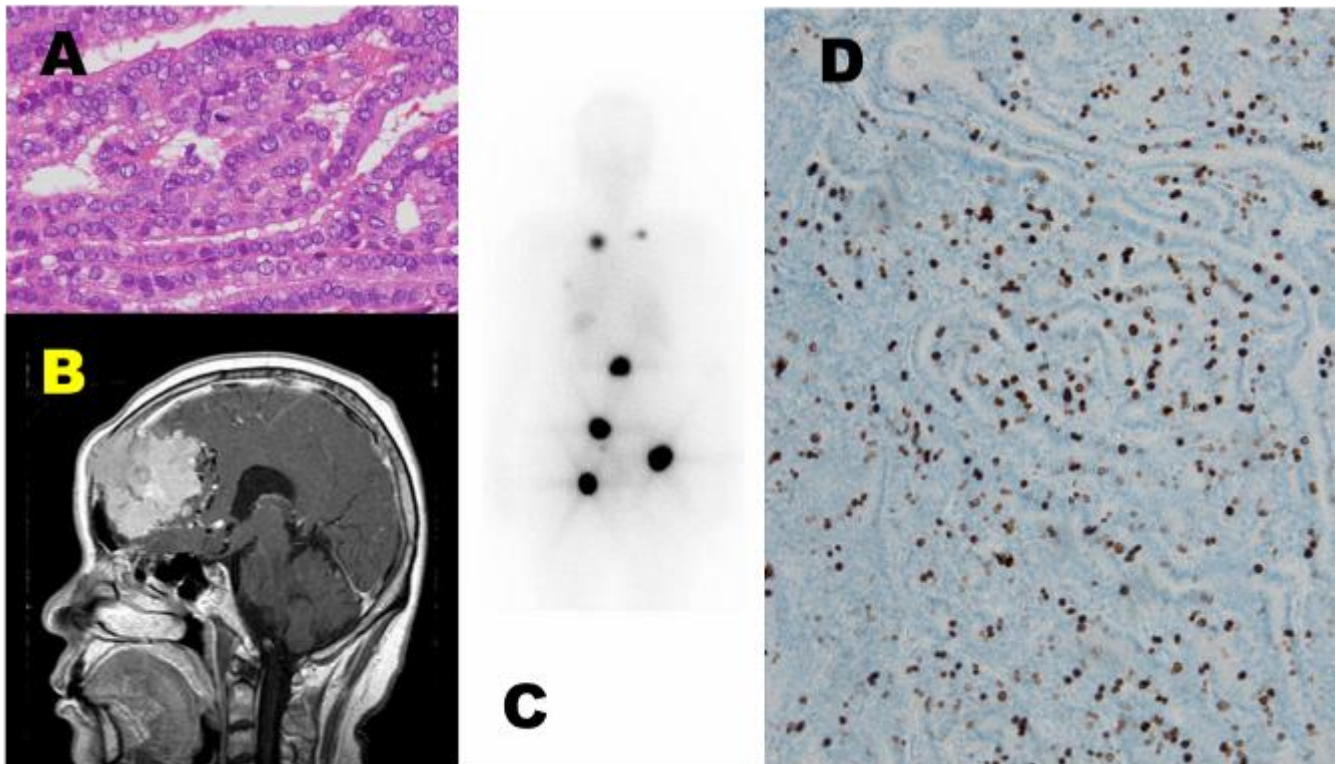
Fig. 12. A high-risk thyroid carcinoma from a 65-year-old female patient. Mild nuclear pleomorphism and follicular cell differentiation was discernible (A: HE stain, x20). Therefore, by the WHO definition, this tumor is not a PDC but well-differentiated carcinoma (FTC or PTC). The patient developed brain (B) and bone (C) metastases. Increased mitoses (A) and a high Ki-67 labeling index (D: Ki-67 immuno-peroxidase stain, x10) are unusual for well-differentiated carcinoma with a favorable prognosis.

Other histological features in follicular cell carcinomas indicating high-risk thyroid carcinoma

Although cases of papillary carcinoma or follicular carcinoma differentiation were excluded from PDC in the WHO classification, there were thyroid carcinomas with poor outcomes. I will introduce 2 other morphological methods to identify biologically high-risk thyroid carcinomas among cases of PTC or FTC differentiation. Histological grading based on the identification of necrosis or increased mitotic activity was reported as PDC by Hiltzik *et al*. in 2006 (55). This type of high-risk thyroid carcinoma with a poor outcome overlapped considerably with PDC defined by the Turin criteria (51). However, the new WHO classification excludes these from PDC and states that "aggressive forms of papillary or follicular carcinomas should not be included in the PDC category if they retain distinctive tumor differentiation" (Figure 12A) (2). The author of this review believes that high-risk thyroid carcinomas can be found in several histological types of thyroid carcinomas other than solid/trabecular/insular carcinomas. We previously proposed immunohistochemical evaluation of the Ki-67 labelling index in thyroid tumor classification to identify cases with increased growth fraction. Confirmation of a high proliferation index with Ki-67 immunohistochemistry is a more objective method to more confidently diagnosis high-risk carcinomas. Table 2 is our proposed classification published in 2015 (56). This prognostic classification was characterized by risk stratification of thyroid carcinomas with the Ki-67 proliferation index into low-risk, moderate-risk, high-risk or undifferentiated carcinomas, in addition to a borderline tumor category (Table 2).

How to measure the proliferation index by immunohistochemistry

Ki-67 staining is usually not homogeneous, and hot spots may exist (Figure 13). Currently, measurements at the hot spots are recommended on evaluation of tumor growth. A method to count Ki-67-positive cells is not well-established because of limitations in immunohistochemical techniques such as antigen activation, poor fixation and/or delayed fixation. About 500 to 1000 tumor cells are usually counted and expressed as a percentile of positive cells. Selection of densely distributed areas of positive tumor cells (hot spots) is recommended (Table 3). Automated computer assisted measurement is helpful, but pigmentation, non-specific staining and overlapping nuclei remain (57). Several excellent publications have discussed how to measure the Ki-67 labelling index (57-60).

Discrepancy between aggressive histology and the proliferation index

It is important to note that not all morphologically aggressive variants and dedifferentiated thyroid carcinomas have high Ki-67 labeling (Figure 14). Figure 14A is an example of tall cell variant PTC, but only 1% of cells is positive for Ki-67 in Figure 14B. Aggressive thyroid carcinomas may be confidently diagnosed by confirmation with the high Ki-67 labeling index (56, 61).

Confirmation of high-risk thyroid carcinoma with the Ki-67 proliferation index

Figure 12 is an example of a high-risk thyroid carcinoma from a 65-year-old female patient. The patient had invasive thyroid carcinoma with follicular cell differentiation (Figure 12A), and developed metastases in the brain and bones (Figure 12B and 12C). Only mild nuclear pleomorphism and follicular cell

Table 2: Classification of follicular cell tumors using the Ki-67 labeling index (Ki-67 LI) (ref. 56)

A: Benign Tumors (Ki-67 LI: <3%)
1. Follicular Adenoma
B: Borderline Tumors (Ki-67 LI: <3%, and T1, N0, EX0 and M0)
1. Encapsulated tumors
1) Well-Differentiated Tumor of Uncertain Malignant Potential
2) Follicular Tumor of Uncertain Malignant Potential
3) Encapsulated Follicular Variant Papillary Carcinoma without Invasion
4) Encapsulated conventional Papillary Carcinoma without Invasion
5) Capsular Invasion Only Follicular Carcinoma
2. Non-Encapsulated Tumors (formerly called Papillary Micro-Carcinoma)
1) Papillary Micro-Tumor (<1 cm)
C: Malignant Tumors (Invasive Carcinoma and >1 cm)
1. Low Risk (Ki-67 LI: <5%)
2. Moderate Risk (Ki-67 LI: 5-10%)
3. High Risk (Ki-67 LI: 10-30%)
4. Undifferentiated Carcinoma (Ki-67 LI: >30%)

Table 3: How to measure the Ki67 labeling index

1. Hot spots.
2. About 500 to 1000 tumor cells on screen or prints.
3. How to exclude positive non-neoplastic cells?
4. Should we include faintly stained cells?
5. Automated computer counting software is helpful, but it suffers pigments, non-specific staining and overlapping nuclei.

differentiation were noted without solid/trabecular/insular growth (Figure 12A). Increased mitoses (Figure 12A) and a high Ki-67 labeling index (Figure 12D) are unusual for well-differentiated follicular cell carcinoma with a favorable prognosis. However, the new WHO classification excludes this type of high-risk thyroid carcinoma from the PDC category, and stated that "aggressive forms of papillary or follicular carcinomas should not be included in the poorly differentiated category if they retain distinctive tumor differentiation" (2). The author of this review believes that high-risk thyroid carcinomas can be confidently diagnosed by the combination of a high Ki-67 labeling index and histological features of dedifferentiation (loss of cellular polarity/loss of cellular cohesion). Figures 15 and 16 are from a PTC patient (Figure 15A) with a poorly differentiated component with necrosis (Figure 15B) and minor anaplastic component (Figure 15C). The diagnosis was confirmed by the low Ki-67 index (3-5%) in Figure 16A, intermediate (10-15%) index in Figure 16B and extremely high (50-60%) index in Figure 16C. Thus, based on the Ki-67 proliferation index, progression from PTC to anaplastic carcinoma in association with PDC can be confirmed. Spindle cell variant PTC (low-risk carcinoma) can be confidently discriminated, and highly aggressive PTC with anaplastic transformation can be diagnosed. The morphological identification of aggressive variants of PTC, PDC and anaplastic carcinomas and confirmation of high Ki-67 proliferation index help to identify high-risk thyroid cancers. Risk stratification of thyroid carcinomas has become an essential component of pathology reports.
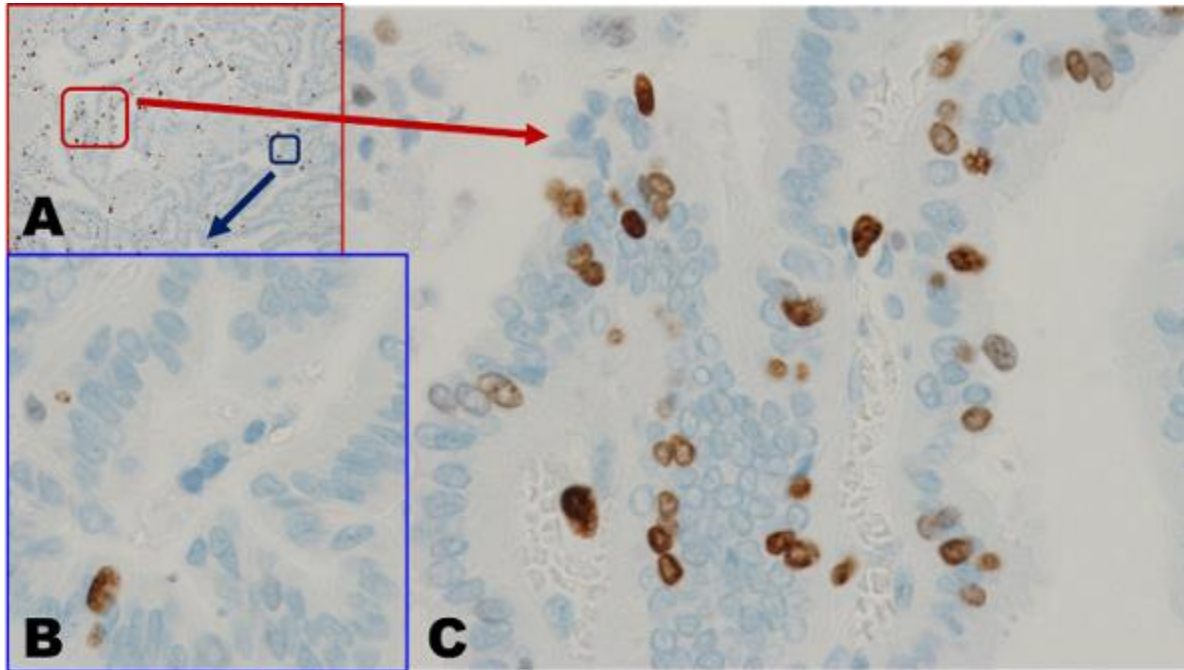
Fig. 13. Hot spots on Ki-67 immunostaining. Ki-67 immunohistochemical staining of tumor tissue is not always homogeneous (A). B, a higher magnification of the blue square in A, showes only a few positive signals, whereas C, a higher magnification of the red square in A, shows a high Ki-67 labeling index of more than 20%. (Immuno-peroxidase staining for Ki-67: A: x10; B: x40; C: x20)
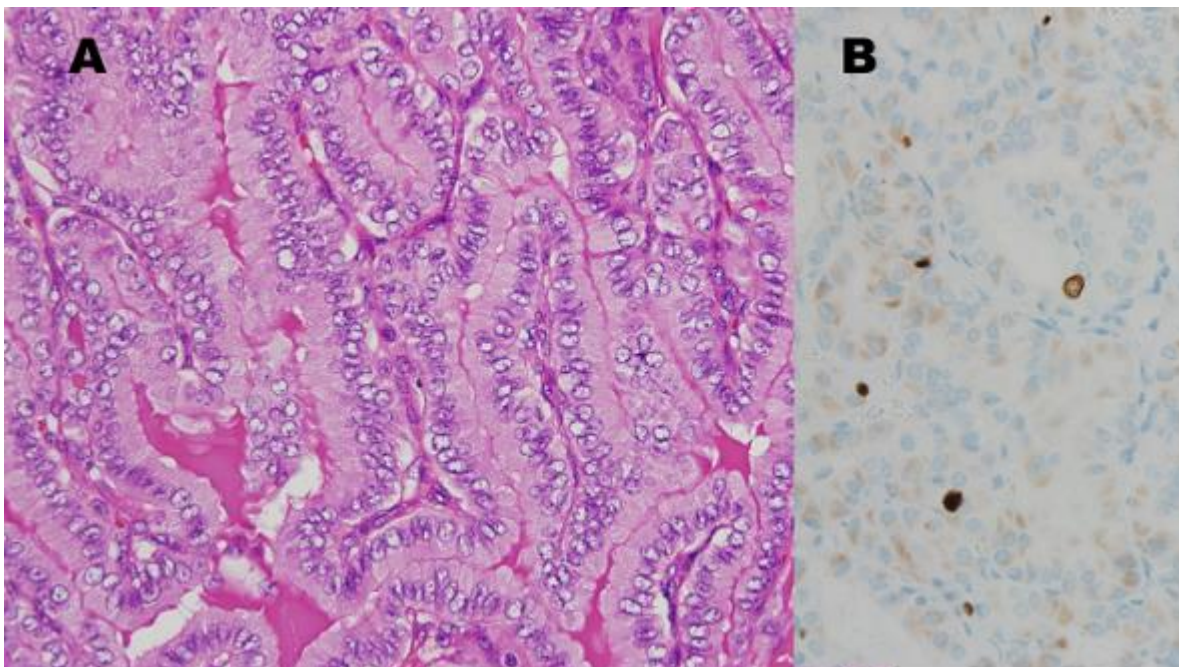

Fig. 14. Tall cell variant PTC with a low Ki-67 proliferation index. This papillary carcinoma has abundant cytoplasm and tall cells (A: HE stain, x40), but Ki-67 immunohistochemistry demonstrated a low (less than 1%) labeling index (B: Ki67 immuno-peroxidase stain, x40).

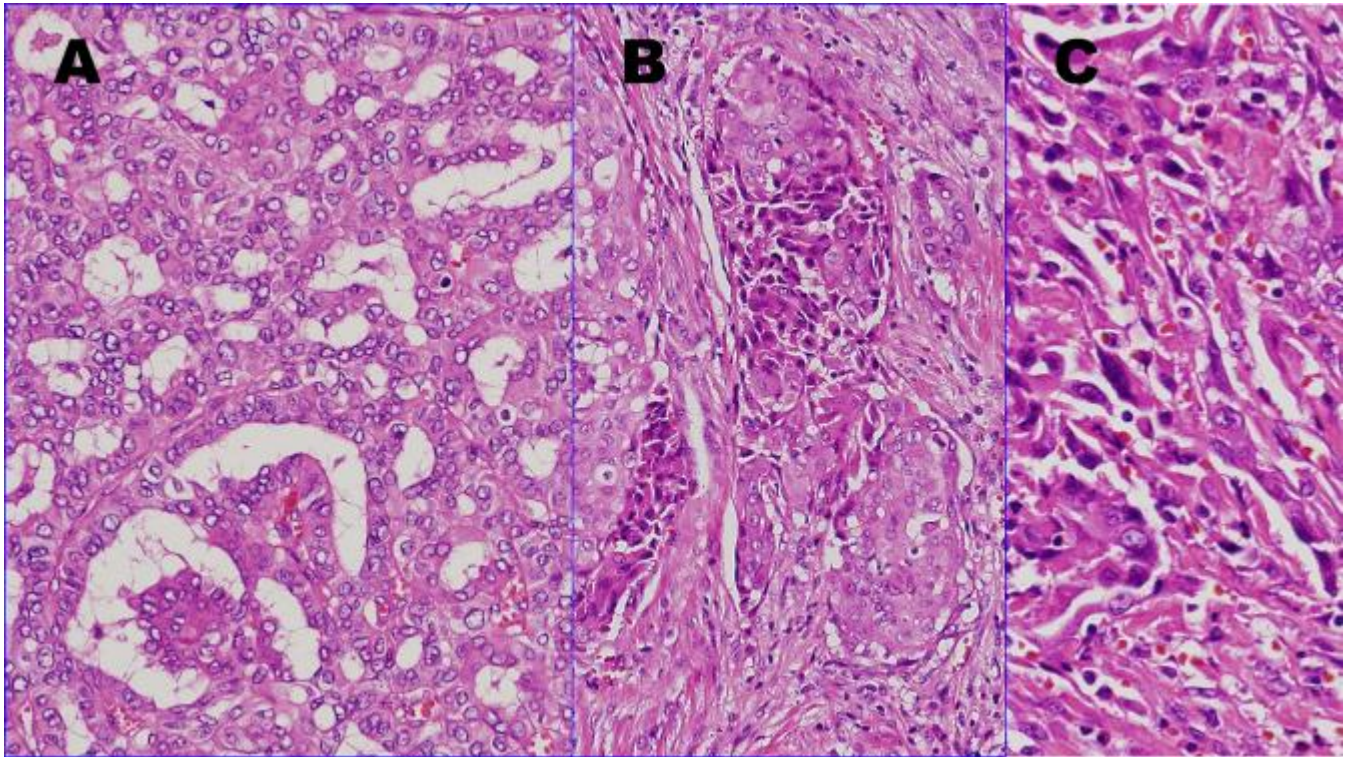Fig. 15. High-risk papillary carcinoma (A) contains a poorly differentiated part with necrosis (B) and a spindle/giant cell anaplastic component (C). (HE stain, x20)
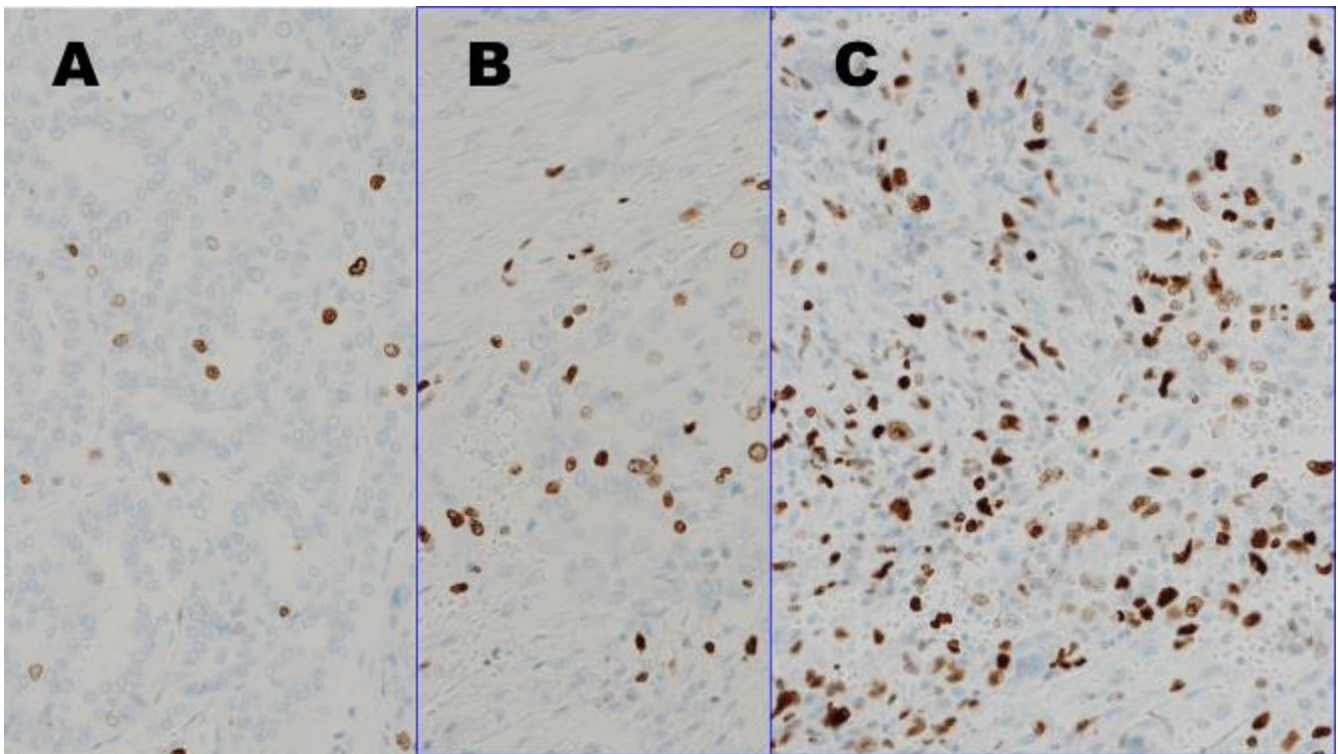

Fig. 16. Immunohistochemistry for Ki-67 on the case illustrated in Fig. 15 demonstrated only 3-5% Ki-67 positivity in A, 10-15% positivity in B and extremely high (50-60%) positivity in C. (Immuno-peroxidase staining for Ki-67, x20)

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