2. THE PATHOLOGY OF THYROID CANCER

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Thyroid nodules are extremely common in the general population; it has been estimated that about 20% of the population has a palpable thyroid nodule and approximately 70% has a nodule that can be detected by ultrasound (1). The prevalence of thyroid nodules is greater in women than in men, and multiple nodules are more common than solitary nodules.

The differential diagnosis of the thyroid nodule includes numerous entities, non-neoplastic and neoplastic, benign and malignant (2–5). The pathologist has an important role to play in their evaluation. The use of fine needle aspiration biopsy has significantly improved our ability to identify specific high-risk disorders and to facilitate their management in an expeditious and cost-effective manner. Patients who require surgery for further confirmation of the disease process rely upon the pathologist to correctly characterise their nodule and pathologists are actively involved in research to clarify the pathogenesis of thyroid disease.

While some of these entities are readily diagnosed based on specific features seen in a routine slide stained with conventional dyes, the morphologic evaluation of many of these lesions is fraught with controversy and diagnostic criteria are highly variable from Pathologist to Pathologist (6). Nevertheless, histology remains the gold standard against which we measure outcomes of cytology, intraoperative consultations, molecular and other studies, and it represents the basis on which we determine patient management and the efficacy of various therapies. Unfortunately, no current morphologic criteria provide adequate information to predict outcome for many follicular nodules of thyroid.

Advances in our understanding of the molecular basis of thyroid cancer will allow more accurate characterisation of specific subtypes of neoplasia and malignancy even on single cells obtained at fine needle aspiration biopsy. This should further enhance the usefulness of this technique and better guide the management of patients with a thyroid nodule.

THYROID FOLLICULAR HYPERPLASIA AND NEOPLASIA

Follicular nodules are the most commonly encountered problems in the surgical pathology of the thyroid. These lesions can be classified along the full spectrum of thyroid pathology from hyperplastic nodules to benign follicular adenomas and malignant follicular carcinomas.

Nodular goitre

Sporadic nodular goitre is characterised by numerous follicular nodules with heterogeneous architecture and cytology, features that have suggested a hyperplastic rather than neoplastic pathogenesis (7–10). The gland may be distorted by multiple bilateral nodules and can achieve weights of several hundred to a thousand grams, but this disorder is often identified as a dominant nodule in what clinically appears to be an otherwise normal gland. Histologically, the nodules are irregular; some are poorly circumscribed while others are surrounded by scarring and condensation of thyroid stroma, creating the appearance of complete encapsulation. They are composed of follicles of variable size and shape. Some follicles are large, with abundant colloid surrounded by flattened, cuboidal or columnar epithelial cells, often with cellular areas composed of small follicles lined by crowded epithelium with scant colloid in a small lumen, alone or pushing into large colloid-filled follicles as "Sanderson's polsters" (Figure 1). There may be focal necrosis, haemorrhage with haemosiderin deposition and cholesterol clefts, fibrosis, and granulation tissue; these degenerative changes are usually found in the centre of large nodules, creating stellate scars.

The morphologic classification of cellular follicular nodules in nodular glands can be extremely difficult. Hyperplasia may be extremely difficult to distinguish from neoplasia. Classical guidelines that allow distinction of a hyperplastic nodule from a follicular adenoma include the following: (i) multiple lesions suggest hyperplasia whereas a solitary lesion is likely to be neoplastic, (ii) a poorly encapsulated nodule is likely hyperplastic; a well developed capsule suggests a neoplastic growth, (iii) variable architecture reflects a polyclonal proliferation whereas uniform architecture suggests a monoclonal neoplastic growth, (iv) cytologic heterogeneity suggests hyperplasia; monotonous cytology is characteristic of neoplasia, (v) the presence of multiple lesions in hyperplasia means that areas similar to the lesion in question will be present in the adjacent gland; in contrast, neoplasms have a distinct morphology compared with the surrounding parenchyma, (vi) classically hyperplastic nodules are said not to compress the surrounding gland whereas neoplasms result in compression of the adjacent parenchyma. For the most part, large nodules in multinodular glands tend to be incompletely encapsulated and poorly demarcated from the internodular tissue. However, in some glands, large encapsulated lesions with relatively monotonous architecture

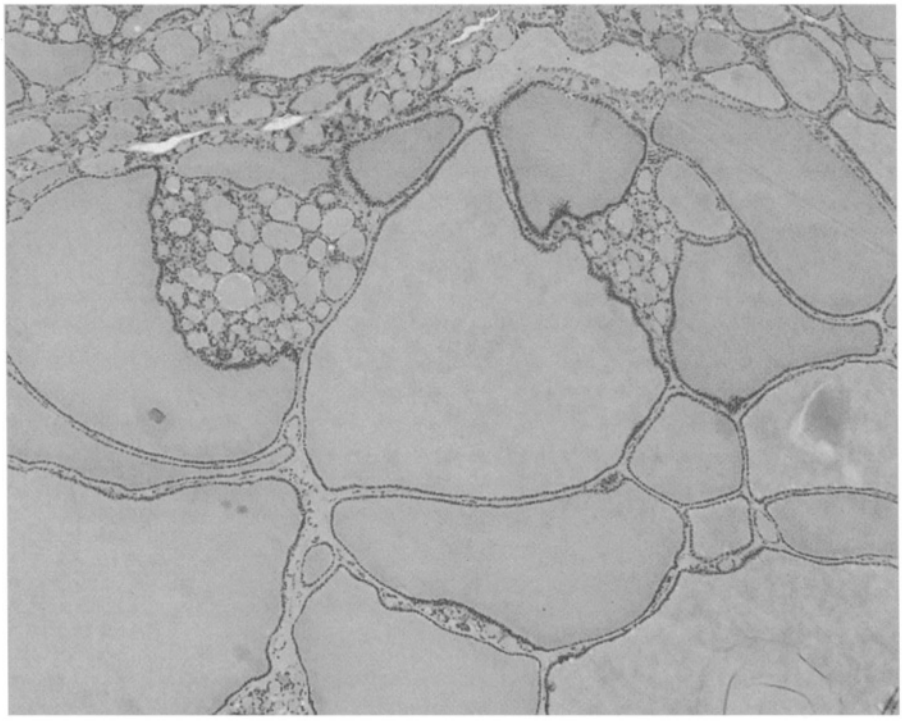

Figure 1. A hyperplastic nodule of thyroid is characterized by architectural and cytologic heterogeneity, usually with abundant colloid and often with subfollicle formation within larger follicles.

and cytology make distinction of hyperplasia from adenoma difficult. Many pathologists have applied nonspecific terms such as "adenomatoid nodules" to describe such lesions.

The pathophysiology of nodule formation remains poorly understood. The aetiology of this disorder has long remained elusive, since the goitres do not appear to be TSH-dependent (9). The work of Stüder suggests that the initial proliferation is a polyclonal one involving cells that are intrinsically more rapidly growing than their neighbours (7,10,11). While the stimulus for growth is not certain, high levels of circulating thyroid growth-stimulating immunoglobulins (TGI) and defects in T suppressor cell function have been documented in patients with sporadic nodular goitre (12,13), implicating autoimmunity in the pathogenesis of this disease. Drexhage and colleagues (12) compared immunoglobulin preparations of patients who have goitrous Graves' disease with those of patients who have sporadic nodular goitre and have found that the former are approximately 10-fold more potent in inducing growth than the latter. It has been postulated that the weaker stimuli result in proliferation of only the most sensitive of the heterogeneous follicular epithelial cell population, hence the nodularity, and that "toxic" nodular goitre results from preferential replication of cells which

are highly responsive to TSH stimulation (14,15). These data implicating an autoimmune pathogenesis explain the presence of chronic inflammation that is usually focally associated with nodular hyperplasia.

In contrast, molecular studies have indicated that the dominant nodules of multinodular goitres are monoclonal proliferations, and therefore represent benign neoplasms (8,16,17). It may be that these represent true adenomas arising in the background of a hyperplastic process that is mediated by growth stimulating immunoglobulins. Moreover, most hyperfunctioning nodules are also now thought to represent clonal benign neoplasms with activating mutations of the TSH receptor or Gsα (18–22). The evidence of clonal proliferation in sporadic nodular goitre and the identification of ras mutations as early events in morphologically classified hyperplastic nodules in this disorder (23) indicates that the thyroid is a site for the hyperplasia-neoplasia sequence. Nevertheless, clinical experience has shown us that the vast majority of these lesions remain entirely benign.

Follicular adenoma

Solitary follicular nodules have been unequivocally shown to be monoclonal (24,25,26) and in the absence of invasive behaviour or of markers of papillary carcinoma, these lesions are considered to be benign. Follicular adenomas are described as solitary encapsulated follicular lesions that exhibit a uniform architectural and cytologic pattern. However, the inclusion of nodules in sporadic nodular goitre in this category alters these criteria.

On aspiration cytology, the diagnosis of "follicular lesion" covers both follicular adenoma and follicular carcinoma, which are difficult if not impossible to distinguish because the diagnostic criteria do not rest on cytologic characteristics. The aspirate of a follicular lesion is usually cellular with follicular cells in sheets or microfollicular arrangements. The follicular cells are monotonous with elongated, bland nuclei and micronucleoli. Worrisome features include nuclear crowding, altered polarity, pleomorphism, macronucleoli and coarse chromatin. The main practical role of cytology is to distinguish a colloid nodule or papillary carcinoma from a follicular neoplasm.

Follicular adenomas are well delineated and usually thickly encapsulated neoplasms that can be classified histologically according the size or presence of follicles and degree of cellularity, each adenoma tending to have a consistent microscopic pattern (Figure 2). The subclassification of follicular adenomas into simple, microfollicular, trabecular, oxyphil, atypical, papillary and signet ring cell types has no prognostic significance.

Atypical adenomas are highly cellular tumours with unusual gross and/or histologic appearances that suggest the possibility of malignancy but these tumours lack evidence of invasion. They may have necrosis, infarction, numerous mitoses or unusual cellularity. Many so-called "atypical adenomas" are indeed papillary carcinomas. The distinction of an encapsulated follicular variant papillary carcinoma from follicular adenoma relies on cytologic characteristics. The presence of the cytologic features of papillary carcinoma described below should indicate that diagnosis, despite lack of invasion. Whether some follicular nodules classified histologically as adenomas have the biologic potential to become carcinoma is not clear; aneuploid cell populations

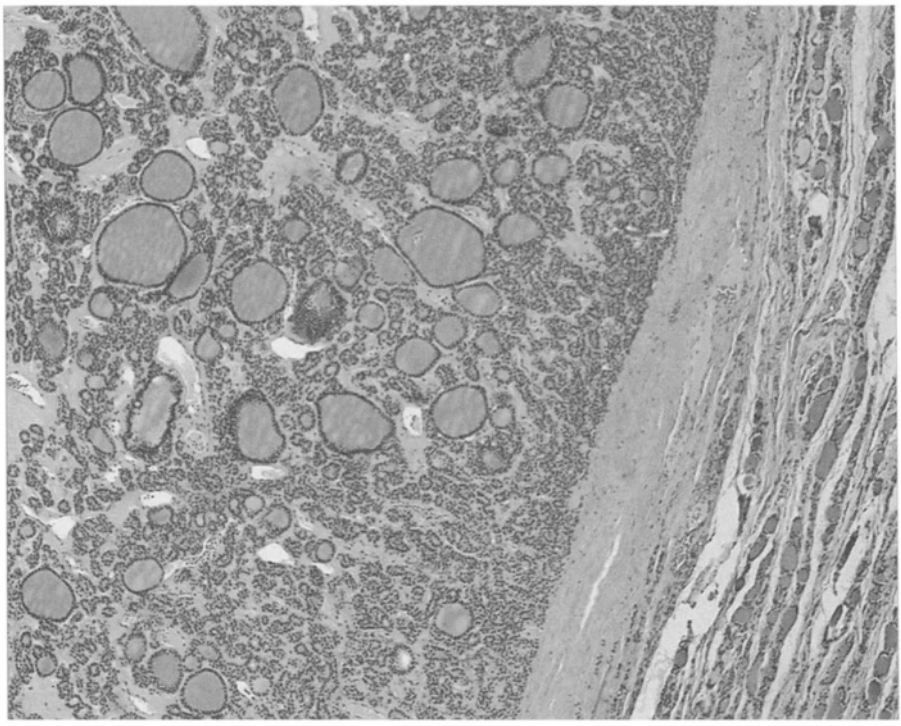

Figure 2. A follicular adenoma is usually well delineated and often surrounded by a thick fibrous capsule. The lesion is generally characterized by uniformity of architecture and cytology.

have been described in a significant percentage of these lesions, suggesting that some of these may represent carcinoma in situ.

Follicular carcinoma

Follicular adenoma and most follicular carcinomas are indistinct with respect to their clinical presentation, radiographic appearance, cytologic findings and microscopic features. In most cases, the parenchymal component of both tumour types is essentially the same histomorphologically. The distinction between these two conditions has been considered possible only by recognition of invasion or metastasis. As indicated above, some encapsulated follicular adenomas exhibit evidence of aneuploidy and may in fact represent in situ follicular carcinomas.

Nuclear and cellular atypia and mitotic figures may be present in adenomas as well as in carcinomas and therefore cytologic characteristics are not helpful. Most follicular tumours are composed of cells with nuclei that are round to oval with uniformly speckled chromatin; the nuclei are evenly spaced and lack the crowded, overlapping appearance found in papillary carcinoma. As stated previously, these lesions cannot be

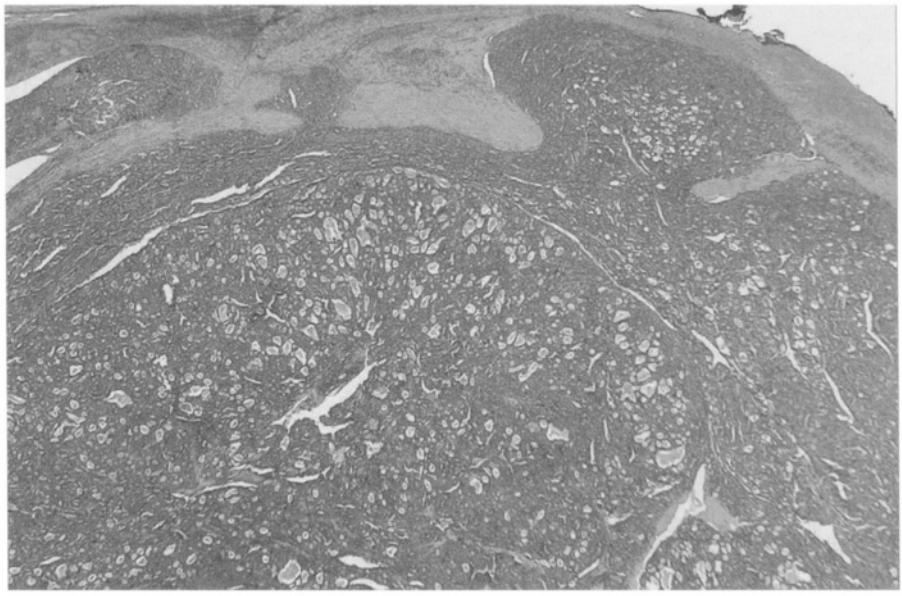

Figure 3. The distinction of follicular carcinoma from follicular adenoma relies on unequivocal evidence of invasive behaviour, as in the multifocal mushrooming capsular penetration exhibited by this lesion.

diagnosed as benign or malignant by fine needle aspiration; the diagnosis should be restricted to "follicular lesion".

Follicular carcinoma can only be diagnosed by the pathologist on high quality sections of well-fixed tissues that demonstrate capsular and/or vascular invasion (Figure 3). At the time of intraoperative consultation, frozen section will reveal only a very small number of these lesions, since the likelihood of identifying microinvasive foci on a single frozen section are low. The use of multiple frozen sections is not cost effective in the evaluation of these lesions (27).

Follicular carcinomas are divided into groups that reflect the biology of tumour growth and metastasis. *Widely invasive follicular carcinomas,* which are usually identifiable as invasive grossly, and certainly are not difficult to recognise as invasive microscopically, carry a poor prognosis with a 25–45% ten year survival (28,29). However, such lesions tend to be insular carcinomas (see below). In contrast, the more common scenario is that of minimal capsular invasion and patients with these tumors have an excellent prognosis. The diagnosis of follicular neoplasms requires very careful and thorough examination of the entire capsule of the follicular neoplasm by the pathologist (30). *Minimally invasive follicular carcinoma* is identified by invasion through but not widely beyond the capsule. Borderline lesions include those with invasion into the capsule beyond the bulk of the lesion but not through the full thickness of the capsule or situations in which islands of tumor are trapped within a capsule, associated with perpendicular rupture of collagen. The finding of nests, cords, or individual tumour

cells within a tumour capsule leads some pathologists to the diagnosis of minimally invasive follicular carcinoma, however, this may represent an artefact in a patient who has undergone fine needle aspiration biopsy, with trapping by fibrosis or displacement of tumour cells into the capsule. The pathologist is therefore advised to carefully search for evidence of fine needle aspiration biopsy in the adjacent tissue. This would include finding focal haemorrhage, deposition of haemosiderin-laden macrophages, the presence of granulation tissue and/or fibrosis, all of which would indicate a needle biopsy site and the possibility of artifactual invasion rather than genuine invasion.

The concept of unencapsulated follicular carcinoma was raised by the identification of tumours that lack a capsule. In one report of four such cases, one patient developed metastases, and this gave rise to citations of a 25% metastatic rate by such lesions (31). However, this has not been substantiated in larger series and this concept has largely been abandoned.

Patients with minimally invasive follicular carcinomas are on average about 10 years younger than those with widely infiltrative carcinomas and since traces of capsule are found in about 24% of widely invasive lesions, it is possible that encapsulated follicular carcinoma is a precursor of the widely invasive lesion (32). Minimally invasive carcinomas have ten year survival rates of 70–100% (33) and therefore some argue that this disease does not warrant the painstaking search for microscopic invasion that distinguishes it from follicular adenoma. Nevertheless, the investigators that have reported these promising data have treated their patients for carcinoma rather than for benign disease (34).

Vasculoinvasive follicular carcinomas are aggressive and require management accordingly. While vascular invasion is more reliable for the diagnosis of malignancy, again the criteria are vague. Vascular invasion cannot be evaluated within the tumour and therefore again the circumference of the lesion is the site that warrants careful examination. Bulging of tumour under endothelium does not qualify as vascular invasion if the endothelium is intact. Nests of tumour cells within an endothelial lumen generally are accepted as representing invasion, however, it is recognised that artefactual implantation of tumour cells into blood vessels can occur during the surgical procedure or sectioning. Therefore, invasive tumour cells infiltrating the wall of an endothelial-lined space and thrombus adherent to intravascular tumour are required to distinguish true invasion from artefact.

Elastin stains are of little value in assessing vascular invasion, since the involved vessels are usually thin-walled veins with little if any elastic tissue. Immunohistochemical markers such as factor-8 related antigen, type IV collagen, CD31 and CD34 can be used to improve the recognition of vascular invasion in follicular carcinoma.

It is obvious that the diagnosis of malignancy in well-differentiated encapsulated follicular tumours rests on subjective criteria. The search for objective markers of malignancy has yielded only one candidate thus far; HBME-1, a marker of mesothelial cells, is immunohistochemically detected in 40% of thyroid follicular malignancies of papillary or follicular differentiation (35–37) and has been used successfully in cytology studies as well as histopathologic evaluation (Figure 4) of thyroid nodules (36,37). Recent studies have advocated the use of galectin-3 as another marker of malignancy

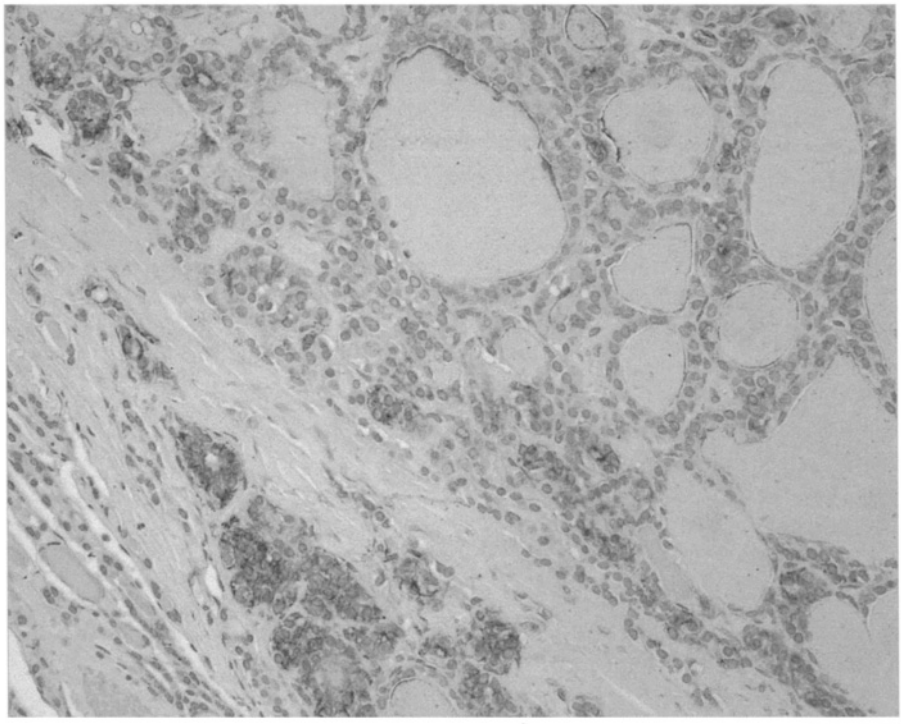

Figure 4. Immunoreactivity for HBME-1 is a feature of thyroid malignancies of epithelial cell derivation, such as this follicular carcinoma with superficial capsular invasion.

(38–40). While this marker also stains normal, hyperplastic and inflamed thyroid tissue, positivity in malignancies is more diffuse and strong. These data should limit the application of this technique for cytology but this has not been widely recognized (41).

Another molecular marker with application to follicular carcinoma is a gene rearrangement that involves the thyroid transcription factor Pax 8 and the peroxisome proliferator-activated receptor γ (PPARγ) gene (42). Normal thyroid follicular cells express Pax 8 at high levels; this transcription factor is essential for thyroid development, involved in regulating expression of the endogenous genes encoding thyroglobulin, thyroperoxidase, and the sodium/iodide symporter. PPARγ, a transcription factor that is implicated in the inhibition of cell growth and promotion of cell differentiation, is also expressed by normal thyroid follicular epithelium. However, this in-frame rearrangement results in a fusion protein that likely interferes with the normal function of both differentiating factors, thereby explaining its potential role in thyroid tumorigenesis. The rearrangement is most reliably detected using fluorescence in situ hybridization (FISH) technology to identify the translocation of the two genes that are normally localized on chromosomes 2q13 (Pax 8) and 3p25 (PPARγ). The presence of overexpressed

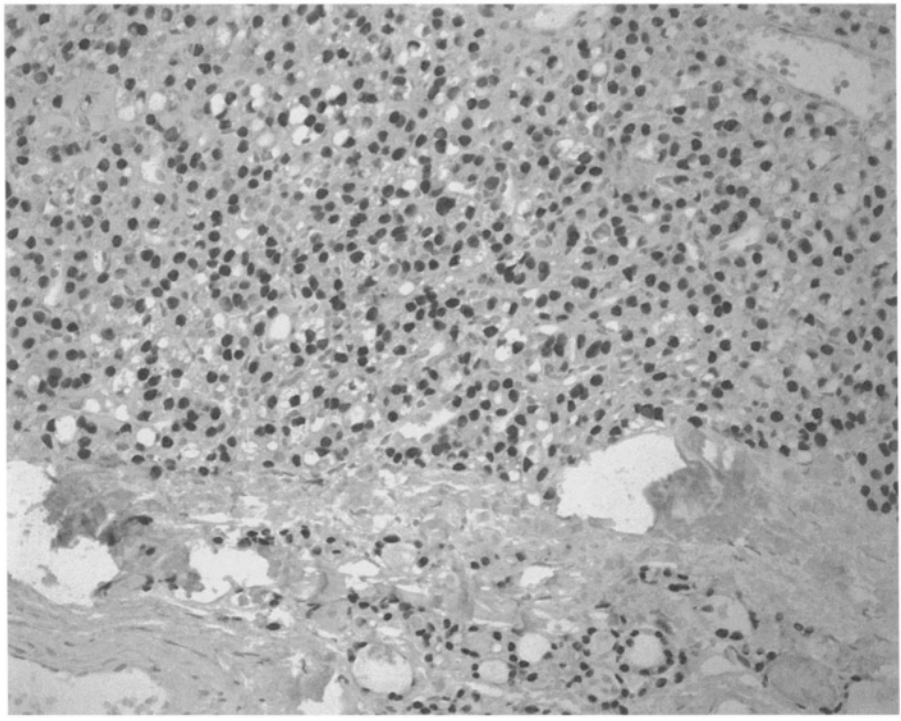

Figure 5. Overexpression of PPAR-γ due to a Pax 8-PPAR-γ gene rearrangement is detectable by immunohistochemistry. This finding has been correlated with aggressive behaviour, usually with vascular invasion.

protein can also be identified using immunostains for PPARγ where strong nuclear staining identifies tumours harboring a translocation (Figure 5). Although follicular carcinomas of thyroid are rare (43), and the numbers of cases studied has been small, it appears to be a useful tool for the diagnosis of malignancy in thyroid follicular lesions, particularly to predict vascular spread and aggressive behaviour (44).

DNA aneuploidy is a well-recognised feature of human malignant tumors and it was initially hoped that ploidy analyses could help to distinguish adenomas from carcinomas of the thyroid. However, it has now been recognised that about 27% of follicular adenomas are aneuploid and about 40% of follicular carcinomas are diploid (45). Therefore such measurements are of limited diagnostic value for the individual patient. In contrast, however, ploidy may be a useful adjunct in determining prognosis.

The significance of this diagnosis must be interpreted in light of clinical data that assess the behaviour of this disorder. The dominant determinant of cause-specific mortality in patients with follicular carcinoma is the presence of distant metastases (46–48). Most studies have indicated that morbidity and mortality for patients with non-metastatic encapsulated follicular carcinoma is very low and correlates better with

patient age than with any other parameter. Some have suggested that capsular invasion alone does not alter the incidence of distant metastases or cancer-related death (33).

Since the incidence of follicular carcinoma is low (43), most investigators still advocate total thyroidectomy and radioactive iodine therapy (34,49,50). The rationale for total thyroidectomy is not bilateral carcinoma; multifocal disease in follicular carcinoma is exceedingly rare and the identification of occult papillary carcinoma in the contralateral lobe is not an indication for further surgery (51). The only logical rationale for completion thyroidectomy is to allow selective uptake of radioactive iodine by metastatic tumour deposits rather than by residual thyroid gland. Uptake of radioactive iodine by distant metastases is a favourable prognostic factor and is improved by pretherapeutic total thyroidectomy, resulting in improved survival (52–54). In contrast, external beam radiotherapy is not thought to be of use in patients with differentiated thyroid carcinoma, apart from those with locally advanced tumours such as widely invasive follicular carcinomas that involve extrathyroidal soft tissues of the neck and cannot be completely resected (54).

The last few decades have seen a decrease in the incidence of follicular thyroid carcinoma, probably due to dietary iodine supplementation (43). However, misdiagnosis of this tumour continues. Benign lesions, such as partly encapsulated hyperplastic nodules or nodules exhibiting pseudoinvasion after fine needle aspiration (55), are often overdiagnosed as malignant; papillary carcinomas with follicular architecture are often misinterpreted as follicular carcinoma. The clinical features, pathophysiology and biological behaviour of follicular cancer differ significantly from those of the entities with which it is often confused. Only careful histopathologic classification will allow correct evaluation of treatment options and prognosis.

PAPILLARY LESIONS OF THYROID

Hyperplastic nodules and adenomas with papillary architecture

The "papillary hyperplastic nodule" of the thyroid is usually identified in girls, usually teenagers in and around the age of menarche. These present as solitary nodules and it is unusual for them to be associated with clinical hyperfunction, although that might occur. These lesion are distinguished from papillary carcinoma in that they are totally encapsulated, often show central cystic change, have subfollicle formation in the centres of broad oedematous papillae, and do not show nuclear features of papillary carcinoma (Figure 6). Although one analysis of clonality has suggested that these are polyclonal hyperplasias (56), the detection of Gsα or TSH receptor activating mutations in such nodules suggests that they are neoplasms (18–22). Their behaviour is almost always benign. Some have advocated the name "papillary adenoma" for these tumours; while scientifically appropriate, this term carries historical connotations that some feel are unacceptable (5).

In adults, one can have a similar histologic appearance in a "hot" nodule, that is, a thyroid nodule that is associated with clinical toxicity or subclinical hyperthyroidism and iodine uptake on scan. These lesions may be solitary but are often seen in the setting of sporadic nodular goitre (see above).

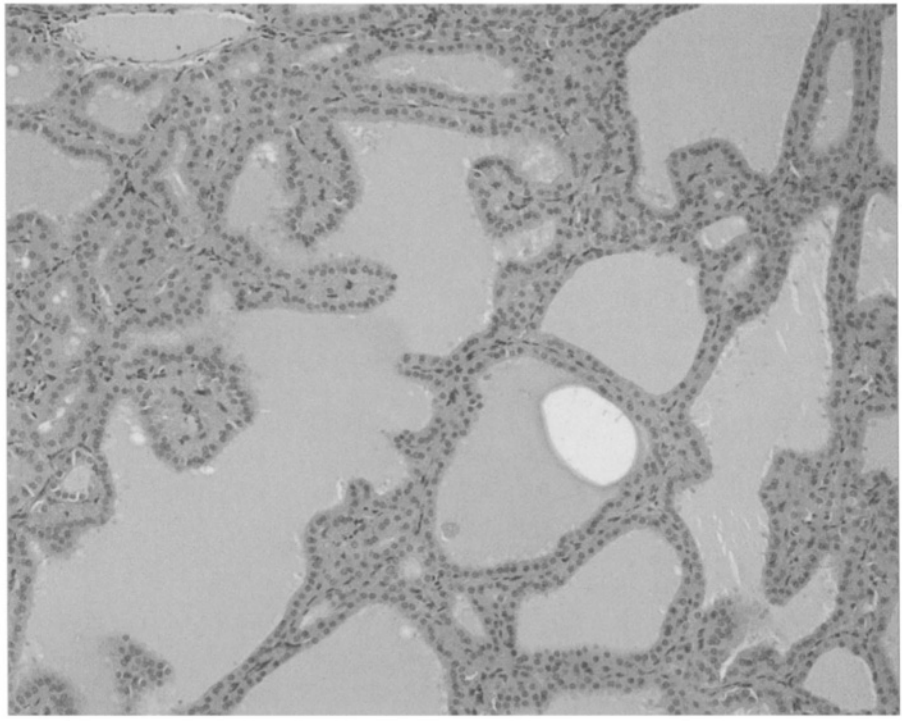

Figure 6. A benign adenoma with true papillary architecture has an organized centripedal orientation of the papillae that are lined by cells with bland nuclei that lack the atypia of papillary carcinoma.

On fine needle aspiration and on histologic evaluation, particularly at frozen section, papillary hyperplastic nodules or adenomas can be very alarming and lead to a false positive diagnosis of papillary carcinoma. Indeed, these entities give rise to well formed papillae but on higher magnification, the cytologic criteria for the diagnosis of papillary carcinoma, including powdery nuclear chromatin, multiple micro- and/or macronucleoli, intranuclear cytoplasmic inclusions, and linear chromatin grooves (57), are lacking.

Papillary carcinoma

Papillary carcinoma comprises at least 80% of thyroid epithelial malignancies diagnosed in regions of the world where goitres are not endemic. The terminology is misleading; papillary carcinomas can exhibit papillary architecture (Figure 7) but they may also have follicular (Figure 8) or mixed papillary and follicular patterns (58–62). It is now recognised that the diagnosis of papillary carcinoma is based on what the WHO has described as "a distinctive set of nuclear characteristics" (63). In contrast to true follicular carcinomas, these lesions are usually more indolent and most have an excellent prognosis with a 20 year survival rate of 90% or better (64,65).

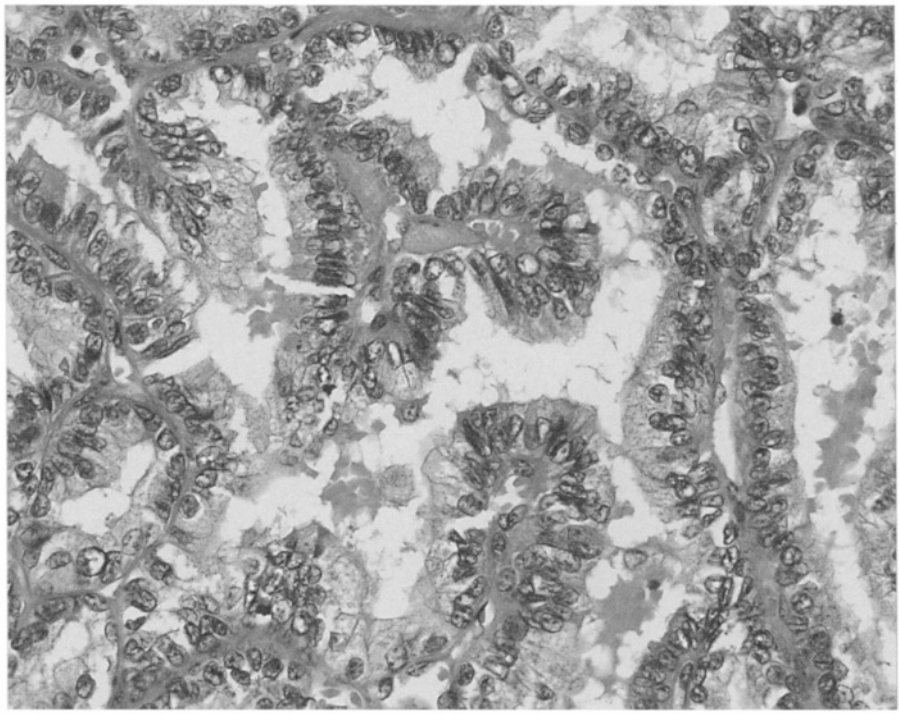

Figure 7. Papillary carcinoma was named as such because many of these lesions have complex papillary architecture. The papillae are lined by crowded cells with nuclear atypia.

The defining nuclear features are readily seen on cytology of fine needle aspirates as well as on histologic sections (Figure 9). They include an alteration of the size and the roundness of the normal follicular cell nucleus to one that is large and oval. Due to peripheral margination of chromatin, the centre of the nucleus has an empty appearance, which when pronounced has been termed "ground glass" (66). The chromatin and nucleolus are pushed to the edge of the nucleus. The nuclear contour is strikingly irregular, resulting in a "crumpled paper" appearance, intranuclear cytoplasmic pseudoinclusions and nuclear grooves (67,68). No one specific feature is absolutely diagnostic of papillary carcinoma; a constellation or combination of nuclear features is required for the diagnosis.

Papillary carcinomas may be multifocal; this has been interpreted as reflective of intraglandular lymphatic dissemination, but the identification of such microcarcinomas in up to 24% of the population (69) and the detection of different clonal rearrangements in multifocal lesions (70) support the interpretation of multifocal primary lesions in most patients. Nevertheless, when these lesions do invade, they show preference for lymphatic involvement with a high percentage of regional lymph node metastases.

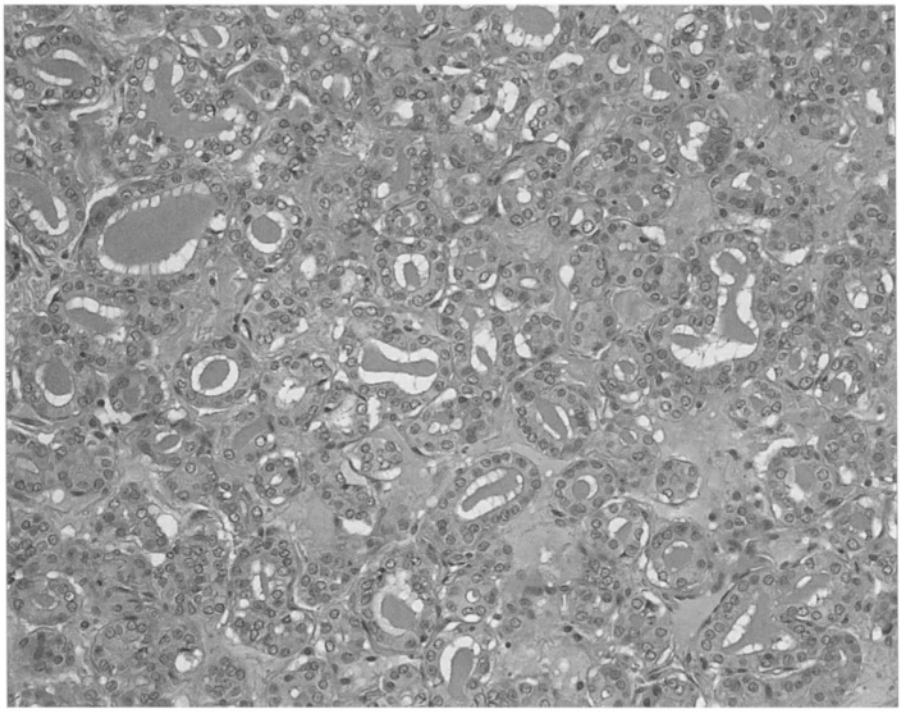

Figure 8. Papillary carcinoma may have partial or complete follicular architecture. The follicles usually harbour hypereosinophilic colloid that has peripheral scalloping. The nuclei exhibit characteristic atypia.

Metastases beyond the neck are unusual in common papillary carcinoma and probably only occur in about 5 to 7% of cases.

The most useful prognostic markers in papillary carcinoma are patient variables, tumour size and extent of disease (28,29,53,71). Patients under the age of 45 usually have an excellent prognosis; in contrast those over 45 years of age generally have a poorer outlook. Sex has also been said in the past to be an important determinant of tumour biology but more recent studies have suggested that there is no major difference in the behaviour of papillary carcinoma in men compared to women. Tumour size is exceedingly important (72). Tumours less than 1 cm are common and appear to be different biologically than larger tumours (73–75); a recent study has shown that occult papillary carcinomas are identified in up to 24% of the population in thyroids that are removed for non-malignant or unrelated disease (69). In contrast, tumours greater than 1 cm are thought to be of clinical significance and those larger than 3 cm generally have a poorer prognosis than do smaller tumours. The presence of cervical lymph node metastasis, whether microscopic or identified clinically, is thought to increase the risk of recurrence of disease but has been shown to have no impact on mortality.

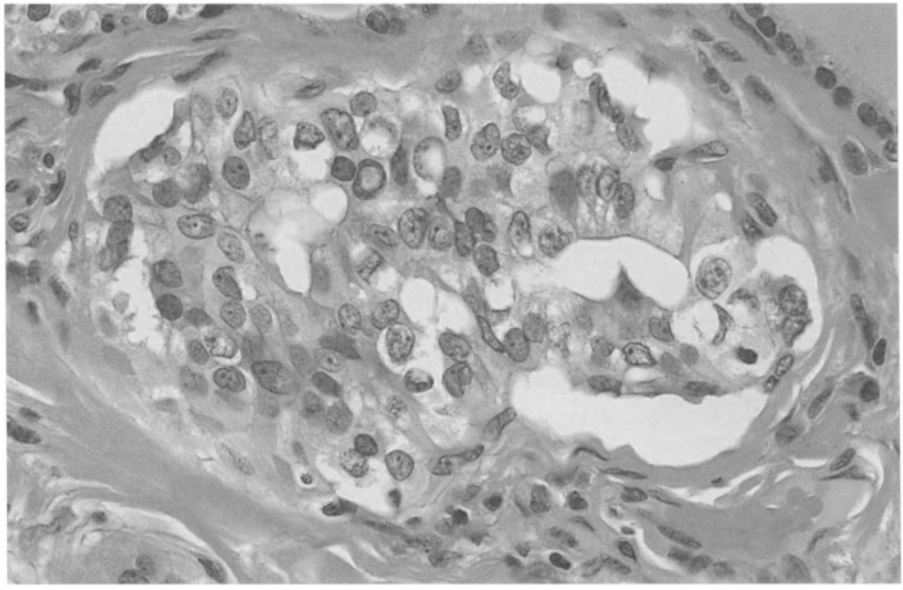

Figure 9. The nuclear features of papillary carcinoma encompass clearing of nucleoplasm and peripheral margination of chromatin, prominent and often multiple nucleoli, and irregular nuclear contours that result in formation of linear grooves and cytoplasmic pseudoinclusions.

Extrathyroidal extension, in contrast, predicts a worse prognosis and the presence of distant metastases is the hallmark of an aggressive tumour that will bear the potential for high mortality.

Grossly, papillary carcinomas vary in size from microcarcinomas (also called small, tiny, occult and minute), which are defined as lesions measuring less than 1 cm (usually 4 to 7 mm) to large neoplasms that extend extrathyroidally beyond the thyroid capsule into surrounding soft tissue. The bulk of clinical papillary carcinomas are intrathyroidal tumours confined within the capsule of the thyroid and may have an encapsulated appearance (this is usual for the follicular variant) or an irregularly infiltrative appearance. One can see gross cystic change but usually papillary carcinoma is a firm tumor and some are calcified or even ossified.

Microscopically, papillary carcinomas classically are composed of papillae but virtually all contain follicular elements. Ghosts of dead papillae or infarcted papillae calcify with a concentric whorled pattern that is characteristic of psammoma bodies (Figure 10); these are found in 40 to 50% of classical papillary carcinomas, either in the tumour stroma or in the surrounding non-tumourous thyroid, but they are distinctively uncommon in lesions with follicular architecture.

Inflammatory infiltrates within papillary carcinomas and in the surrounding thyroid parenchyma have been noted by several authors, although the prognostic significance of this is not clear (76,77). Some people have postulated that this inflammatory infiltrate

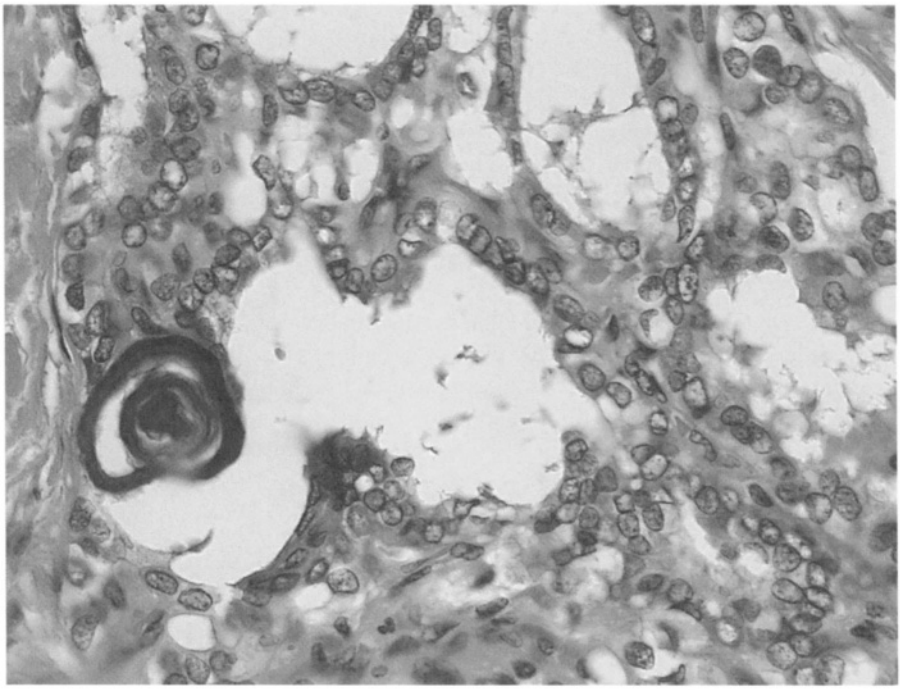

Figure 10. A minority of papillary carcinomas form psammoma bodies, concentric calcifications.

may indicate host-tumour immune interactions that are responsible for the general indolence of this type of thyroid carcinoma (76).

Variants

Although there are reports to the contrary, the exact histological variant of papillary carcinoma usually cannot be predicted from the appearance of the fine needle aspirate (78). Nevertheless, the histologic distinctions, which are characteristic (3,5,63,79–81), are of prognostic value.

Papillary microcarcinoma (75), *cystic and encapsulated variants of papillary carcinoma* (82) have an apparently better prognosis than usual papillary carcinoma.

The follicular variant has been recognized more frequently in the past 20 years (5,63,83,84). It has either been misdiagnosed as follicular carcinoma or underdiagnosed as follicular adenoma or atypical adenoma. Any lesion with follicular architecture and characteristic nuclear features of papillary carcinoma should be classified as this tumor. Infiltrating areas and metastases may exhibit a more striking papillary appearance and may even have psammoma bodies. It is unclear what the ultimate biological and clinical behaviour of follicular variant is, since some of these may be underdiagnosed as atypical adenomas and it is likely that the initial reports of this tumour included the aggressive biological spectrum of this variant.

The presence of cytologic atypia may raise the possibility of papillary carcinoma without being sufficiently convincing for unequivocal diagnosis. In some cases the changes may be induced by previous needle biopsy. The presence of haemorrhage, granulation tissue and hemosiderin laden-macrophages, inflammation and foreign body giant cells and even foreign material should point to this possibility. There may be calcification that can be mistaken for psammoma bodies. Various metaplastic changes occur. These changes have been described with the acronym WHAFFT which stands for *"Worrisome Histological Alterations Following FNA of Thyroid"* (55). The diagnosis of papillary carcinoma should not be made in this situation unless the lesion is entirely unequivocal.

In cases where the features are suggestive of papillary carcinoma but not entirely diagnostic, specific markers of this tumour as well as other markers if malignancy may be useful. A proportion of malignancies of thyroid follicular epithelium stain for HBME-1 (35–37)and some investigators have advocated the use of galectin-3 as a marker of thyroid carcinoma (38–41). Stains for high molecular weight cytokeratins may be useful. This technique, also considered controversial in the past, has recently been shown to be useful when applied to paraffin sections with microwave antigen retrieval (85). The results of these studies indicate that moderate to strong diffuse staining is confined to papillary carcinoma (Figure 11) whereas follicular neoplasms and hyperplastic nodules are negative or show only focal staining in areas of reaction to degeneration or previous fine needle aspiration biopsy. Nevertheless, only approximately 60% of papillary carcinomas are positive; a positive stain is therefore helpful, but negative stains are unable to assist in the diagnostic process.

The diagnosis of this entity has been further advanced by the recognition of a family of gene rearrangements that are specific to papillary carcinoma (86). The *ret*/PTC oncogenes (1 through 15, depending on the site of rearrangement, reviewed in (87)) are the result of DNA damage with rearrangements that transpose various cellular genes adjacent to the gene encoding the intracellular tyrosine kinase domain of the *ret* protooncogene (88–92). The rearrangements result in constitutive tyrosine kinase activation and translocation of the fusion protein to the cytoplasm (93). Animal models have shown the tumorigenicity of these fusion proteins (94–96); the rearrangements are common in radiation-induced tumors (97–101) but are also found in sporadic papillary carcinomas (102–105) and appear to be an early event in tumour development (106). Immunohistochemical staining with antisera directed against the carboxy terminus of *ret* allows rapid and clinically useful detection of this marker of papillary carcinoma which is present in almost 80% of occult papillary microcarcinomas and approximately 50% of clinically detected lesions (70). Again, a negative stain is not useful, however, the combination of high molecular weight cytokeratins and *ret* provides a set of immunohistochemical markers that aids in the diagnosis of papillary carcinoma in equivocal cases (107). At the moment, antisera or antibodies to *ret* offer inconsistent detection of these rearrangements and molecular diagnostics using RT-PCR remain the gold standard of this diagnostic tool. This methodology has been applied to FNA specimens when collected in suspension (108) and application of this technique enhances the cytological diagnosis of papillary carcinoma.

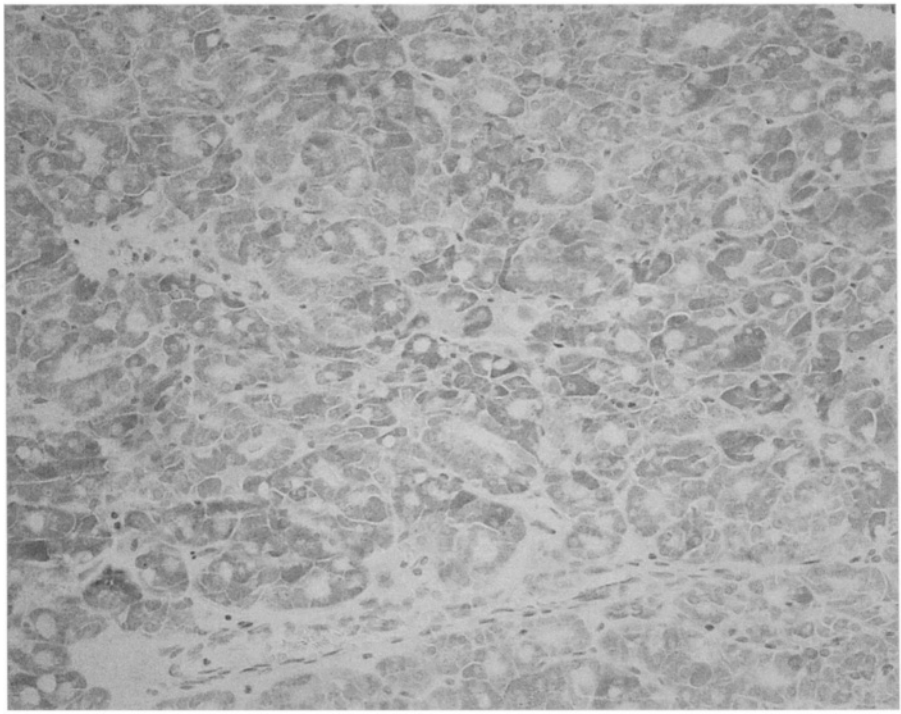

Figure 11. A diffuse cytoplasmic staining pattern for high molecular weight cytokeratins and cytokeratin-19 are the hallmark of papillary carcinomas of all types.

An unusual variant of papillary carcinoma is the *hyalinizing trabecular tumour.* This tumour was originally described by pioneers such as Zipkin in 1905 (109), Masson in 1922 (110), and Ward et al. in 1982 (111). The terminology "hyalinizing trabecular adenoma" (HTA) was defined by Carney et al. in 1987 (112). This lesion has also been designated "paraganglioma-like adenoma of thyroid" (PLAT) by Bronner et al (113) because of its unusual histologic pattern (Figure 12). Since the original descriptions, a malignant counterpart, hyalinizing trabecular carcinoma (HTC), has been described (114–116) and both HTA and HTC are now incorporated under the umbrella of hyalinizing trabecular tumors (HTT). Their main importance lies in the fact that they are sometimes mistaken for other entities such as paraganglioma or medullary carcinoma (112). Immunohistochemical stains for neuroendocrine markers will easily discriminate between HTT and paraganglioma or medullary carcinoma. However, it was noted that many features of HTT were also seen in papillary carcinoma; both lesions are of thyroid follicular epithelial origin and therefore both express thyroglobulin; several cases of HTT have been reported in patients with Hashimoto's thyroiditis or who have had a history of neck irradiation (117); HTT can co-exist with papillary carcinoma (5); HTT can often exhibit papillary carcinoma-like histologic features such as

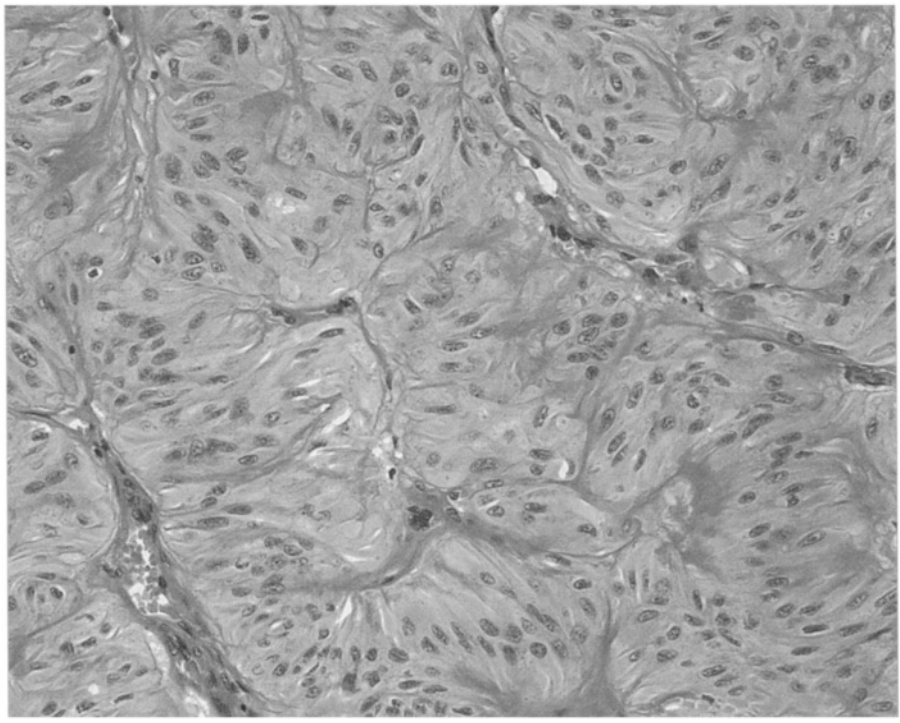

Figure 12. The hyalinizing trabecular tumour of thyroid is characterized by elongated spindle-shaped cells with hyaline cytoplasm, as well as stromal hyaline fibrosis. The tumour cells exhibit the nuclear atypia of papillary carcinoma.

psammoma-body formation, and characteristic nuclear changes including elongation, hypochromasia, grooves and pseudoinclusions (112). Based on these observations, a number of authors have hypothesized that these two entities are related and may in fact share a similar pathogenesis (118). These lesions are generally well delineated tumors characterised architecturally by trabecular and nesting architecture and elongated tumor cells which can have abundant pale eosinophilic cytoplasm and scattered "yellow bodies" (112,113,117,119). There is perivascular hyaline fibrosis and the cytoplasmic hyaline is usually identified as cytoplasmic filaments of cytokeratin. Occasional cases are immunoreactive for S100 protein. Most importantly, the tumour cells harbour large clear nuclei with irregular and elongated contours, grooves and inclusions as well as micronucleoli, features of papillary carcinoma. Application of *ret*/PTC analysis identified rearrangements in these lesions at a rate identical to that found in other papillary carcinomas (120,121) and many pathologists now consider this to be a variant of papillary carcinoma. However, some continue to maintain that these are distinct lesions (122,123) .

The *diffuse sclerosis variant* occurs in young individuals and often presents as goitre without a specific mass lesion (124–127). This tumour microscopically involves thyroid

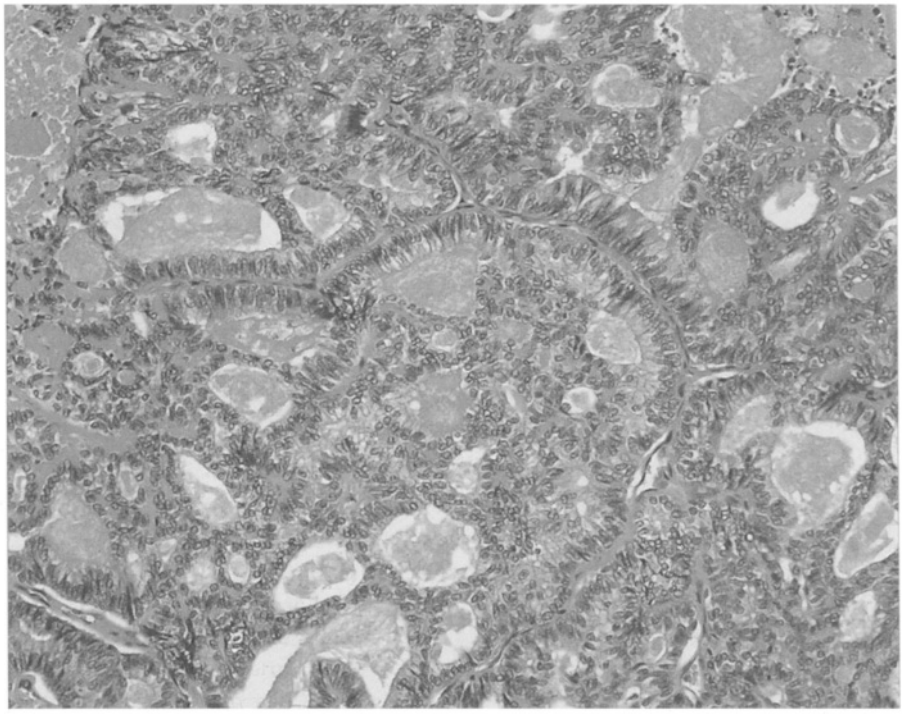

Figure 13. Patients with a family history of familial adenomatous polyposis and a germline mutation of the APC gene develop a type of papillary thyroid carcinoma that is characterized by a prominent cribriform and/or morular architecture.

lymphatics, exhibits squamous metaplasia and forms numerous psammoma bodies, giving it a very gritty appearance when examined grossly. These tumours almost always have lymph node metastases at presentation and 25% have lung metastases as well. It is interesting that about 10% of the paediatric thyroid cancers that occurred following the Chernobyl nuclear accident in 1986 were of the diffuse sclerosis type (128).

An unusual variant of papillary thyroid carcinoma known as the *cribriform-morular variant* has been identified in patients who harbour mutations of the APC gene that is responsible for familial adenomatous polyposis (FAP) syndrome (25,62,129). These lesions have unusual architecture as their name implies; they exhibit intricate admixtures of cribriform, follicular, papillary, trabecular, and solid patterns of growth (Figure 13), with morular or squamoid areas. Cribriform structures are prominent. The tumor cells are generally cuboidal or tall, with nuclear pseudostratification. Vascular and capsular invasion are common in these lesions, and while they may exhibit lymph node metastasis, there are no data to suggest that they have worse outcomes than other conventional forms of papillary carcinoma. They harbour *ret*/PTC gene rearrangements and do not exhibit loss of heterozygosity of the normal allele of the APC gene to explain an independent mechanism of tumorigenesis. Alterations in the APC

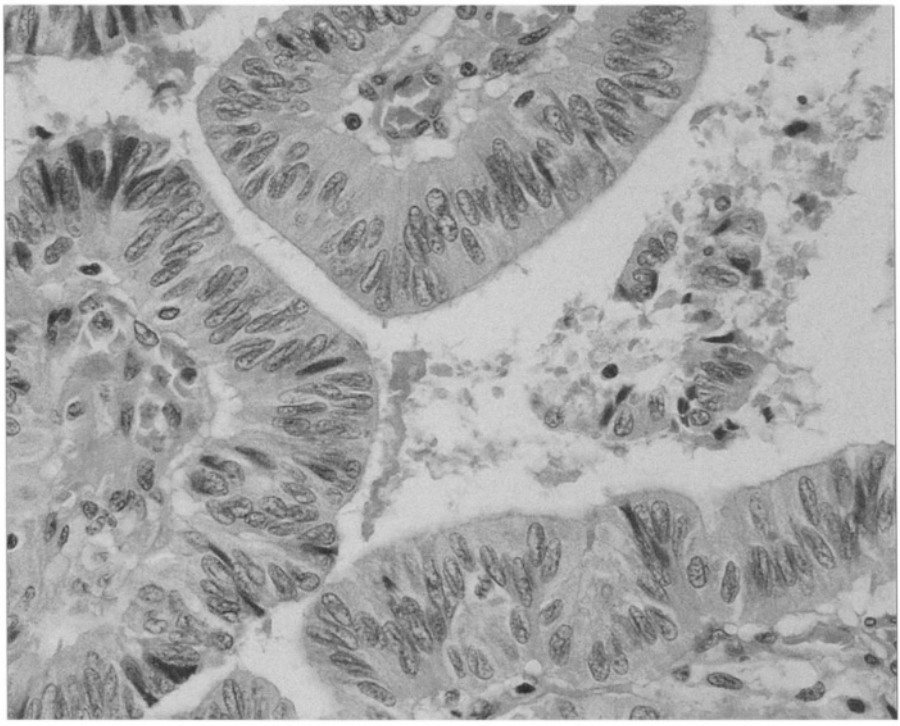

Figure 14. Tall cell papillary carcinoma is composed of a majority of tumour cells that have a height-to-width ratio that exceeds 3:1. These lesions are usually more aggressive than conventional papillary carcinomas.

gene are not thought to underlie the more common sporadic thyroid carcinomas (130,131).

Aggressive variants of papillary carcinoma include *the tall cell variant* and probably related lesions, the *trabecular and columnar cell variant* (132–137). The tall cell variant is defined as a tumor composed of cells that have a height to width ratio that exceeds 3:1 (Figure 14). They usually have complex papillary architecture and may show focal tumor cell necrosis. Tall cells generally have abundant eosinophilic cytoplasm. Columnar cells are similar to tall cells but generally are more crowded with pseudostratification and resemble endometrial lining. The two cell types tend to be found in the same tumours. Tumors that exhibit this feature in more than 30% of the tumor mass generally tend to occur in older individuals with a median age at diagnosis of 20 years older than usual papillary carcinoma, are often large lesions greater than 5 cm and often extend extrathyroidally (134). In addition to lymphatic invasion, vascular invasion is not uncommonly found in these lesions. Tumor mortality rates vary up to 25% for tall cell tumors and 90% for columnar cell carcinoma (136,138).

The management of the less aggressive forms of papillary thyroid carcinoma is controversial. Most experts advocate total thyroidectomy and radioactive iodine therapy (34,50). The rationale for total thyroidectomy is twofold, based on the frequency of bilateral carcinoma and on the need for enhancement of uptake of radioactive iodine by metastatic tumor deposits rather than residual thyroid tissue. However, as shown by the studies of Sugg et al (70), the identification of occult papillary carcinoma in the contralateral lobe is usually not attributable to intrathyroidal dissemination, which would justify further surgery for local disease. Therefore, the major indications for total thyroidectomy are the enhancement of uptake of radioactive iodine and the more sensitive use of thyroglobulin to detect persistent disease (52–54). The controversy involves the management of patients with low risk clinical and pathological parameters; some have advocated less aggressive management with unilateral thyroidectomy and no radioiodine therapy in this setting (49). Recent studies have identified potential markers of those more aggressive tumors that will metastasise to local lymph nodes, including loss of nuclear p27 and upregulation of cyclin D1 (139–141) and these may prove valuable to stratify patients for completion thyroidectomy and radioiodine therapy, but more studies are needed to validate these data. Since there are no controlled clinical trials that address this issue, the answer remains an empirical one. As for follicular carcinoma, external beam radiotherapy is not used in patients with papillary thyroid carcinoma, apart from those with locally advanced tumors that involve extrathyroidal soft tissues of the neck and cannot be completely resected (54,71).

HÜRTHLE CELL LESIONS

Hürthle cells in the thyroid represent a misnomer in that Dr. Hürthle originally described the parafollicular cell. The first description of oxyphilic cells in the thyroid is actually attributed to Askenazy. However, the term Hürthle cell is ingrained in the literature and it is unlikely that the historical error will even be corrected.

The Hürthle cell is derived from the follicular epithelium by metaplasia and possesses the capacity to produce thyroglobulin (142). Morphologically, Hürthle cells are characterised by large size, polygonal to square shape, distinct cell borders, voluminous granular and eosinophilic cytoplasm, prominent nucleus with “cherry-pink” macronucleoli. With the Papanicolau stain, the cytoplasm may be orange, green or blue. By electron microscopy, the cytoplasmic granularity is produced by large mitochondria filling the cell, consistent with oncocytic transformation (143,144). Hürthle cells have been studied by enzyme histochemistry and have been shown to contain a high level of oxidative enzymes (145,146). Somatic mutations and sequence variants of mitochondrial DNA (mtDNA) have been identified in oncocytic thyroid carcinomas (147,148). Similar changes have been found in the nontumorous thyroid tissue of patients with oncocytic neoplasms (148), suggesting that certain polymorphisms predispose to this cytologic alteration.

Hürthle cells are sometimes considered to be a cause of concern in needle biopsies (57). When they are not the major component in a thyroid aspirate, they are not diagnostic of any given lesion. Hürthle cells are found in patients with thyroiditis as

well as in several forms of thyroid neoplasia. Confusion and concern also arises with the histologic diagnosis of Hürthle cell nodules in the thyroid. Hürthle cell nodules found in the setting of thyroiditis or nodular goitre may be hyperplastic. Those lesions that arise in otherwise normal glands are usually encapsulated and are considered to be neoplastic. They can have microfollicular, macrofollicular, trabecular or solid architecture. On occasion, especially with the solid pattern and since these lesions can be extremely vascular, they may resemble medullary thyroid carcinomas and it may be necessary to resort to immunoperoxidase stains for thyroglobulin and calcitonin to obtain the correct diagnosis.

Hürthle cell adenomas and Hürthle cell follicular carcinomas are diagnosed when more than 75% of a lesion is composed of this cell type; the criteria for the diagnosis of lesions that are composed predominantly of Hürthle cells are the same as those applied to follicular lesions that do not contain Hürthle cells (149). The diagnosis of Hürthle cell papillary carcinoma (see below) is possible when the minimal cytologic criteria for papillary carcinoma are present (150).

FNA of Hürthle cell tumors may cause them to partially or totally infarct (151). This probably occurs because of the high metabolic activity of these cells and the delicate blood supply of these lesions that may readily become inadequate after direct trauma. A solitary tumor of the thyroid which occurs in a patient without thyroiditis and which is purely or predominantly composed of Hürthle cells on FNA should be excised, since Hürthle cell tumors show an average of 30% malignancy rate based on histology (149).

Hürthle cell hyperplasia

Hürthle cells are found in the thyroid in a variety of conditions and therefore are not specific for any particular disease. Individual cells, follicles or groups of follicles may show Hürthle cell features in irradiated thyroids, in ageing thyroids, in nodular goitre and in thyroiditis as well as in long-standing autoimmune hyperthyroidism (142). One can see these cells in chronic lymphocytic thyroiditis, in Graves' disease and in nodular goitre, where one can often find an entire nodule composed of oncocytes.

Hürthle cell adenoma and carcinoma

For many years it was felt that all Hürthle cell neoplasms of the thyroid (Figure 15) should be considered malignant since it was felt that the histology could not predict clinical behaviour. However, numerous studies have indicated that the criteria that apply to all follicular neoplasms of the thyroid also distinguish malignant from benign Hürthle cell lesions (149,152–158) . The larger the Hürthle cell lesion, however, the more likely it is to show invasive characteristics; a Hürthle cell tumour which is 4 cm or greater has an 80% chance of showing histologic evidence of malignancy (149). Nuclear atypia, which is the hallmark of the Hürthle cell, multinucleation, and mitotic activity are not useful to predict prognosis and therefore should not be used as diagnostic criteria for malignancy.

A subgroup of Hürthle cell neoplasm has been described which show some atypical features including marked nuclear anaplasia, mitoses, spontaneous infarction and

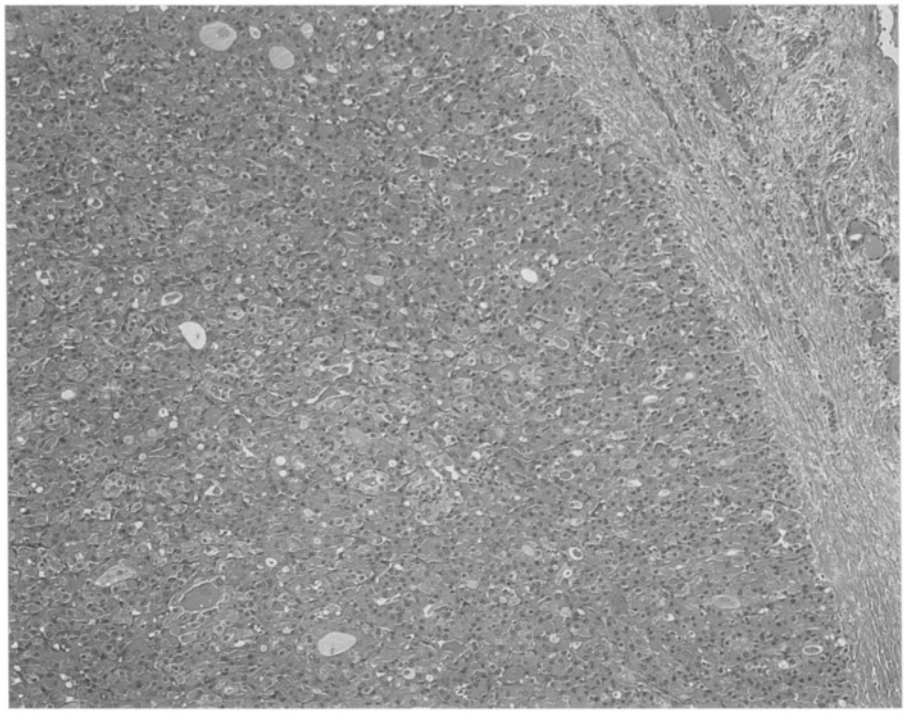

Figure 15. Hürthle cell tumours of thyroid are usually well delineated or encapsulated lesions in which more than 75% of the tumor cells have abundant eosinophilic granular cytoplasm due to the accumulation of spherulated and dilated mitochondria. These cells are derived from follicular epithelium and the criteria used to classify them should be identical to those used for non-oncocytic lesions.

trapping of tumor cells within the capsule in the absence of a preoperative FNA. Some authors have called these "atypical Hürthle cell adenoma" or "tumour of indeterminate malignancy". The great majority of these behave in a clinically benign fashion.

Flow cytometric analyses document aneuploid cell populations in 10 to 25% of Hürthle cell neoplasms that are clinically and histologically classified as adenomas (159–161). Virtually all of these tumours behave in a benign fashion after excision. Among histologically confirmed carcinomas, patients with thyroid tumors that have diploid DNA content tend to have a better prognosis than those with aneuploid values (159,161,162). Oncocytic neoplasms show frequent chromosomal DNA imbalance, with numerical chromosomal alterations being the dominant feature (163). Activating ras mutations are infrequent in oncocytic tumors (163).

The management of Hürthle cell carcinoma is controversial (155,156,164–167). In most institutions patients undergo total thyroidectomy followed by radioactive iodine. Iodine uptake by these lesions tends to be poor. External beam radiotherapy is advocated only for locally invasive disease.

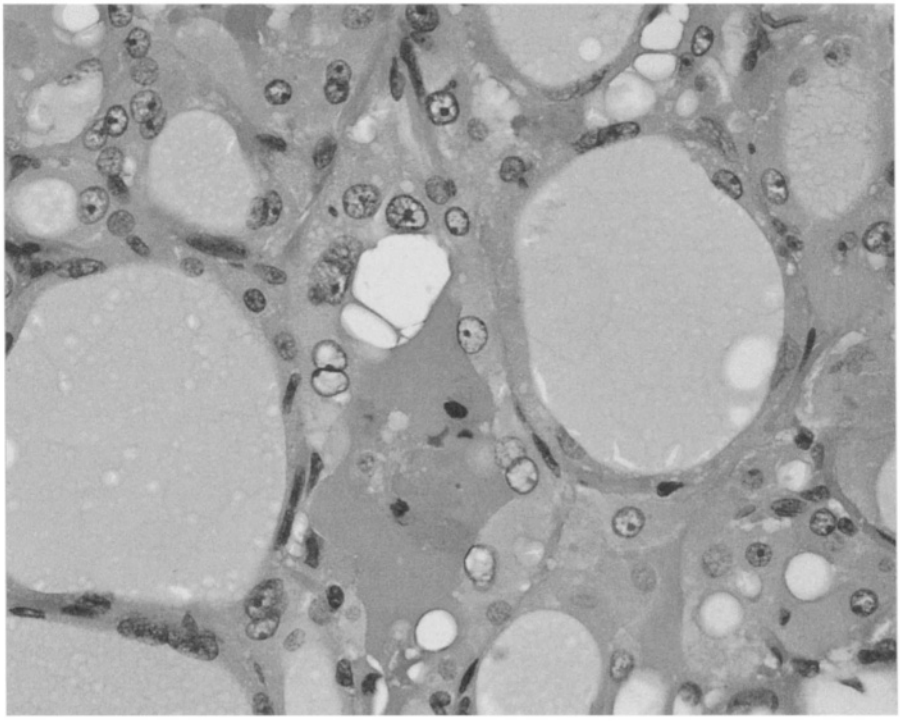

Figure 16. Oncocytic tumours with or without papillae that exhibit the nuclear features of papillary carcinoma represent Hürthle cell or oncocytic papillary carcinomas. This is an example of a follicular lesion that was not invasive, mimicking adenoma, but that harboured a ret/PTC gene rearrangement and metastasized to a local lymph node.

Hürthle cell papillary carcinoma

Many Hürthle cell tumors, whether benign or malignant, show papillary change which is really a pseudopapillary phenomenon, since Hürthle cell neoplasms have only scant stroma and may fall apart during manipulation, fixation and processing.

True oxyphilic or Hürthle cell variant of papillary carcinoma has been reported to comprise from 1 to 11% of all papillary carcinomas (144,168–173). These tumors have papillary architecture, but are composed predominantly or entirely of Hürthle cells (144,174). The nuclei may exhibit the characteristics of usual papillary carcinoma (169,175) (Figure 16), or they may instead resemble the pleomorphic nuclei of Hürthle cells, being large, hyperchromatic and pleomorphic (63,170). The clinical behaviour of this rare subtype is controversial; some authors have reported that they behave like typical papillary carcinomas (63,150,172,174,175), while others maintain that the Hürthle cell morphology confers a more aggressive behaviour (176,177) with higher rates of 10 year tumor recurrence and cause-specific mortality (170). This suggestion

of aggressive behaviour may be attributed to inclusion of tall cell variant papillary carcinoma in the group of Hürthle cell carcinomas.

One morphologic subtype of Hürthle cell papillary carcinoma which, because of a characteristic cystic change and extensive lymphocytic infiltration into the cores of the papillae of the tumour, has a striking histological resemblance to papillary cystadenoma lymphomatosum of the salivary gland and has been called "Warthin-like tumour of the thyroid" (178). This lesion occurs in the setting of chronic lymphocytic thyroiditis, predominantly in women, and is associated with a similar prognosis to usual papillary carcinoma.

The diagnosis of Hürthle cell follicular variant papillary carcinoma remains controversial. Many of these lesions have been diagnosed in the past as Hürthle cell adenoma, however, reports of aggressive behaviour suggested that this diagnosis could not be trusted (156,179). The application of *ret*/PTC analysis by RT-PCR allowed recognition of a follicular variant of Hürthle cell papillary carcinoma as a group of lesions with no invasive behaviour at the time of diagnosis but that harboured a *ret*/PTC gene rearrangement (180,181). Many of these lesions exhibit irregularity of architecture with hypereosinophilic colloid and nuclear features of papillary carcinoma, but these can be obscured by the hyperchromasia and prominent nucleoli of oncocytic change. Nevertheless, they can be recognised when there is a high index of suspicion and with the addition of immunohistochemistry for HBME-1, galectin-3, CK19 and ret or by RT-PCR studies of ret rearrangements. These tumours have the potential to metastasise (182), explaining the occurrence of malignancy in patients with a histopathological diagnosis of adenoma.

Nodules associated with hashimoto's thyroiditis

In 1912, Hashimoto described a well-defined clinicopathologic syndrome consisting of goitre, hypothyroidism, and lymphocytic thyroiditis. It is now generally accepted that the form of lymphocytic thyroiditis known as Hashimoto's thyroiditis is of autoimmune aetiology (183,184). Patients have antibodies to thyroglobulin and to thyroid peroxidase (also know as "microsomal antigen") (185). Some patients also have antibodies to a colloid component other than thyroglobulin "second colloid antigen") and, occasionally, to thyroid hormones. Patients with this disorder are most often women (female-male ratio is 10:1) between 30 and 50 years of age. They typically develop a diffuse, lobulated, asymmetrical, nontender goitre. Most patients with long-standing disease are hypothyroid. Occasionally there is a transient episode of hyperthyroidism known as "Hashitoxicosis" early in the course of the disease; this has been attributed to release of stored hormone during tissue destruction or to stimulation by antibodies to the TSH receptor (185).

The presence of thyroid growth-stimulating immunoglobulins (TGI) in these patients and/or compensatory TSH excess due to tissue destruction and hypothyroidism have been implicated in the development of hyperplastic nodules that present as discrete masses in patients with this disorder. Aspiration of these lesions yields an admixture of epithelial cells and inflammatory cells (57). The hallmark is the Hürthle

cell, a follicular epithelial cell that is characterised by abundant granular cytoplasm and a nucleus often with prominent "cherry pink" nucleolus. The background is composed of small and large lymphocytes, plasma cells, germinal centre fragments and macrophages with or without tangible bodies. Follicular cells and colloid are usually scant but may show nuclear atypia with irregular nuclear contours and prominent grooves.

The appearance of the thyroid involved by Hashimoto's thyroiditis is variable. The gland is usually enlarged and can weigh more than 200 g. It is composed of firm, lobulated, rubbery tissue with a homogeneous, pale grey, fleshy cut surface that lacks colloid translucence and resembles lymphoid tissue. Microscopically, the gland is diffusely infiltrated by mononuclear inflammatory cells, including lymphocytes, plasma cells, immunoblasts, and macrophages. Lymphoid follicles contain well-formed germinal centres. The glandular epithelium exhibits variable degrees of damage. Residual follicles are either atrophic, with sparse colloid and flattened epithelium or exhibit oxyphilic metaplasia, the accumulation of abundant eosinophilic granular cytoplasm characteristic of Hürthle cells (142). Follicular epithelial cells may also exhibit marked cytologic atypia that can be characterised by irregular nuclear membranes, grooves and even clearing of nucleoplasm. These features which in the face of inflammation are considered reactive, mimic papillary carcinoma (3,5). Areas of squamous metaplasia may be found (186). As the disease evolves, fibrosis becomes more conspicuous and in some patients, there is progression to the "fibrous variant" with less prominent lymphocytic infiltration, more prominent squamous metaplasia, and intense fibrosis that almost totally replaces thyroid tissue (187).

The nodules that usually precipitate surgical intervention are cellular areas composed of follicles with variable colloid storage. It is not uncommon for them to be composed predominantly of Hürthle cells and they may be difficult to distinguish from adenomas. The cytologic atypia that resembles that of papillary carcinoma and the fibrosis that can trap follicular epithelium create difficult diagnostic problems. The distinction of thyroid cancer from a reactive process or hyperplasia can be extremely difficult. Application of special techniques is particularly important in this setting. Stains such as HBME-1, galectin-3, CK 19 and ret can be of assistance.

Recent data indicate that glands with Hashimoto's disease express *ret*/PTC gene rearrangements (188). In the author's experience, this is the case when there are nodules of Hürthle cells or micropapillary carcinomas in the tissue submitted for examination, but not if these lesions are carefully excluded from the inflamed tissue examined (70). In general, *ret*/PTC expression in Hürthle cell nodules in this setting identifies gene rearrangements that correlate with other features of papillary carcinoma.

Sudden and rapid enlargement of a nodule in a patients with Hashimoto's thyroiditis may indicate the development of primary thyroid lymphoma which occurs usually in this setting.

POORLY DIFFERENTIATED (INSULAR) CARCINOMA

Poorly differentiated or insular carcinoma is a tumour of follicular cell origin which mimics the architecture of medullary thyroid carcinoma (189–191). The tumour may

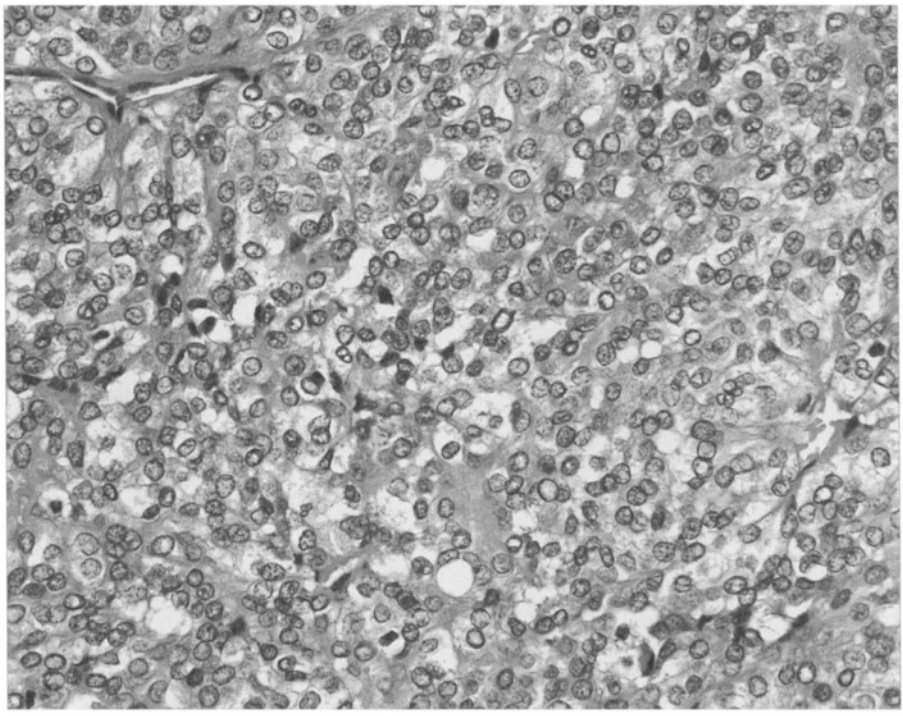

Figure 17. Insular or poorly differentiated carcinoma derived from follicular epithelium can mimick medullary carcinoma since it has a solid nesting architecture. Individual tumour cell necrosis is usually present.

have a central nidus that is encapsulated but usually the lesion exhibits frank capsular invasion and forms satellite nodules in the surrounding thyroid. The tumour architecture is characterised by large well-defined solid nests; it is largely devoid of follicular architecture and devoid of colloid (Figure 17). The tumor cells are usually small and uniform in size and there is a variable degree of mitotic activity. Sclerosis can mimic amyloid, however, congo red stains are negative and immunohistochemical stains for calcitonin, chromogranin and CEA are negative. In contrast the tumors are uniformly positive for thyroglobulin, confirming the follicular cell differentiation of this neoplasm. In contrast to anaplastic carcinomas, there is little pleomorphism and no bizarre, giant, or multinucleated cells are found, however, mitotic activity is identified. Single cell necrosis is a defining feature, but geographic necrosis is unusual.

Insular carcinoma behaves in an aggressive fashion and is often lethal. This is the lesion that most often is identified in cases that have been diagnosed as “widely invasive follicular carcinoma”. Most aggressive Hürthle cell lesions show insular growth and focal tumor cell necrosis. Vascular invasion and or metastases are frequent at the time of diagnosis. Insular carcinoma therefore occupies a position both morphologically

and biologically between differentiated papillary or follicular carcinoma and anaplastic thyroid carcinoma. These tumors are not uncommonly found associated with well differentiated carcinoma (either papillary or follicular) and the insular growth is thought to represent a dedifferentiation phenomenon. Since this entity has only been recognised relatively recently and the clinical literature does not include studies of this tumor type as a separate entity, appropriate clinical management remains to be established.

Clear cell carcinoma is a rare finding in the thyroid and raises important differential diagnoses. The identification of any clear cell lesion should alert the pathologist to the possibility of metastasis, particularly from renal or adrenal tumors (5). However, primary clear cell tumors of thyroid follicular cells occur and are thought to be due to accumulation of glycogen, lipid or even mucin (5). Proof that these represent follicular cells is obtained from thyroglobulin and TTF-1 staining. The term "clear cell tumor" should be restricted to lesions in which more than 75% of the tumor cells show this change.

ANAPLASTIC CARCINOMA

Anaplastic or undifferentiated carcinoma accounts for 5% to 10% of all primary malignant tumors of the thyroid (192) but in many centres this is decreasing with earlier detection of disease. These tumors are rapidly growing, with massive local invasion that usually overshadows the early metastases, most frequently to lung, adrenals and bone (4,5). They are highly lethal with a 5 year survival rate of 7.1% (193) and a mean survival period of 6.2 to 7.2 months (193,194).

Microscopically, anaplastic carcinomas exhibit wide variation. Three general patterns are recognised but most tumours manifest mixed morphology:

The most common type is the *giant cell variant*; as the name suggests, these tumors are composed predominantly of large cells with abundant amphophilic or eosinophilic, often granular cytoplasm and bizarre, often multiple, hyperchromatic nuclei (Figure 18). Some have round, densely acidophilic intracytoplasmic hyaline globules. These tumors grow in solid sheets; artefactual tissue fragmentation may simulate an alveolar pattern.

The squamoid variant is composed of large, moderately pleomorphic epithelial cells that form nests, resembling squamous carcinoma (Figure 19). They may even form keratin pearls.

Spindle cell anaplastic carcinomas have a fascicular architecture and dense stromal collagen with spindle-shaped tumor cells. They may resemble fibrosarcoma; the presence of scattered atypical cells and inflammatory infiltrates may suggest malignant fibrous histiocytoma. Prominent vascularization may suggest hemangioendothelioma (3,5,195).

In all three variants, mitotic figures and atypical mitoses are frequent. There is usually extensive necrosis and in some cases, necrosis may be so extensive that the only viable tumour is around blood vessels. Inflammatory infiltrates are associated with necrosis and the osteoclast-like giant cells that are occasionally found in these tumors have been shown by immunohistochemical studies to be reactive cells of monocytic/histiocytic lineage (196,197).

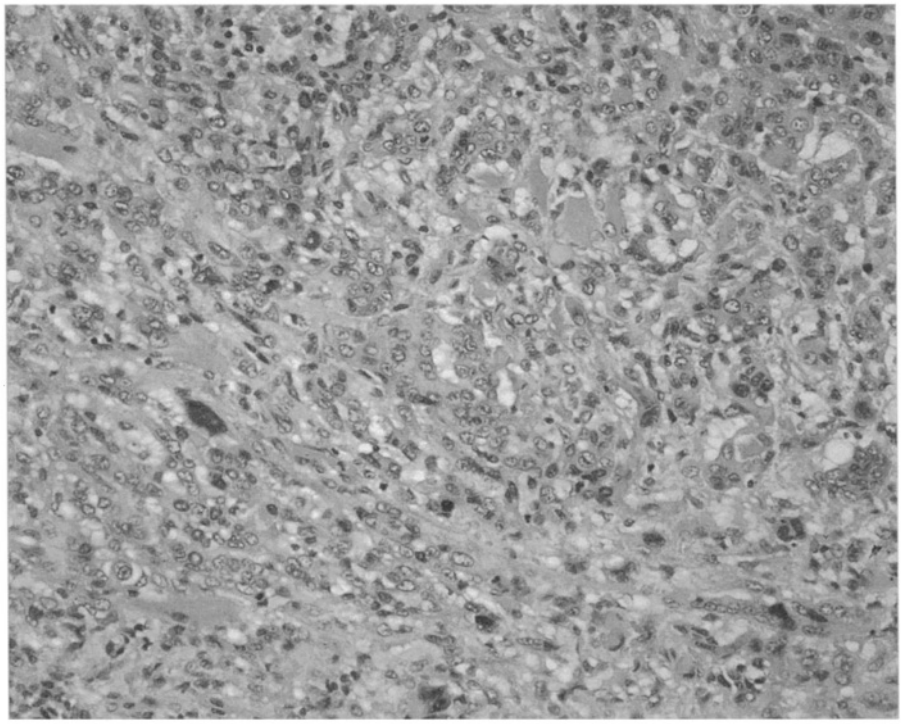

Figure 18. Anaplastic carcinoma may arise in differentiated carcinoma; it is characterized by anaplastic giant cells, prominent mitoses and geographic necrosis (not shown).

Anaplastic carcinomas are highly infiltrative. Malignant cells usually grow between residual thyroid follicles and invade skeletal muscle, adipose tissue and other perithyroidal structures. Blood vessel invasion and thrombosis with or without tumour cell involvement is frequent.

The appearances of anaplastic carcinoma on FNA are quite varied and reflect the histologic type with giant cells or squamoid cells or spindle cells. There is high cellularity, with necrosis, acute inflammation and marked cellular pleomorphism. Mitoses are often atypical and no colloid is seen.

Immunohistochemistry is useful in only a limited fashion in the diagnosis of these lesions. Most anaplastic carcinomas do not contain convincing reactivity for thyroglobulin and the few that are positive have only a weak or focal reaction (194, 197–201). This staining must be interpreted carefully, since it may reflect trapped nontumorous follicles or follicular cells, and since thyroglobulin is known to diffuse into non-follicular cells (5). The epithelial nature of the tumor cells can be verified with stains for cytokeratins but again most undifferentiated lesions are negative for this marker. Squamoid areas may exhibit reactivity for high molecular weight keratins and/or epithelial membrane antigen (EMA) (194, 197–199). CEA reactivity may be found in the centre of squamous

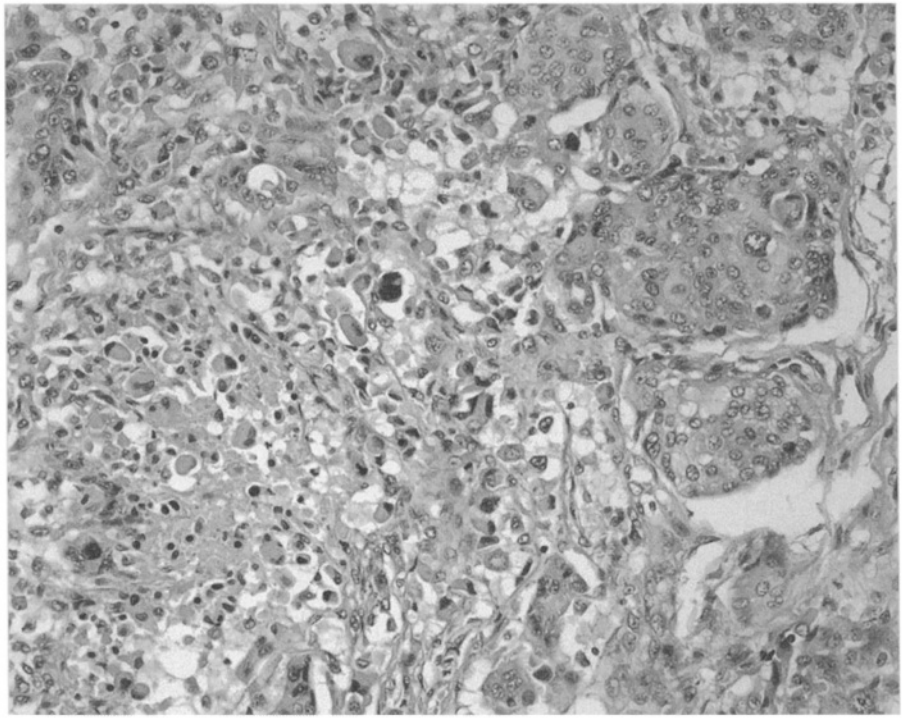

Figure 19. Some anaplastic carcinomas exhibit rhabdoid and/or sqamous morphology.

nests (194,197). Anaplastic tumours have been reported to be positive for calcitonin, but this finding should alter the diagnosis to that of medullary carcinoma (5).

p53 mutations are common in anaplastic thyroid carcinomas (202–208); since mutated forms of this tumour suppressor gene have prolonged half lives, the application of immunohistochemistry has yielded positive results in these tumours (209,210). (Chapter 8).

By electron microscopy (196,198,201,211,212), there may be formation of intercellular junctions, microvilli, and basal lamina, providing evidence of epithelial differentiation. However, many tumors do not exhibit evidence of any differentiation. Their large nuclei have prominent nucleoli and clumped chromatin; usually the cytoplasm contains only poorly developed rough endoplasmic reticulum, scattered dense bodies, lipid droplets, numerous free ribosomes, mitochondria and lysosomes. Intermediate filaments (keratin or vimentin) may form filamentous whorls that correspond to the acidophilic hyaline globules seen by light microscopy. Secretory granules are not seen in these tumours.

Most anaplastic thyroid carcinomas are aneuploid on flow cytometry; this abnormality correlates with poor outcome (162).

Some tumors do not exhibit immunohistochemical or ultrastructural markers that allow classification as epithelial malignancies. Nevertheless, the diagnosis of anaplastic

carcinoma should be favoured for pleomorphic lesions in older patients if they arise in the thyroid.

Small cell carcinomas and lymphomas constitute a common source of diagnostic error, often misclassified as anaplastic carcinomas (3,5,195,198). The former are usually poorly differentiated medullary carcinomas, which can also mimic giant cell or spindle cell anaplastic carcinomas; the latter are readily identified by staining for leukocyte common antigen (LCA) and other markers of lymphoid cells. Rarely, primary intrathyroidal thymoma may be mistaken for anaplastic carcinoma (213,214).

The reported association between well-differentiated thyroid carcinoma and anaplastic carcinoma ranges from 7% to 89% of cases, however, the lower figures are likely underestimates, attributable to inadequate sampling (3,193,194,198,215–217). The data suggest that anaplastic carcinoma originates most often in an abnormal thyroid; the tumor has a higher incidence in regions of endemic goitre and a history of goitre is reported in over 80% of cases (3,193). As stated above, nodular goitre is often the site of monoclonal proliferation, the first step in the hyperplasia-neoplasia sequence. However, it is difficult to document transformation of a benign lesion to a malignant tumor. Insular carcinoma appears to be intermediate in the spectrum, and may represent a transition form (190,217). The association of papillary carcinoma, particularly the more aggressive tall cell variant, with anaplastic tumors has also been described (3,217,218). Thyroid carcinomas can exhibit an entire spectrum of differentiation through insular to anaplastic foci. The significance of microscopic insular or anaplastic change is controversial; some people have suggested that focal microscopic dedifferentiation does not alter prognosis but others have shown that this finding alone is statistically significant as a marker of aggressive behaviour.

The factors underlying dedifferentiation in thyroid tumors remain to be established; age and radiation have been implicated (219,220). Clearly, the vast majority of well differentiated thyroid lesions do not undergo such transformation. A pattern of genetic mutations resulting in oncogene activation or loss of tumour suppressor gene activity has been proposed to correlate with the stepwise progression from adenoma to carcinoma and through the dedifferentiation process in thyroid (202,203).

MEDULLARY CARCINOMA

Medullary carcinoma of the thyroid comprises 5–10% of all thyroid carcinomas (5). This lesion is usually readily recognised because of its unusual cytologic and histologic features but sometimes special investigation is required to distinguish it from follicular lesions or other tumours, including lymphomas and/or anaplastic carcinomas.

The aspirate from medullary carcinoma has a variable appearance. The cells may be spindle-shaped, columnar or plasmacytoid; they may even exhibit oncocytic or clear cell morphology. Nucleoli and nuclear pseudoinclusions are often seen. Amyloid is identified in up to 60% of cases as homogeneous, spherical or rod-shaped extracellular material which polarises with the Pap stain or the Congo Red stain. The diagnosis is confirmed by immunostaining for calcitonin or the demonstration of secretory granules on electron microscopy.

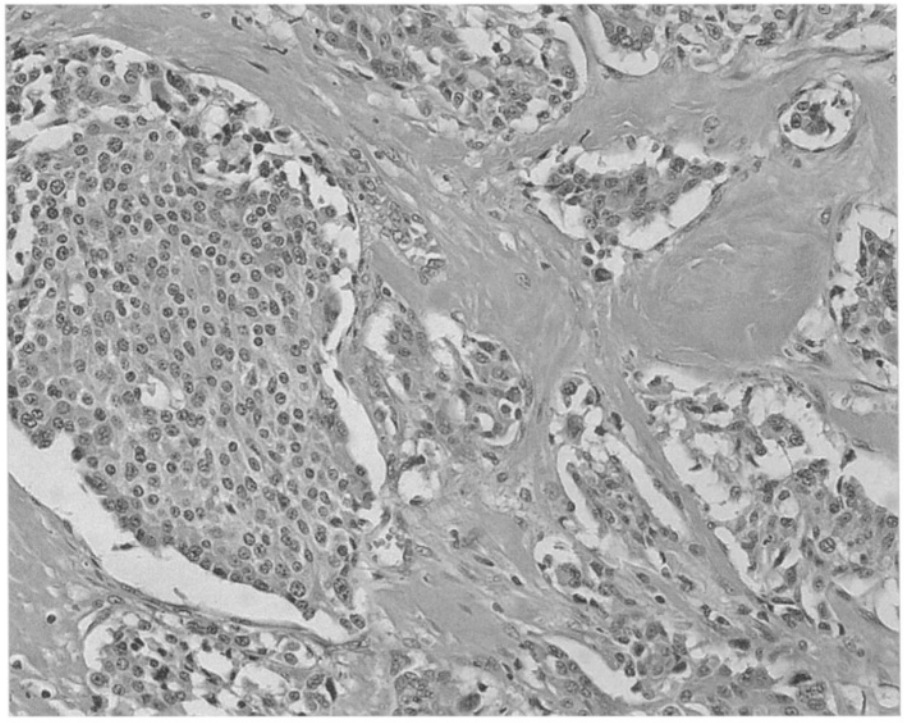

Figure 20. Medullary carcinoma of thyroid is derived from the calcitonin-producing C cells that are neuroendocrine cells. These lesions are composed of solid nests of epithelial cells with poorly defined cell borders. They often have stromal fibrosis and occasionally there is deposition of intensely eosinophilic material, amyloid, derived from the calcitonin precursor molecule.

Medullary carcinoma has a wide range of histologic appearances (2,221). Typically, the tumors are composed of sheets or more usually nests of round, polyhedral or spindle-shaped cells which may exhibit palisading at the periphery (Figure 20). The stroma is vascular. There may be prominent amyloid in the stroma, which, when present, provides a helpful diagnostic marker. However, although amyloid is present in more than half of these tumours, it may be intracytoplasmic and difficult to identify without a high index of suspicion. In addition, amyloid may also be present in occasional non-medullary thyroid carcinomas (222).

Sometimes, fixation artefact produces a pseudopapillary appearance; areas of true papillary architecture may also be found and the distinction of such lesions from papillary carcinoma can be difficult (223). A pseudofollicular appearance frequently results from entrapped nonneoplastic thyroid follicles or rounded masses of amyloid and true glandular variants have been described. Dedifferentiation results in a small cell tumour morphology, which can mimic lymphoma. Oncocytic features may predominate and make the distinction of medullary from oncocytic follicular carcinoma difficult.

Foreign body giant cells may be associated with amyloid deposits and calcification may be identified. These features may result in difficult differential diagnosis. True psammoma bodies are generally not seen in these tumours but have been reported.

Staining for amyloid can be helpful. Congo Red staining is typical and the apple-green birefringence with polarised light is diagnostic. Nevertheless, as indicated, some follicular tumors may also contain amyloid stroma.

Immunohistochemical staining represents the gold standard for the diagnosis of medullary thyroid carcinoma. These tumours express cytokeratins, chromogranin A, and NSE, but the most specific diagnostic marker is calcitonin. The number of calcitonin-positive cells varies from case to case, but the diagnosis should be questioned in the absence of calcitonin staining. The amyloid in these tumours often stains for calcitonin, likely because the amyloid protein represents deposition of a precursor of the calcitonin molecule.

These tumours also stain for carcinoembryonic antigen (CEA) and the inverse relationship between the intensity of staining for calcitonin and that for CEA may be prognostically significant: tumors containing few calcitonin-positive cells and abundant CEA immunoreactivity are said to have a worse prognosis than the well differentiated tumours with strong calcitonin immunoreactivity (224,225). CEA is not identified in follicular thyroid tumors; occasional reports of positivity are attributable to use of antibodies that react with non-specific cross-reacting antigens (226). Therefore CEA positivity indicates the presence of medullary thyroid carcinoma or other lesions such as metastatic carcinomas or thymic carcinomas.

Medullary thyroid carcinomas also produce a number of other peptides including somatostatin, derivatives of the proopiomelanocortin molecule (ACTH, MSH, ß-endorphin and enkephalin), serotonin, glucagon, gastrin, cholecystokinin, VIP, bombesin, and α-HCG (5,227–229). Calcitonin gene-related peptide (CGRP) is also identified in normal C-cells as well as medullary thyroid carcinomas. Individual tumours may express a variety of these various hormones but none have been shown to correlate with altered prognosis (230).

Ultrastructural examination confirms the presence of cells that do not form desmosomes but do show complex interdigitations of cell membranes. The cytoplasm contains characteristic membrane-bound secretory granules which usually are numerous and variable in size.

The importance of distinguishing this tumour from follicular lesions is two-fold. The first is for diagnostic classification and management considerations in the individual patient. These tumors do not preferentially take up iodine and therapy with radioactive iodine is not indicated; in contrast, expression of somatostatin receptors by some of these tumors (231) makes the octreoscan a feasible diagnostic tool to localise the primary lesion and to identify metastatic deposits (232) and somatostatin analoges may have applications in the management of disseminated disease (233). The other aspect of management involves the implications for both the patient and members of his/her family, since many of these tumours are hereditary (234).

The inherited forms of medullary carcinoma are of three types: familial medullary thyroid carcinoma alone (FMTC), multiple endocrine neoplasia (MEN) type IIA in

which MTC is associated with pheochromocytomas, and MEN IIB in which the thyroid and adrenal proliferative disorders are associated with mucosal ganglioneuromas and a Marfanoid habitus. The inheritance of all three syndromes was mapped to the pericentromeric region of chromosome 10 by linkage analysis (235–237). Subsequently, mutations in exons 10 and 11 of the *ret* proto-oncogene in patients with FMTC or MEN IIA and at codon 918 in MEN IIB (238,239) have provided a more accurate marker of germline mutation and predisposition to this disease (240). Current recommendations suggest that family members of FMTC and MEN IIA kindreds have genetic screening early in life and affected members should undergo total thyroidectomy at around the age of 5 years. This age was chosen because of the early onset of medullary thyroid carcinoma in these familial forms of the disease; metastatic tumour has been found in patients as young as 6 years of age. Affected children with MEN IIB undergo surgery even earlier (241, Chapter 24).

Sporadic medullary carcinomas also may have mutations of *ret* in the same codons as the familial disorders (239,242); the mutation involved may have prognostic value (243). The presence of *ret* mutations in sporadic tumours indicates the importance of analysing DNA from white blood cells to establish that a mutation is germ line, therefore potentially hereditary. Other oncogenes and tumor suppressor genes have not been implicated in the pathogenesis of MCT: *ras* mutations are rare, c-*myc,* and c-*erb*B are not amplified (244,245), and p53 mutations are not found in these tumors (246).

Familial forms of medullary thyroid carcinoma usually result in multicentric disease as well as multicentric C-cell hyperplasia (247). Many definitions of C-cell hyperplasia have been offered, all requiring immunohistochemistry since C cells cannot be reliably recognised with routine histologic stains. Quantitation of C cells as well as geographic mapping throughout the gland must be performed (247,248). C cells are usually limited to the central portion of the junction between the upper and middle thirds of the lateral lobes where they are generally distributed singly rather than in clusters. Increased numbers of C cells (>7 cells per cluster), complete follicles surrounded by C cells, and distribution of cells beyond this geographic location are indicative of C-cell hyperplasia. The presence of C-cell hyperplasia usually indicates an inherited disorder rather than a sporadic lesion, however, C-cell hyperplasia can also be associated with chronic hypercalcemia, thyroid follicular nodular disease, and thyroiditis (249–252).

The identification of oncogenic activation of *ret* in familial C cell disease has raised questions about the term “C cell hyperplasia”. In this disorder, unlike other familial cancer syndromes that result from inactivation of tumour suppressor genes, each affected member is born with an activated oncogene. Theoretically, then, every C cell has already undergone transformation, since it does not appear to require a second hit to knock out protective mechanisms. If this proves to be true, it will suggest that the term “C cell hyperplasia” is a misnomer, since each C cell with its activated oncogene is a transformed cell that represents a site of neoplastic potential. This remains to be proven, however, and the mechanism of tumorigenesis in C cells of the thyroid, as it unfolds, will shed further light on the biology of neoplasia.

MIXED FOLLICULAR-C CELL LESIONS

Although controversial, mixed follicular-parafollicular cell carcinomas do occur (253); these rare monomorphous tumours are composed of cells showing dual differentiation (254,255). Composite tumors are composed of two intermixed well differentiated components, one showing thyroglobulin immunoreactivity and either papillary or follicular architecture and cytology, the other with calcitonin and CEA immunopositivity (81,256). The diagnosis of a mixed or composite tumor can be convincing only in cases where metastatic disease is identified, since the identification of thyroglobulin and calcitonin in a primary intrathyroidal tumor may represent the identification of a typical medullary thyroid carcinoma with trapped nontumorous elements containing thyroglobulin, or phagocytosis of thyroglobulin by medullary carcinoma cells. Moreover, the two tumours may occur separately in the same gland and metastasise together to a regional node (257,258).

CONCLUSION

Thyroid nodules are common and their management can be difficult and controversial. Clearly, the pathologist has an important role to play in their evaluation. The use of fine needle aspiration biopsy has significantly improved our ability to identify specific high risk disorders and to facilitate their management in an expeditious and cost-effective manner. Patients who require surgery for further confirmation of the disease process rely upon the pathologist to correctly characterize their nodule and pathologists are actively involved in research to clarify the pathogenesis of thyroid disease. There are other areas of thyroid pathology that have seen uniform advances in our understanding of the pathobiology of disease. Most experts accept tall cell or columnar morphology as predictive of more aggressive variants of papillary carcinoma. The recognition of insular carcinomas as an intermediate category of "poorly differentiated carcinoma" has been validated by clinical and molecular studies. The biology of familial genetic alterations in medullary carcinoma has revolutionised patient care. Advances in our understanding of the molecular basis of thyroid cancer will allow more accurate characterization of specific subtypes of neoplasia and malignancy even on single cells obtained at fine needle aspiration biopsy. This should further enhance the usefulness of this technique and better guide the management of patients with a thyroid nodule.

REFERENCES

1. Ezzat S, Sarti DA, Cain DR, Braunstein GD. Thyroid incidentalomas. Prevalence by palpation and ultrasonography. Arch Intern Med 1994; 154:1838–1840.
2. Hedinger C, Williams ED, Sobin LH. The WHO histological classification of thyroid tumors: A commentary on the second edition. Cancer 1989; 63:908–911.
3. LiVolsi VA. Surgical Pathology of the Thyroid. Philadelphia, P.A.: W.B, Saunders, 1990.
4. Murray D. The thyroid gland. In: Kovacs K, Asa SL, editors. Functional Endocrine Pathology. Boston: Blackwell Science, 1998: 295–380.
5. Rosai J, Carcangiu ML, DeLellis RA. Tumors of the Thyroid Gland. Atlas of Tumor Pathology, Third Series, Fascicle 5. Washington, D.C.: Armed Forces Institute of Pathology, 1992.
6. Lloyd RV, Erickson LA, Sebo TJ. Diagnosis of follicular variant of papillary carcinoma by a panel of endocrine pathologists. Lab Invest, 106A. 2003.

7. Studer H, Peter H-J, Gerber H. Natural heterogeneity of thyroid cells: The basis for understanding thyroid function and nodular goiter growth. Endocr Rev 1989; 10:125–135.
8. Aeschimann S, Kopp PA, Kimura ET et al. Morphological and functional polymorphism within clonal thyroid nodules. J Clin Endocrinol Metab 1993; 77:846–851.
9. Studer H, Ramelli F. Simple goiter and its variants: Euthyroid and hyperthyroid multinodular goiters. Endocr Rev 1982; 3:40–61.
10. Peter HJ, Gerber H, Studer H, Smeds S. Pathogenesis of heterogeneity in human multinodular goiter. A study on growth and function of thyroid tissue transplanted onto nude mice. J Clin Invest 1985; 76:1992–2002.
11. Studer H, Hunziker HR, Ruchti C. Morphologic and functional substrate of thyrotoxicosis caused by nodular goiters. Am J Med 1978; 65:227–234.
12. Drexhage HA, Bottazzo GF, Bitensky L, Chayen J, Doniach D. Evidence for thyroid growth-stimulating immunoglobulin in some goitrous thyroid diseases. Lancet 1980; 2:287–292.
13. Van der Gaag RD, Drexhage HA, Wiersinga WM et al. Further studies on thyroid growth-stimulating immunoglobulins in euthyroid nonendemic goiter. J Clin Endocrinol Metab 1985; 60:972–979.
14. Studer H, Peter H-J, Gerber H. Toxic nodular goitre. J Clin Endocrinol Metab 1985; 14:351–372.
15. Wiener JD, Van der Gaag RD. Autoimmunity and the pathogenesis of localized thyroid autonomy (Plummer's disease). Clin Endocrinol 1985; 23:635–642.
16. Apel RL, Ezzat S, Bapat B, Pan N, LiVolsi VA, Asa SL. Clonality of thyroid nodules in sporadic goiter. Diag Mol Pathol 1995; 4:113–121.
17. Bamberger AM, Bamberger CM, Barth J, Heidorn K, Kreipe H, Schulte HM. Clonal composition of thyroid nodules from patients with multinodular goiters: Determination by X-chromosome inactivation analysis with M27beta. Experimental and Clinical Endocrinology (Leipzig) suppl.1, 73. 1993.
18. Lyons J, Landis CA, Harsh G. Two G protein oncogenes in human endocrine tumors. Science 1990; 249:635–639.
19. Porcellini A, Ciullo I, Laviola L, Amabile G, Fenzi G, Avvedimento VE. Novel mutations of thyrotropin receptor gene in thyroid hyperfunctioning adenomas. Rapid identification by fine needle aspiration biopsy. J Clin Endocrinol Metab 1994; 79:657–661.
20. van Sande J, Parma J, Tonacchera M, Swillens S, Dumont J, Vassart G. Genetic basis of endocrine disease. Somatic and germline mutations of the TSH receptor gene in thyroid disease. J Clin Endocrinol Metab 1995; 80:2577–2585.
21. Parma J, Duprez L, Van SandemH et al. Diversity and prevalence of somatic mutations in the thyrotropin receptor and Gs alpha genes as a cause of toxic thryoid adenomas. J Clin Endocrinol Metab 1997; 82:2695–2701.
22. Krohn D, Fuhrer D, Holzapfel H, Paschke R. Clonal origin of toxic thyroid nodules with constitutively activating thyrotropin receptor mutations. J Clin Endocrinol Metab 1998; 83:180–184.
23. Ezzat S, Zheng L, Kholenda J, Safarian A, Freeman JL, Asa SL. Prevalence of activating *ras* mutations in morphologically characterized thyroid nodules. Thyroid 1996; 6:409–416.
24. Hicks DG, LiVolsi VA, Neidich JA, PuckJM, Kant JA. Clonal analysis of solitary follicular nodules in the thyroid. Am J Pathol 1990; 137:553–562.
25. Cetta F, Toti P, Petracci M et al. Thyroid carcinoma associated with familial adenomatous polyposis. Histopathology 1997; 31(3):23l–236.
26. Namba H, Matsuo K, Fagin JA. Clonal composition of benign and malignant human thyroid tumors. J Clin Invest 1990; 86:120–125.
27. Bronner MP, Hamilton R, LiVolsi VA. Utility of frozen section analysis on follicular lesions of the thyroid. Endocr Pathol 1994; 5:154–161.
28. Treseler PA, Clark OH. Prognostic factors in thyroid carcinoma. Surg Oncol Clin N Am 1997; 6:555–598.
29. Clark OH. Predictors of thyroid tumor aggressiveness. West J Med 1996; 165:131–138.
30. Yamashina M. Follicular neoplasms of the thyroid. Total circumferential evaluation of the fibrous capsule. Am J Surg Pathol 1992; 16:392–400.
31. Kahn NF, Perzin KH. Follicular carcinoma of the thyroid: An evaluation of the histologic criteria used for diagnosis. Pathol Annu 1983; 1:221–253.
32. Jorda M, Gonzalez-Campora R, Mora J, Herrero-Zapatero A, Otal C, Galera H. Prognostic factors in follicular carcinoma of the thyroid. Arch Pathol Lab Med 1993; 117:631–635.
33. van Heerden JA, Hay ID, GoellnerJR et al. Follicular thyroid carcinoma with capsular invasion alone: A nonthreatening malignancy. Surgery 1992; 112:1130–1138.

34. Harness JK, Thompson NW, McLeod MK, Eckhauser FE, Lloyd RV. Follicular carcinoma of the thyroid gland: Trends and treatment. Surgery 1984; 96:972–980.
35. Miettinen M, Karkkainen P. Differential reactivity of HBME-1 and CD15 antibodies in benign and malignant thyroid tumours. Preferential reactivity with malignant tumours. Virchows Arch 1996; 429:213–219.
36. Sack MJ, Astengo-Osuna C, Lin BT, Battifora H, LiVolsi VA. HBME-1 immunostaining in thyroid fine-needle aspirations: a useful marker in the diagnosis of carcinoma. Mod Pathol 1997; 10:668–674.
37. van Hoeven KH, Kovatich AJ, Miettinen M. Immunocytochemical evaluation of HBME-1, CA 19-9, and CD-15 (Leu-Mi) in fine-needle aspirates of thyroid nodules. Diagn Cytopathol 1997; 18:93–97.
38. Fernandez PL, Merino MJ, Gomez M et al. Galectin-3 and laminin expression in neoplastic and non-neoplastic thyroid tissue. J Pathol 1997; 181:80–86.
39. Orlandi F, Saggiorato E, Pivano G et al. Galectin-3 is a presurgical marker of human thyroid carcinoma. Cancer Res 1998; 58:3015–3020.
40. Cvejic D, Savin S, Paunovic I, Tatic S, Havelka M, Sindinovic J. Irnmuhohistochemical localization of galectin-3 in malignant and benign human thyroid tissue. Anticancer Res 1998; 18:2637–2642.
41. Inohara H, Honjo Y, Yoishii T et al. Expression of galectin-3 in fine-needle aspirates as a diagnostic marker differentiating benign from malignant thyroid neoplasms. Cancer 1999; 85:2475–2484.
42. Kroll TG, Sarraf P, Pecciarini L et al. PAX8-PPARγ1 fusion oncogene in human thyroid carcinoma. Science 2000; 289(5483):1357–1360.
43. LiVolsi VA, Asa SL. The demise of follicular carcinoma of the thyroid gland. Thyroid 1994; 4:233–235.
44. Nikiforova MN, Biddinger PW, Caudill CM, Kroll TG, Nikiforov YE. PAX8-PPAR gamma rearrangement in thyroid tumors: RT-PCR and immunohistochemical analyses. Am J Surg Pathol 2002; 26(8):1016–1023.
45. Zedenius J, Auer G, Bäckdahl M et al. Follicular tumors of the thyroid gland: Diagnosis, clinical aspects and nuclear DNA analysis. World J Surg 1992; 16:589–594.
46. Samaan NA, Schultz PN, Haynie TP, Ordonez NG. Pulmonary metastasis of differentiated thyroid carcinoma: Treatment results in 101 patients. J Clin Endocrinol Metab 1985; 65:376–380.
47. DeGroot LJ, Kaplan EL, Shukla MS, Salti G, Straus FH. Morbidity and mortality in follicular thyroid cancer. J Clin Endocrinol Metab 1995; 80:2946–2953.
48. Mizukami Y, Michigishi T, Nonomura A et al. Distant metastases in differentiated thyroid carcinomas: A clinical and pathologic study. Hum Pathol 1990; 21:283–290.
49. Shah JP, Loree TR, Dharker D, Strong EW. Lobectomy versus total thyroidectomy for differentiated carcinoma of the thyroid: a matched-pair analysis. Am J Surg 1993; 166:331–335.
50. Singer PA, Cooper DS, Daniels GH et al. Treatment guidelines for patients with thyroid nodules and well-differentiated thyroid cancer. American Thyroid Association. Arch Intern Med 1996; 156:2165–2172.
51. Pasieka JL, Thompson NW, McLeod MK, Burney RE, Macha M. The incidence of bilateral well-differentiated thyroid cancer found at completion thyroidectomy. World J Surg 1992; 16:711–717.
52. Benker G, Olbricht Th, Reinwein D et al. Survival rates in patients with differentiated thyroid carcinoma. Influence of postoperative external radiotherapy. Cancer 1990; 65:1517–1520.
53. Mazzaferri EL, Jhiang SM. Long-term impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994; 97:418–428.
54. Tsang RW, Brierley JD, Simpson WJ, Panzarella T, Gospodarowicz MK, Sutcliffe SB. The effects of surgery, radioiodine, and external radiation therapy on the clinical outocme of patients with differentiated thyroid carcinoma. Cancer 1998; 82:375–388.
55. LiVolsi VA, Merino MJ. Worrisome histologic alterations following fine needle aspiration of the thyroid. Pathol Annu 1994; 29(2):99–120.
56. Namba H, Ross JL, Goodman D, Fagin JA. Solitary polyclonal autonomous thyroid nodule: A rare cause of childhood hyperthyroidism. J Clin Endocrinol Metab 1991; 72:1108–1112.
57. Kini SR. Thyroid. 2 ed. New York: Igaku-Shoin Ltd., 1996.
58. Vickery AL. Thyroid papillary carcinoma. Pathological and philosophical controversies. Am J Surg Pathol 1983; 7:797–807.
59. Rosai J, Zampi G, Carcangiu ML, Pupi A, et al. Papillary carcinoma of the thyroid. Am J Surg Pathol 1983; 7:809–817.
60. Vickery AL, Carcangiu ML, Johannessen JV, et al. Papillary carcinoma. Semin Diagn Pathol 1985; 2:90–100.
61. LiVolsi VA. Papillary neoplasms of the thyroid. Pathologic and prognostic features. Am J Clin Pathol 1992; 97:426–434.

62. Soravia C, Sugg SL, Berk T et al. Familial adenomatous polyposis-associated thyroid cancer. Am J Pathol 1999; 154:127–135.
63. Hedinger C, Williams ED, Sobin LH. Histological typing of thyroid tumours. World Health Organization International Histological Classification of Tumours. 2 ed. Berlin: Springer-Verlag, 1988.
64. Hay ID. Papillary thyroid carcinoma. Endocrinol Metab Clin North Am 1990; 19:545–576.
65. Mazzaferri E, Jhiang S. Long-term follow-up impact of initial surgical and medical therapy on papillary and follicular thyroid cancer. Am J Med 1994; 97:418–428.
66. Hapke MR, Dehner LP. The optically clear nucleus. A reliable sign of papillary carcinoma of the thyroid? Am J Surg Pathol 1979; 3:31–38.
67. Chan JKC, Saw D. The grooved nucleus: A useful diagnostic criterion of papillary carcinoma of the thyroid. Am J Surg Pathol 1986; 10:672–679.
68. Deligeorgi-Politi H. Nuclear crease as a cytodiagnostic feature of papillary thyroid carcinoma in fine-needle aspiration biopsies. Diagn Cytopathol 1987; 3:307–310.
69. Fink A, Tomlinson G, Freeman JL, Rosen IB, Asa SL. Occult micropapillary carcinoma associated with benign follicular thyroid disease and unrelated thyroid neoplasms. Mod Pathol 1996; 9(8):816–820.
70. Sugg SL, Ezzat S, Rosen IB, Freeman J, Asa SL. Distinct multiple *ret*/PTC gene rearrangements in multifocal papillary thyroid neoplasia. J Clin Endocrinol Metab 1998; 83:4116–4122.
71. Brierley JD, Panzarella T, Tsang RW, Gospodarowicz MK, O'Sullivan B. A comparison of different staging systems predictability of patient outcome. Thyroid carcinoma as an example. Cancer 1997; 79:2414–2423.
72. Noguchi M, Tanaka S, Akiyama T, et al. Clinicopathological studies of minimal thyroid and ordinary thyroid cancers. Jpn J Surg 1984; 13:110–117.
73. Harach HR, Franssila KO, Wasenius V-M. Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study. Cancer 1985; 56:531–538.
74. Yamashita H, Nakayama I, Noguchi S et al. Thyroid carcinoma in benign thyroid diseases. An analysis from minute carcinoma. Acta Pathol Jpn 1985; 35(4):781–788.
75. Yamashita H, Noguchi S, Murkama N, et al. Prognosis of minute carcinoma of the thyroid. Follow-up study of 48 patients. Acta Pathol Jpn 1986; 36:1469–1475.
76. Schröder S, Schwarz W, Rehpenning W, Löning T, Böocker W. Dendritic/Langerhans cells and prognosis in patients with papillary thyroid carcinomas. Am J Clin Pathol 1988; 89:295–300.
77. Volpé R. Immunology of human thyroid disease. In: Volpé R, editor. Autoimmune diseases of the endocrine system. Boca Raton: CRC Press, 1990: 73–240.
78. Leung CS, Hartwick RWJ, Bédard YC. Correlation of cytologic and histologic features in variants of papillary carcinoma of the thyroid. Acat Cytol 1993; 37:645–650.
79. Hawk WA, Hazard JB. The many appearances of papillary carcinoma of the thyroid. Cleve Clin Q 1976; 43:207–216.
80. Chan JK. Papillary carcinoma of the thyroid: classical and variants. Histol Histopathol 1990; 5:241–257.
81. Mizukami Y, Michigishi T, Nonomura A et al. Mixed medullary-follicular carcinoma of the thyroid occurring in familial form. Histopathology 1993; 22:284–287.
82. Evans HL. Encapsulated papillary neoplasms of the thyroid. A study of 14 cases followed for a minimum of 10 years. Am J Surg Pathol 1987; 11:592–597.
83. Chen KTK, Rosai J. Follicular variant of thyroid papillary carcinoma: A clinicopathologic study of six cases. Am J Surg Pathol 1977; 1:123–130.
84. Tielens ET, Sherman SI, Hruban RH, et al. Follicular variant of papillary thyroid carcinoma; a clinicopathologic study. Cancer 1994; 73:424–431.
85. Raphael SJ, Apel RL, Asa SL. Detection of high-molecular-weight cytokeratins in neoplastic and non-neoplastic thyroid tumors using microwave antigen retrieval. Mod Pathol 1995; 8:870–872.
86. Santoro M, Carlomagno F, Hay ID et al. Ret oncogene activation in human thyroid neoplasms is restricted to the papillary cancer subtype. J Clin Invest 1992; 89:1517–1522.
87. Tallini G, Asa SL. RET oncogene activation in papillary thyroid carcinoma. Adv Anat Pathol 2001; 8(6):345–354.
88. Fusco A, Grieco M, Santoro M et al. A new oncogene in human thyroid papillary carcinomas and their lymph-nodal metastases. Nature 1987; 328:170–172.
89. Pierotti MA, Santoro M, Jenkins RB et al. Characterization of an inversion on the long arm of chromosome 10 juxtaposing D10S170 and RET and creating the oncogenic sequence RET/PTC. Proc Natl Acad Sci USA 1992; 89:1616–1620.
90. Minoletti F, Butti Mg, Coronelli S et al. The two genes generating RET/PTC3 are localized in chromosomal band 10q11.2. Genes, Chromosmes & Cancer 1994; 11:51–57.

91. Sozzi G, Bongarzone I, Miozzo M et al. A t(10;17) translocation creates the RET/PTC2 chimeric transforming sequence in papillary thyroid carcinoma. Genes, Chromosomes & Cancer 1994; 9: 244–250.
92. Jhiang SM, Mazzaferri EL. The ret/PTC oncogene in papillary thyroid carcinoma. J Lab Clin Med 1994; 123:331–337.
93. Ishizaka Y, Shima H, Sugimura T, Nagao M. Detection of phosphorylated *ret*/TPC oncogene product in cytoplasm. Oncogene 1992; 7:1441–1444.
94. Jhiang SM, Sagartz JE, Tong Q et al. Targeted expression of the ret/PTC1 oncogene induces papillary thyroid carcinomas. Endocrinology 1996; 137:375–378.
95. Santoro M, Chiappetta G, Cerrato A et al. Development of thyroid papillary carcinomas secondary to tissue-specific expression of the RET/PTC1 oncogene in transgenic mice. Oncogene 1996; 12:1821–1826.
96. Powell DJJr, Russell J, Nibu K et al. The RET/PTC3 oncogene: metastatic solid-type papillary carcinomas in murine thyroids. Cancer Res 1998; 58:5523–5528.
97. Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM. High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. Oncoogene 1995; 11:2459–2467.
98. Nishisho I, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinoma in children. Cancer Res 1997; 57:1690–1694.
99. Klugbauer S, Lengfelder E, Demidchik EP, Rabes HM. A new form of RET rearrangement in thyroid carcinomas of children after the Chernobyl reactor acident. Oncogene 1996; 13:1099–1102.
100. Fugazzola L, Pierotti MA, Vigano E, Pacini E Vorontsova TV, Bongarzone I. Molecular and biochemical analysis of RET/PTC4, a novel oncogenic rearrangement between RET and ELE1 genes, in a post-Cherynobyl papillary thyroid cancer. Oncogene 1996; 13:1093–1097.
101. Klugbauer S, Demidchik EP, Lengfelder E, Rabes HM. Detection of a novel type of RET rearrangement (PTC5) in thyroid carcinomas after Chernobyl and analysis of the involved RET-fused gene RFG5. Cancer Res 1998; 58:198–203.
102. Williams GH, Rooney S, Thomas GA, Cummins G, Williams ED. RET activation in adult and childhood papillary thyroid carcinoma using a reverse transcriptase-polymerase chain reaction approach on archival-nested material. Br J Cancer 1996; 74:585–589.
103. Jhiang SM, Caruso DR, Gilmore E et al. Detection of the PTC/retTPC oncogene in human thyroid cancers. Oncogene 1992; 7:1331–1337.
104. Sugg SL, Zheng L, Rosen IB, Freeman JL, Ezzat S, Asa SL. *ret*/PTC-1,-2 and -3 oncogene rearrangements in human thyroid carcinomas: Implications for metastatic potential? J Clin Endocrinol Metab 1996; 81:3360–3365.
105. Mayr B, Brabant G, Goretzki P, Ruschoff J, Dietmaier W, Dralle H. ret/Ptc-1, -2, and -3 oncogene rearrangements in human thyroid carcinomas: implications for metastatic potential? J Clin Endocrinol Metab 1997; 82:1306–1307.
106. Viglietto G, Chiappetta G, Martinez-Tello FJ et al. RET/PTC oncogene activation is an early event in thyroid carcinogenesis. Oncogene 1995; 11:1207–1210.
107. Cheung CC, Ezzat S, Freeman JL, Rosen IB, Asa SL. Immunohistochemical diagnosis of papillary thyroid carcinoma. Mod Pathol 2001; 14(4):338–342.
108. Cheung CC, Carydis B, Ezzat S, Bedard YC, Asa SL. Analysis of ret/PTC gene rearrangements refines the fine needle aspiration diagnosis of thyroid cancer. J Clin Endocrinol Metab 2001; 86(5):2187–2190.
109. Zipkin P. Hyalinahniliche collagene kugeln als produkte epitelialer zellen in malignen strumen. Virchows Arch 1905; 182:374–406.
110. Masson P. Cancers thyroidiens a polarite alternative. Bull Cancer 1922; 11:350–355.
111. Ward JV, Murray D, Horvath E, Kovacs K, Bauman A. Hyaline cell tumor of the thyroid with massive accumulation of cytoplasmic microfilaments. Laboratory Investigation 46, 88A. 1982.
112. Carney JA, Ryan J, Goellner JR. Hyalinizing trabecular adenoma of the thyroid gland. Am J Surg Pathol 1987; 11:583–591.
113. Bronner MP, LiVolsi VA, Jennings TA. PLAT: Paraganglioma-like adenomas of the thyroid. Surg Pathol 1988; 1:383–389.
114. Sambade C, Franssila K, Cameselle-Teijeiro J, Nesland J, Sobrinho-Simoes M. Hyalinizing trabecular adenoma: A misnomer for a peculiar tumor of the thyroid gland. Endocr Pathol 1991; 2:83–91.
115. Molberg K, Albores-Saavedra J. Hyalinizing trabecular carcinoma of the thyroid gland. Hum Pathol 1994; 25:192–197.

116. McCluggage WG. Sloan JM. Hyalinizing trabecular carcinoma of the thyroid gland. Histopathology 1996; 28:357–362.
117. Katoh R, Jasani B, Williams ED. Hyalinizing trabecular adenoma of the thyroid. A report of three cases with immunohistochemical and ultrastructural studies. Histopathology 1989; 15:211–224.
118. Li M, Carcangiu ML, Rosai J. Abnormal intracellular and extracellular distribution of base membrane material in papillary carcinoma and hyalinizing trabecular tumors of the thyroid: implication for deregulation secretory pathways. Hum Pathol 1997; 28:1366–1372.
119. Chan JKC, Tse CCH, Chiu HS. Hyalinizing trabecular adenoma-like lesion in multinodular goitre. Histopathology 1990; 16:611–614.
120. Cheung CC, Boerner SL, MacMillan CM, Ramyar L, Asa SL. Hyalinizing trabecular tumor of the thyroid: a variant of papillary carcinoma proved by molecular genetics. Am J Surg Pathol 2000; 24(12): 1622–1626.
121. Papotti M, Volante M, Giuliano A et al. RET/PTC activation in hyalinizing trabecular tumors of the thyroid. Am J Surg Pathol 2000; 24(12):1615–1621.
122. Hirokawa M, Carney JA, Ohtsuki Y. Hyalinizing trabecular adenoma and papillary carcinoma of the thyroid gland express different cytokeratin patterns. Am J Surg Pathol 2000; 24(6):877–881.
123. Hirokawa M, Carney JA. Cell membrane and cytoplasmic staining for MIB-1 in hyalinizing trabecular adenoma of the thyroid gland. Am J Surg Pathol 2000; 24(4):575–578.
124. Chan JKC, Tsui MS, Tse CH. Diffuse sclerosing variant of papillary carcinoma of the thyroid: a histological and immunohistochemical study of three cases. Histopathology 1987; 11:191–201.
125. Carcangiu ML, Bianchi S. Diffuse sclerosing variant of papillary thyroid carcinoma: Clinicopathologic study of 15 cases. Am J Surg Pathol 1989; 13:1041–1049.
126. Soares J, Limbert E, Sobrinho-Simoes M. Diffuse sclerosing variant of papillary thyroid carcinoma. A clinicopathologic study of 10 cases. Path Res Pract 1989; 185:200–206.
127. Fujimoto.Y., Obara T, Ito Y, et al. Diffuse sclerosing variant of papillary carcinoa of the thyroid. Cancer 1990; 66:2306–2312.
128. Nikiforov Y, Gnepp DR. Pediatric thyroid cancer after the Chernobyl disaster: Pathomorphologic study of 84 cases (1991–1992) from the Republic of Belarus. Cancer 1994; 74:748–766.
129. Cameselle-Teijeiro J, Chan JK. Cribiform-morular variant of papillary carcinoma: a distinct variant representing the sporadic counterpart of familial adenomatous polyposis-associated with thyroid carcinoma. Mod Pathol 1999; 12:400–411.
130. Zeki K, Spambalg D, Sharifi N, Gonsky R, Fagin JA. Mutations of the adenomatous polyposis coli gene in sporadic thyroid neoplasms. J Clin Endocrinol Metab 1994; 79:1317–1321.
131. Colletta G, Sciacchitano S, Palmirotta R et al. Analysis of adenomatous polyposis coli gene in thyroid tumours. Br J Cancer 1994; 70(6):1085–1088.
132. Flint A, Davenport RD, Lloyd RV. The tall cell variant of papillary carcinoma of the thyroid gland. Arch Pathol Lab Med 1991; 115:169–171.
133. Hicks MJ, Batsakis JG. Tall cell carcinoma of the thyroid gland. Ann Otol Rhinol Laryngol 1993; 102:402–403.
134. Van den Brekel MWM, Hekkenberg RJ, Asa SL, Tomlinson G, Rosen IB, Freeman JL. Prognostic features in tall cell papillary carcinoma and insular thyroid carcinoma. Laryngoscope 1997; 107:254–259.
135. Sobrinho-Simoes M, Nesland JM, Johannessen JV. Columnar cell carcinoma: another variant of poorly differentiated carcinoma of the thyroid. Am J Clin Pathol 1988; 89:264–267.
136. Evans HL. Columnar-cell carcinoma of the thyroid. A report of two cases of an aggressive variant of thyroid carcinoma. Am J Clin Pathol 1986; 85:77–80.
137. Akslen L, Varhaug JE. Thyroid carcinoma with mixed tall cell and columnar cell features. Am J Clin Pathol 1990; 94:442–445.
138. Johnson TL, Lloyd RV, Thompson NW, Beierwaltes WH, Sisson JC. Prognostic implications of the tall cell variant of papillary thyroid carcinoma. Am J Surg Pathol 1988; 12:22–27.
139. Khoo ML, Ezzat S, Freeman JL, Asa SL. Cyclin D1 protein expression predicts metastatic behavior in thyroid papillary microcarcinomas but is not associated with gene amplification. J Clin Endocrinol Metab 2002; 87(4):1810–1813.
140. Khoo ML, Beasley NJ, Ezzat S, Freeman JL, Asa SL. Overexpression of cyclin D1 and underexpression of p27 predict lymph node metastases in papillary thyroid carcinoma. J Clin Endocrinol Metab 2002; 87(4):1814–1818.

141. Khoo ML, Freeman JL, Witterick IJ et al. Underexpression of p27/Kip in thyroid papillary microcarcinomas with gross metastatic disease. Arch Otolaryngol Head Neck Surg 2002; 128(3):253–257.
142. Friedman NB. Cellular involution in thyroid gland: significance of Hürthle cells in myxedema, exhaustion atrophy, Hashimoto's disease and reaction in irradiation, thiouracil therapy and subtotal resection. J Clin Endocrinol 1949; 9:874–882.
143. Nesland JM, Sobrinho-Simoes M, Holm R, Sambade MC, Johannessen JV. Hürthle cell lesions of the thyroid: a combined study using transmission electron microscopy, scanning electron microscopy and immunocytochemistry. Ultrastructrual Pathol 1985; 8:131–142.
144. Sobrinho-Simoes M, Nesland JM, Holm R, Sambade MC, Johannessen JV. Hürthle cell and mitochondrion-rich papillary carcinomas of the thyroid gland: An ultrastructural and immunocytochemical study. Ultrastruct Pathol 1985; 8:131–142.
145. Triggs SM, Pearse AGE. Histochemistry of oxidate enzyme systems in the human thyroid with special reference to Askanazy cells. J Pathol Bacteriol 1960; 80:353–358.
146. Clark OH, Gerend PL. Thyrotropin receptor-adenylate cyclase system in Hürthle cell neoplasms. J Clin Endocrinol Metab 1985; 39:719–723.
147. Yeh JJ, Lunetta KL, van Orsouw NJ et al. Somatic mitochondrial DNA (mtDNA) mutations in papillary thyroid carcinomas and differential mtDNA sequence variants in cases with thyroid tumours. Oncogene 2000; 19(16):2060–2066.
148. Maximo V, Soares P, Lima J, Cameselle-Teijeiro J, Sobrinho-Simoes M. Mitochondrial DNA somatic-mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology: a study with emphasis on Hurthle cell tumors. Am J Pathol 2002; 160(5):1857–1865.
149. Bronner MP, LiVolsi VA. Oxyphilic (Askenasy/Hürthle cell) tumors of the thyroid. Microscopic-features predict biologic behavior. Surg Pathol 1988; 1:137–150.
150. Chen KTK. Fine-needle aspiration cytology of papillary Hürthle-cell tumors of thyroid: A report of three cases. Diagn Cytopathol 1991; 7:53–56.
151. Kini SR, Miller JM, Abrash MP, Γαβα A, Johnson T. Post tine needle apsiration biopsy infarction in thyroid nodules. Modem Pathology 1, 48A. 1988.
152. Johnson TL, Lloyd RV, Burney RE, Thompson NW. Hürthle cell thyroid tumors: an immunohistochemical study. Cancer 1987; 59:107–112.
153. Arganini M, Behar R, Wi TC et al. Hürthle cell tumors: a twenty-five year experience. Surgery 1986; 100:1108–1114.
154. Gosain AK, Clark OH. Hürthle cell neoplasms: malignant potential. Arch Surg 1984; 119:515–519.
155. Har-El G, Hadar T, Segal K, Levy R, Sidi J. Hurthle cell carcinoma of the thyroid gland. A tumor of moderate malignancy. Cancer 1986; 57:1613–1617.
156. Thompson NW, Dunn EL, Batsakis JG, Nishiyama RH. Hürthle cell lesions of the thyroid gland. Surg Gynecol Obstet 1974; 139:555–560.
157. Flint A, Lloyd RV. Hürthle cell neoplasms of the thyroid gland. Pathol Annu 1990; 25:37–52.
158. Carcangiu ML, Bianchiu S, Savino D, et al. Follicular Hürthle cell tumors of the thyroid gland. Cancer 1991; 68:1944–1953.
159. McLeod MK, Thompson NW, Hudson JL et al. Flow cytometric measurements of nuclear DNA and ploidy analysis in Hürthle cell neoplasms of the thyroid. Arch Surg 1988; 123:849–854.
160. Ryan J J, Hay ID, Grant CS, Rainwater LM, Farrow GM, Goellner JR. Flow cytometric DNA measurements in benign and malignant Hürthle cell tumors of the thyroid. World J Surg 1988; 12:482–487.
161. Galera-Davidson H, Bobbo M, Bartels PH, Dytch HE, Puls JH, Wied GL. Correlation between automated DNA ploidy measurements of Hürthle cell tumors and their histopathologic and clinical features. Anal Quant Cytol Histol 1986; 8:158–167.
162. Klemi PJ, Joensuu H, Eerola E. DNA aneuploidy in anaplastic carcinoma of the thyroid gland. Am J Clin Pathol 1988; 89:154–159.
163. Tallini G, Hsueh A, Liu S, Garcia-Rostan G, Speicher MR, Ward DC. Frequent chromosomal DNA unbalance in thyroid oncocytic (Hurthle cell) neoplasms detected by comparative genomic hybridization. Lab Invest 1999; 79(5):547–555.
164. Bondeson L, Bondeson A-G, Ljungberg O. Treatment of Hürthle cell neoplasms of the thyroid. Arch Surg 1983; 118:1453.
165. Bondeson L, Bondeson AG, Ljungberg O, Tibblin S. Oxyphil tumors of the thyroid. Ann Surg 1981; 194:677–680.
166. Gundry SR, Burney RE, Thompson NW, Lloyd R. Total thyroidectomy for Hürthle cell neoplasm of the thyroid. Arch Surg 1983; 118:529–532.

167. Watson RG, Brennan MD, Goellner JR, van Heerden JA, McConahey WM, Taylor WF. Invasive Hürthle cell carcinoma of the thyroid: Natural history and management. Pathol Annu 1984; 59:851–855.
168. Gardner LW. Hürthle-cell tumors of the thyroid. Arch Pathol 1955; 59:372–381.
169. González-Campora R, Herrero-Zapatero A, Lerma E, Sanchez F, Galera H. Hürthle cell and mitochondrion-rich cell tumors. A clinicopathologic study. Cancer 1986; 57:1154–1163.
170. Herrera MF, Hay ID, Wu PS et al. Hürthle cell (oxyphilic) papillary thyroid carcinoma: A variant with more aggressive biologic behavior. World J Surg 1992; 16:669–675.
171. Meissner WA, Adler A. Papillary carcinoma of the thyroid. A study of the pathology of two hundred twenty-six cases. Arch Pathol 1958; 66:518–525.
172. Tscholl-Ducommun J, Hedinger C. Papillary thyroid carcinomas. Morphology and Prognosis. Virchows Arch [Pathol Anat] 1982; 396:19–39.
173. Beckner ME, Heffess CS, Oertel JE. Oxyphilic papillary thyroid carcinoma. Am J Clin Pathol 1995; 103:280–287.
174. Hill JH, Werkhaven JA, DeMay RM. Hürthle cell variant of papillary carcinoma of the thyroid gland. Otolaryngol Head Neck Surg 1988; 98:338–341.
175. Berho M, Suster S. The oncocytic variant of papillary carcinoma of the thyroid. A clinicopathologic study of 15 cases. Hum Pathol 1997; 28:47–53.
176. Barbuto D, Carcangiu ML, Rosai J. Papillary Hürthle cell neoplasms of the thyroid gland: A study of 20 cases (abstract). Lab Invest 1990; 62:7A.
177. Wu P-C, Hay ID, Herrmann MA et al. Papillary thyroid carcinoma (PTC), oxyphilic cell type: A tumor misclassified by the World Health Organization (WHO)? Clinical Research 39, 279A. 1991.
178. Apel RL, Asa SL, LiVolsi VA. Papillary Hürthle cell carcinoma with lymphocytic stroma. "Warthin-like tumor" of the thyroid. Am J Surg Pathol 1995; 19:810–814.
179. Grant CS, Barr D, Goellner JR, Hay ID. Benign Hürthle cell tumors of the thyroid: A diagnosis to be trusted? World J Surg 1988; 12:488–495.
180. Cheung CC, Ezzat S, Ramyar L, Freeman JL, Asa SL. Molecular basis of Hurthle cell papillary thyroid carcinoma. J Clin Endocrinol Metab 2000; 85(2):878–882.
181. Chiappetta G, Toti P, Cetta F et al. The RET/PTC oncogcne is frequently activated in oncocytic thyroid tumors (Hurthle cell adenomas and carcinomas), but not in oncocytic hyperplastic lesions. J Clin Endocrinol Metab 2002; 87(1):364–369.
182. Belchetz G, Cheung CC, Freeman J, Rosen IB, Witterick IJ, Asa SL. Hurthle cell tumors: using molecular techniques to define a novel classification system. Arch Otolaryngol Head Neck Surg 2002; 128(3):237–240.
183. Volpé R. Lymphocytic (Hashimoto's) thyroiditis. In: Werner SC, Ingbar SC, editors. The Thyroid. New York: Harper and Row, 1978: 996–1008.
184. Jansson R, Karlsson A, Forsum U. Intrathyroidal HLA-DR expression and T lymphocyte phenotypes in Graves' thyrotoxicosis, Hashimoto's thyroiditis and nodular colloid goitre. Clin Exp Immunol 1984; 58:264–272.
185. Asa SL. The pathology of autoimmune endocrine disorders. In: Kovacs K, Asa SL, editors. Functional Endocrine Pathology. Boston: Blackwell Scientific Publications, 1991: 961–978.
186. Dube VE, Joyce GT. Extreme squamous metaplasia in Hashimoto's thyroiditis. Cancer 1971; 27:434–437.
187. Katzmann JA, Vickery AL. The fibrosing variant of Hashimoto's thyroiditis. Hum Pathol 1974; 5:161–170.
188. Wirtschafter A, Schmidt R, Rosen D et al. Expression of the RET/PTC fusion gene as a marker for papillary carcinoma in Hashimoto's thyroiditis. Laryngoscope 1997; 107:95–100.
189. Carcangiu ML, Zampi G, Rosai J. Poorly differentiated "insular") thyroid carcinoma. A reinterpretation of Langhans' "wuchernde Struma". Am J Surg Pathol 1984; 8:655–668.
190. Sakamoto A, Kasai N, Sugano H. Poorly differentiated carcinoma of the thyroid. A clinicopathologic entity for a high-risk group of papillary and follicular carcinomas. Cancer 1983; 52:1849–1855.
191. Papotti M, Botto Micca F, Favero A, Palestini N, Bussolati G. Poorly differentiated thyroid carcinomas with primordial cell component. A group of aggressive lesions sharing insular, trabecular, and solid patterns. Am J Surg Pathol 1993; 17:291–301.
192. Samaan NA, Ordoñez NG. Uncommon types of thyroid cancer. Endocrinol Metab Clin North Am 1990; 19:637–648.
193. Aldinger KA, Samaan NA, Ibanez M, Hill CS, Jr. Anaplastic carcinoma of the thyroid. A review of 84 cases of spindle and giant cell carcinoma of the thyroid. Cancer 1978; 41:2267–2275.

194. Venkatesh YSS, Ordoñez NG, Schultz PN, Hickey RC, Goepfert H, Samaan NA. Anaplastic carcinoma of the thyroid. A clinicopathologic study of 121 cases. Cancer 1990; 66:321–330.
195. Shvero J, Gal R, Avidor I, Hadar T, Kessler E. Anaplastic thyroid carcinoma. A clinical, histologic, and immunohistochemical study. Cancer 1988; 62:319–325.
196. Gaffey MJ, Lack EE, Christ ML, Weiss LM. Anaplastic thyroid carcinoma with osteoclast-like giant cells. A clinicopathologic, immunohistochemical, and ultrastructural study. Am J Surg Pathol 1991; 15:160–168.
197. Ordóñez NG, El-Naggar AK, Hickey RC, Samaan NA. Anaplastic thyroid carcinoma. Immunocytochemical study of 32 cases. Am J Clin Pathol 1991; 96:15–24.
198. Carcangiu ML, Steeper T, Zampi G, Rosai J. Anaplastic thyroid carcinoma. A study of 70 cases. Am J Clin Pathol 1985; 83:135–158.
199. Hurlimann J, Gardiol D, Scazziga B. Immunohistology of anaplastic thyroid carcinoma. A study of 43 cases. Histopathology 1987; 11:567–580.
200. LiVolsi VA, Brooks JJ, Arendash-Durand B. Anaplastic thyroid tumors. Immunohistology. Am J Clin Pathol 1987; 87:434–442.
201. Pilotti S, Collini P, Del Bo R, Cattoretti G, Pierotti MA, Rilke F. A novel panel of antibodies that segregates immunocytochemically poorly differentiated carcinoma from undifferentiated carcinoma of the thyroid gland. Am J Surg Pathol 1994; 18:1054–1064.
202. Fagin JA. Genetic basis of endocrine disease 3. Molecular defects in thyroid gland neoplasia. J Clin Endocrinol Metab 1992; 75:1398–1400.
203. Farid NR, Shi Y, Zou M. Molecular basis of thyroid cancer. Endocr Rev 1994; 15:202–232.
204. Fagin JA, Matsuo K, Karmakar A, Chen DL, Tang S-H, Koeffler HP. High prevalence of mutations of the p53 gene in poorly differentiated human thyroid carcinomas. J Clin Invest 1993; 91:179–184.
205. Donghi R, Longoni A, Pilotti S, Michieli P, Delia Porta G, Piarotti MA. Gene p53 mutations are restricted to poorly differentiated and undifferentiated carcinomas of the thyroid gland. J Clin Invest 1993; 91:1753–1760.
206. Nakamura T, Yana I, Kobayashi T et al. p53 gene mutations associated with anaplastic transformation of human thyroid carcinomas. Jpn J Cancer Res 1992; 83:1293–1298.
207. Ito T, Seyama T, Mizuno T et al. Unique association of p53 mutations with undifferentiated but not with differentiated carcinomas of the thyroid gland. Cancer Res 1992; 52:1369–1371.
208. Wyllie FS, Lemoine NR, Williams ED, Wynford-Thomas D. Structure and expression of nuclear oncogenes in multi-stage thyroid tumorigenesis. Br J Cancer 1989; 60:561–565.
209. Hosal SA, Apel RL, Freeman JL et al. Immunohistochemical localization of p53 in human thyroid neoplasms: correlation with biological behavior. Endocr Pathol 1997; 8:21–28.
210. Jossart GH, Epstein HD, Shaver JK et al. Immunocytochemical detection of p53 in human thyroid carcinomas is associated with mutation and immortalization of cell lines. J Clin Endocrinol Metab 1996; 81:3498–3504.
211. Gaal JM, Horvath E, Kovacs K. Ultrastructure of two cases of anaplastic giant cell tumor of the human thyroid gland. Cancer 1975; 35:1273–1279.
212. Jao W, Gould VE. Ultrastructure of anaplastic (spindle and giant cell) carcinoma of the thyroid. Cancer 1975; 35:1280–1292.
213. Asa SL, Dardick I, Van Nostrand AWP, Bailey DJ, Gullane PJ. Primary thyroid thymoma: a distinct clinicopathologic entity. Hum Pathol 1988; 19:1463–1467.
214. Chan JKC, Rosai J. Tumors of the neck showing thymic or related branchial pouch differentiation: A unifying concept. Hum Pathol 1991; 22:349–367.
215. Nishiyama RH, Dunn EL, Thompson NW Anaplastic spindle-cell and giant-cell tumors of the thyroid gland. Cancer 1972; 30:113–127.
216. Spires JR, Schwartz MR, Miller RH. Anaplastic thyroid carcinoma. Association with differentiated thyroid cancer. Arch Otolaryngol Head Neck Surg 1988; 114:40–44.
217. van der Laan BFAM, Freeman JL, Tsang RW, Asa SL. The association of well-differentiated thyroid carcinoma with insular or anaplastic thyroid carcinoma: Evidence for dedifferentiation in tumor progression. Endocr Pathol 1993; 4:215–221.
218. Bronner MP, LiVolsi VA. Spindle cell squamous carcinoma of the thyroid: An unusual anaplastic tumor associated with tall cell papillary cancer. Mod Pathol 1991; 4:637–643.
219. Yoshida A, Kamma H, Asaga T et al. Proliferative activity in thyroid tumors. Cancer 1992; 69:2548–2552.
220. Kapp DS, LiVolsi VA, Sanders MM. Anaplastic carcinoma following well-differentiated thyroid cancer: Etiological considerations. Yale J Biol Med 1982; 5:521–528.

221. Uribe M, Fenoglio-Preiser CM, Grimes M, Feind C. Medullary carcinoma of the thyroid gland. Clinical, pathological, and immunohistochemical features with review of the literature. Am J Surg Pathol 1985; 9:577–594.
222. Valenta LJ, Michel-Bechet M, Mattson JC, Singer FR. Microfollicular thyroid carcinoma with amyloid rich stroma, resembling the medullary carcinoma of the thyroid (MCT). Cancer 1977; 39:1573–1586.
223. Harach HR, Williams ED. Glandular (tubular and follicular) variants of medullary carcinoma of the thyroid. Histopathology 1983; 7:83–97.
224. Nelkin BD, de Bustros AC, Mabry M, Baylin SB. The molecular biology of medullary thyroid carcinoma. A model for cancer development and progression. JAMA 1989; 261:3130–3135.
225. Mendelsohn G, Wells SA, Baylin SB. Relationship of tissue carcinoembryonic antigen and calcitonin to tumor virulence in medullary thyroid carcinoma. An immunohistochemical study in early, localized and virulent disseminated stages of disease. Cancer 1984; 54:657–662.
226. Schröder S, Klöppel G. Carcinoembryonic antigen and nonspecific cross-reacting antigen in thyroid cancer. An immunocytochemical study using polyclonal and monoclonal antibodies. Am J Surg Pathol 1987; 11:100–108.
227. Williams ED, Morales AM, Horn RC. Thyroid carcinoma and Cushing's syndrome. A report of two cases with a review of the common features of the non-endocrine tumours associated with Cushing's syndrome. J Clin Patho 1968; 21:129–135.
228. Birkenhäger JC, Upton GV, Seldenrath HJ, Kreiger DT, Tashjian AHJr. Medullary thyroid carcinoma: ectopic production of peptides with ACTH-like, corticotrophin releasing factor-like and prolactin production-stimulating activities. Acta Endocrinol (Copen) 1976; 83:280–292.
229. Goltzman D, Huang S-N, Browne C, Solomon S. Adrenocorticotropin and calcitonin in medullary thyroid carcinoma: frequency of occurrence and localization in the same cell type by immunohistochemistry. J Clin Endocrinol Metab 1979; 49:364–369.
230. Takami H, Bessho T, Kameya T et al. Immunohistochemical study of medullary thyroid carcinoma: Relationship of clinical features to prognostic factors in 36 patients. World J Surg 1988; 12:572–579.
231. Reubi JC, Chayvialle JA, Franc B, Cohen R, Calmettes C, Modigliani E. Somatostatin receptors and somatostatin content in medullary thyroid carcinomas. Lab Invest 1991; 64:567–573.
232. Lamberts SWJ, Bakker WH, Reubi JC, Krenning EP. Somatostatin-receptor imaging in the localization of endocrine tumors. N Engl J Med 1990; 323:1246–1249.
233. Lamberts SWJ, Krenning EP, Reubi JC. The role of somatostatin and its analogs in the diagnosis and treatment of tumors. Endocr Rev 1991; 12:450.
234. Schimke RN, Hartmann WH. Familial amyloid-producing medullary thyroid carcinoma and pheochromocytoma: A distinct genetic entity. Ann Intern Med 1965; 63:1027–1037.
235. Goodfellow PJ. Mapping the inherited defects associated with multiple endocrine neoplasia type 2A, multiple endocrine neoplasia type 2B, and familial medullary thyroid carcinoma to chromosome 10 by linkage analysis. Endocrinol Metab Clin North Am 1994; 23:177–185.
236. Carson NL, Wu J, Jackson CE, Kidd KK, Simpson NE. The mutation for medullary thyroid carcinoma with parathyroid tumors (mTC with PTs) is closely linked to the centromeric region of chromosome 10. Am J Hum Genet 1990; 47:946–951.
237. Nelkin BD, Nakamura N, White RW et al. Low incidence of loss of chromosome 10 in sporadic and hereditary human medullary thyroid carcinoma. Cancer Res 1989; 49:4114–4119.
238. Mulligan LM, Kwok JBJ, Healey CS et al. Germ-line mutations of the *RET* proto-oncogene in multiple endocrine neoplasia type 2A. Nature 1993; 363:458–460.
239. Hofstra RMW, Landsvater RM, Ceccherini I et al. A mutation in the RET proto-oncogene associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 1994; 367:375–376.
240. Marsh DJ, Robinson BG, Andrew S et al. A rapid screening method for the detection of mutations in the RET proto-oncogene in multiple endocrine neoplasia type 2A and familial medullary thyroid carcinoma families. Genomics 1994; 23:477–479.
241. Brandi ML, Gagel RF, Angeli A et al. Guidelines for diagnosis and therapy of MEN type 1 and type 2. J Clin Endocrinol Metab 2001; 86(12):5658–5671.
242. Santoro M, Rosati R, Grieco M et al. The *ret* proto-oncogene is consistently expressed in human pheochromocytomas and thyroid medullary carcinomas. Oncogene 1990; 5:1595–1598.
243. Zedenius J, Larsson C, Bergholm U et al. Mutations of codon 918 in the RET proto-oncogene correlate to poor prognosis in sporadic medullary thyroid carcinomas. J Clin Endocrinol Metab 1995; 80:3088–3090.

244. Molcy JF, Brother MB, Wells SA, Spengler BA, Biedler JL, Brodeur GM. Low frequency of *ras* gene mutations in neuroblastomas, pheochromocytomas, and medullary thyroid cancers. Cancer Res 1991; 51:1596–1599.
245. Yang KP, Castillo SG, Nguyen CV. C-myc, N-ras, c-erb B: lack of amplification or rearrangement in human medullary thyroid carcinoma and a derivative cell line. Anticancer Res 1990; 10:189–192.
246. Yana I, Nakamura T, Shin E. Inactivation of the p53 gene is not required for tumorigenesis of medullary thyroid carcinoma or pheochromocytoma. Jpn J Cancer Res 1992; 83:1113–1116.
247. Wolfe HJ, Melvin KEW, Cervi-Skinner SJ. C-cell hyperplasia preceding medullary thyroid carcinoma. N Engl J Med 1973; 289:437–441.
248. DeLellis RA, Wolfe HJ. The pathobiology of the human calcitonin (C)-cell: a review. Pathol Annu 1981; 16:25–52.
249. Albores-Saavedra J, Monforte H, Nadji M, Morales AR. C-cell hyperplasia in thyroid tissue adjacent to follicular cell tumors. Hum Pathol 1988; 19:795–799.
250. Biddinger PW, Brennan MF, Rosen PP. Symptomatic C-cell hyperplasia associated with chronic lymphocytic thyroiditis. Am J Surg Pathol 1991; 15:599–604.
251. Scopsi L, Di Palma S, Ferrari C, Holst JJ, Rehfeld JF, Rilke F. C-cell hyperplasia accompanying thyroid diseases other than medullary carcinoma: an immunocytochemical study by means of antibodies to calcitonin and somatostatin. Mod Pathol 1991; 4:297–304.
252. Libbey NP, Nowakowski KJ, Tucci JR. C-cell hyperplasia of the thyroid in a patient with goitrous hypothyroidism and Hashimoto's thyroiditis. Am J Surg Pathol 1989; 13:71–77.
253. LiVolsi VA. Mixed Thyroid Carcinoma: A real entity? Lab Invest 1987; 57:237–239.
254. Holm R, Sobrinho-Simoes M, Nesland JM, Johannessen J-V. Concurrent production of calcitonin and thyroglobulin by the same neoplastic cells. Ultrastruct Pathol 1986; 10:241–248.
255. Holm R, Sobrinho-Simoes M, Nesland JM, Sambade C, Johannessen J-V. Medullary thyroid carcinoma with thyroglobulin immunoreactivity. A special entity? Lab Invest 1987; 57:258–268.
256. Apel RL, Alpert LC, Rizzo A, LiVolsi VA, Asa SL. A metastasizing composite carcinoma of the thyroid with distinct medullary and papillary components. Arch Pathol Lab Med 1994; 118:1143–1147.
257. González-Cámpora R, Lopez-Garrido J, Martin-Lacave I, Miralles-Sánchez EJ, Villar JL. Concurrence of a symptomatic encapsulated follicular carcinoma, an occult papillary carcinoma and a medullary carcinoma in the same patient. Histopathology 1992; 21:380–382.
258. Pastolero GC, Coire CI, Asa SL. Concurrent medullary and papillary carcinomas of thyroid with lymph node metastases. Am J Surg Pathol 1996; 20:245–250.