



Overview of the 2022 WHO Classification of Parathyroid Tumors

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Accepted: 29 January 2022 / Published online: 17 February 2022

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Abstract

The 2022 WHO classification reflects increases in the knowledge of the underlying pathogenesis of parathyroid disease. In addition to the classic characteristic features of parathyroid neoplasms, subtleties in histologic features which may indicate an underlying genetic abnormality reflect increased understanding of the clinical manifestations, histologic, and genetic correlation in parathyroid disease. The importance of underlying genetic aberrancies is emphasized due to their significance to the care of the patient. Traditionally, the term “parathyroid hyperplasia” has been applied to multiglandular parathyroid disease; however, the concept of hyperplasia is generally no longer supported in the context of primary hyperparathyroidism since affected glands are usually composed of multiple “clonal” neoplastic proliferations. In light of these findings and management implications for patient care, the 2022 WHO classification endorses primary hyperparathyroidism-related multiglandular parathyroid disease (multiglandular multiple parathyroid adenomas) as a germline susceptibility-driven multiglandular parathyroid neoplasia. From such a perspective, pathologists can provide additional value to genetic triaging by recognizing morphological and immunohistochemical harbingers of *MEN1*, *CDKN1B*, *MAX*, and *CDC73*-related manifestations. In the current WHO classification, the term “parathyroid hyperplasia” is now used primarily in the setting of secondary hyperplasia which is most often caused by chronic renal failure. In addition to expansion in the histological features, including those that may be suggestive of an underlying genetic abnormality, there are additional nomenclature changes in the 2022 WHO classification reflecting increased understanding of the underlying pathogenesis of parathyroid disease. The new classification no longer endorses the use of “atypical parathyroid adenoma”. This entity is now being replaced with the term of “atypical parathyroid tumor” to reflect a parathyroid neoplasm of uncertain malignant potential. The differential diagnoses of atypical parathyroid tumor are discussed along with the details of worrisome clinical and laboratory findings, and also features that define atypical histological and immunohistochemical findings to qualify for this diagnosis. The histological definition of parathyroid carcinoma still requires one of the following findings: (i) angioinvasion (vascular invasion) characterized by tumor invading through a vessel wall and associated thrombus, or intravascular tumor cells admixed with thrombus, (ii) lymphatic invasion, (iii) perineural (intranural) invasion, (iv) local malignant invasion into adjacent anatomic structures, or (v) histologically/cytologically documented metastatic disease. In parathyroid carcinomas, the documentation of mitotic activity (e.g., mitoses per 10mm²) and Ki67 labeling index is recommended. Furthermore, the importance of complete submission of parathyroidectomy specimens for microscopic examination, and the crucial role of multiple levels along with ancillary biomarkers have expanded the diagnostic workup of atypical parathyroid tumors and parathyroid carcinoma to ensure accurate characterization of parathyroid neoplasms. The concept of parafibromin deficiency has been expanded upon and term “parafibromin deficient parathyroid neoplasm” is applied to a parathyroid neoplasm showing complete absence of nuclear parafibromin immunoreactivity. Nucleolar loss is considered as abnormal finding that requires further molecular testing to confirm its biological significance. The 2022 WHO classification emphasizes the role of molecular immunohistochemistry in parathyroid disease. By adopting a question–answer framework, this review highlights advances in knowledge of histological features, ancillary studies, and associated genetic findings that increase the understanding of the underlying pathogenesis of parathyroid disease that are now reflected in the updated classification and new entities in the 2022 WHO classification.

Keywords Parathyroid adenoma · Parathyroid hyperplasia · Atypical parathyroid tumor · Parathyroid carcinoma · Inherited hyperparathyroidism · Hyperparathyroidism · Multiglandular parathyroid disease · Parafibromin · WHO classification

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Introduction

Advances in the understanding of parathyroid disorders have significantly improved the understanding of parathyroid neoplasia and are reflected in a spectrum of clinicopathological entities, including specific genetic and pathological changes that manifest with multiglandular parathyroid disease. Ancillary biomarkers have also improved the clinical practice and helped to better characterize parathyroid proliferations. This practical review adopted a question–answer framework to provide an overview of the new WHO classification of parathyroid disorders in respect to relevant clinical, pathologic, and molecular features. This review serves as a primer for multidisciplinary team members dealing with parathyroid disorders.

Question 1. Are There Any Nomenclature Changes or New Entities in the 2022 WHO Classification of Parathyroid Tumors?

In addition to expansion in the histological features, including those that may be suggestive of an underlying genetic abnormality, ancillary studies, in more extensive genetic correlates of disease, there are important nomenclature changes in the 2022 WHO classification reflecting increased understanding of the underlying pathogenesis of parathyroid disease.

Primary hyperparathyroidism involving multiple parathyroid glands historically has been traditionally referred to as “parathyroid hyperplasia”. However, it is now known that the multiglandular parathyroid involvement in cases of primary hyperparathyroidism appears to be due to multiple clonal proliferations [1–3]. Thus, terms such as multiglandular parathyroid disease and multiple multiglandular parathyroid adenomas have now replaced the historical term “parathyroid hyperplasia”. A characteristic example of this is the involvement of multiple parathyroid glands in the setting of multiple endocrine neoplasia (MEN) type 1 (MEN1) [4–6], MEN4 [7], and MEN5 [8] syndromes. Involvement of the parathyroid glands in MEN syndromes may be asymmetrical and can occur in an asynchronous manner [4, 5]. Histologically, the hypercellular parathyroid glands can show nodular proliferations of chief cells as well as other cell types and often lack of rim of normal or atrophic appearing nonlesional parathyroid tissue. As hyperparathyroidism-related multiglandular parathyroid disease appears to be a germline susceptibility-driven multiglandular parathyroid neoplasia, underlying genetic testing may be helpful in identifying underlying possible genetic abnormalities that may affect patient care.

The concept of parafibromin deficiency has been expanded upon, and the term “parafibromin deficient parathyroid neoplasm” is new to the 2022 WHO classification.

This term is applied to a parathyroid neoplasm (e.g., parathyroid adenoma, atypical parathyroid tumor, parathyroid carcinoma) showing complete absence of nuclear parafibromin immunoreactivity. Nucleolar loss is considered as abnormal finding that requires further molecular testing to confirm its biological significance. As *CDC73* encodes parafibromin, complete loss of nuclear parafibromin immunoreactivity indicates biallelic *CDC73* inactivation, often arising in the setting of germline mutation/deletion [9–12]. The presence of parafibromin expression does not, however, completely exclude the possibility of underlying *CDC73* inactivation as point mutations of *CDC73* can result in preserved parafibromin expression [13, 14]. A “parafibromin deficient” parathyroid neoplasm is significant as it suggests an underlying germline or somatic *CDC73* mutation, and the identification of a “parafibromin deficient” parathyroid neoplasm is also an indication for germline *CDC73* mutation testing [9, 11, 12]. Parathyroid adenomas usually retain parafibromin nuclear immunoreactivity unless they are in the setting of underlying germline pathogenic *CDC73* variants leading to either hyperparathyroidism jaw tumor (HPT-JT) or familial isolated hyperparathyroidism syndromes. Still, long-term follow-up is recommended as metachronous disease can occur in other glands [13]. In addition to indicating the possibility of underlying germline *CDC73* disorder such as HPT-JT syndrome, parafibromin may be helpful in predicting outcome in atypical parathyroid tumors [15, 16]. In addition to the high life risk of parathyroid carcinoma in the setting of HPT-JT syndrome, somatic only *CDC73* mutations are also associated with sporadic parathyroid carcinoma [10, 13, 17, 18]. Although parathyroid adenomas account for most parathyroid disease in HPT-JT, the lifetime risk of parathyroid carcinoma is thought to be up to 15% [19, 20]. Parathyroid carcinoma is rare overall but occurs in up to 15% of individuals with HPT-JT. Thus, germline *CDC73* mutation testing is recommended for all individuals diagnosed with parathyroid carcinoma. Up to 30% of apparently sporadic parathyroid carcinomas are associated with underlying germline *CDC73* inactivation (Fig. 1) [9, 11–13, 21–23].

Atypical parathyroid tumor is new to the 2022 WHO classification. Atypical parathyroid tumor defined as a parathyroid neoplasm that demonstrates atypical cytological and architectural features that are worrisome for parathyroid carcinoma, but lacks unequivocal capsular, vascular, or perineural invasion or invasion into adjacent structures or metastases. These tumors can have histologic features often seen in parathyroid carcinoma including adherence to adjacent structures, monotonous sheet-like or trabecular growth, cytologic atypia, fibrosis, including band-like fibrosis, and cells extending into but not through the capsule, but they do not show unequivocal invasion as would be required for diagnosis of parathyroid carcinoma (Figs. 2

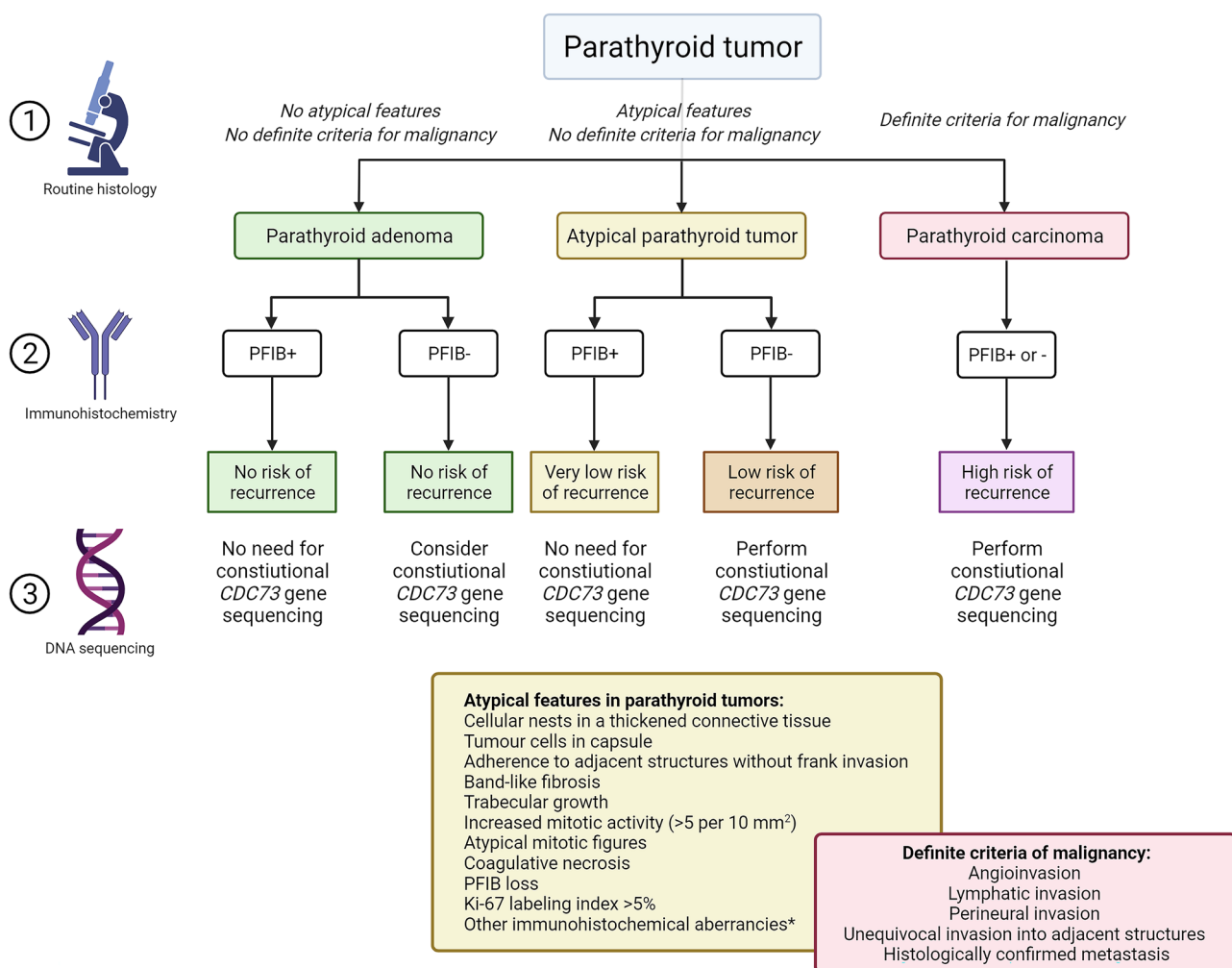


Fig. 1 Proposed scheme for triaging parathyroid tumors using histology, immunohistochemistry, and molecular genetics. Parathyroid tumor classification is based on histopathology, in which several factors are weighed before a diagnosis is made. Tumors lacking (or exhibiting only few) atypical features are diagnosed as parathyroid adenoma, while atypical parathyroid tumors are lesions with several worrisome histological and/or immunohistochemical features in which a differential diagnosis of parathyroid carcinoma is considered, yet definite criteria of malignancy are lacking. Parathyroid carcinoma is a diagnosis reserved for cases displaying one or several definite

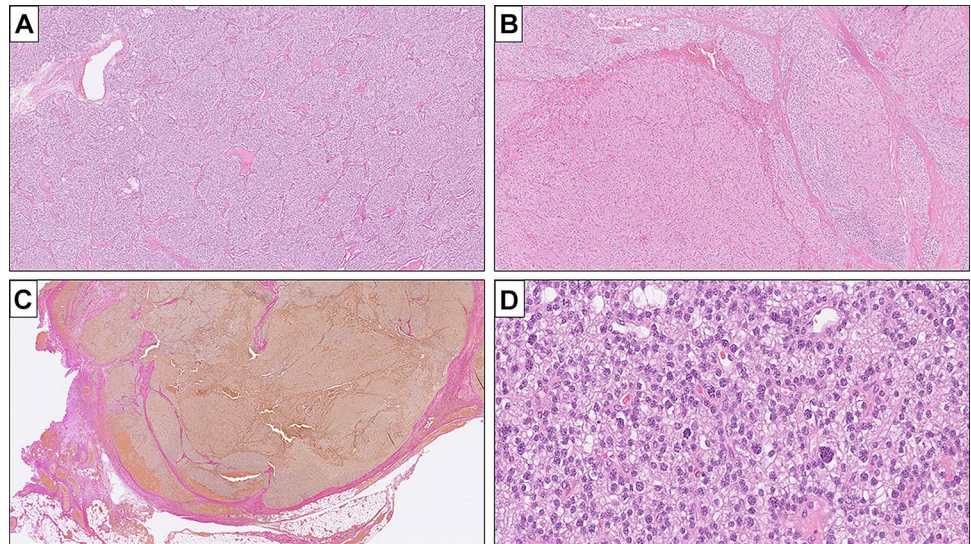
criteria of malignancy. Parafibromin (PFIB) immunohistochemistry is recommended for atypical parathyroid tumors and parathyroid carcinoma in order to appreciate the risk of tumor recurrence and to indicate the possibility of an underlying *CDC73* gene mutation. PFIB triaging of parathyroid adenoma is not recommended in clinical routine, but could be considered in specific instances (young patients with multiple tumors) in order to increase the likelihood of identifying an unknown germline *CDC73* gene mutation. *Other immunohistochemical aberrancies that are also a recurrent feature of parathyroid carcinoma, apart from PFIB. Created with BioRender.com

and 3). Conventional parathyroid adenomas can show areas of fibrosis particularly if they are large or if there has been prior fine needle aspiration or trauma (including ethanol injection). A focal area of fibrosis or an occasional mitotic figure is insufficient for designation of parathyroid adenoma as an atypical parathyroid tumor. Therefore, the term atypical parathyroid tumor is reserved for a parathyroid neoplasm with atypical features unrelated to a previous manipulation for which differential diagnosis is parathyroid carcinoma (Fig. 1). Atypical parathyroid tumors are considered as a neoplasm of uncertain malignant potential and usually warrant long-term follow-up.

Question 2. What Are the Clinicopathological Correlates of Hyperparathyroidism in the 2022 WHO Classification? What Clinical Information Is Critical for Pathologist to be Provided in the Evaluation of Parathyroidectomy Specimens?

The hallmark of hyperparathyroidism is the presence of autonomous PTH secretion. With the advent of screening programs, patients are more frequently diagnosed in the absence of classical signs (e.g., nephrolithiasis, osteitis fibrosis cystica, ileus, constipation, polyuria, polydipsia) of

Fig. 2 Histological features of atypical parathyroid tumors. **A** Trabecular, nested and solid areas of chief cells are commonly noted in atypical parathyroid tumors. **B** Irregular fibrous bands can be visualized, creating a nodular appearance of tumor cells. **C** Fibrosis highlighted by a van Gieson stain. Note the difficulties in interpreting the tumor cell relationship to the true tumor capsule in tumors with extensive, intercalating fibrosis. **D** Nuclear pleomorphism and an increased mitotic count are features coupled to atypical parathyroid tumors



hyperparathyroidism and also with normocalcemic manifestations [1, 24, 25].

Based on clinical and laboratory findings, autonomous PTH secretion is subtyped into three distinct clinicopathological forms including (i) primary hyperparathyroidism due to clonal (neoplastic) proliferation(s), (ii) secondary hyperparathyroidism due to underlying chronic disorder (e.g., chronic renal failure) or chronic stimulation (e.g., lithium administration) that interferes with PTH secretion, and (iii) tertiary hyperparathyroidism due to clonal expansion in

refractory secondary hyperparathyroidism [1]. The distinction between clinicopathological subtypes is of significance since secondary and tertiary hyperparathyroidism almost always manifest with multiglandular parathyroid disease, whereas primary hyperparathyroidism often manifests with a uniglandular parathyroid disease [1].

Autonomous PTH secretion is reflected in enlarged and heavy parathyroid gland(s). A normal parathyroid gland measures up to 6–8 mm and does not exceed a weight of 40–60 mg [1]; therefore, any figure beyond the upper limit of

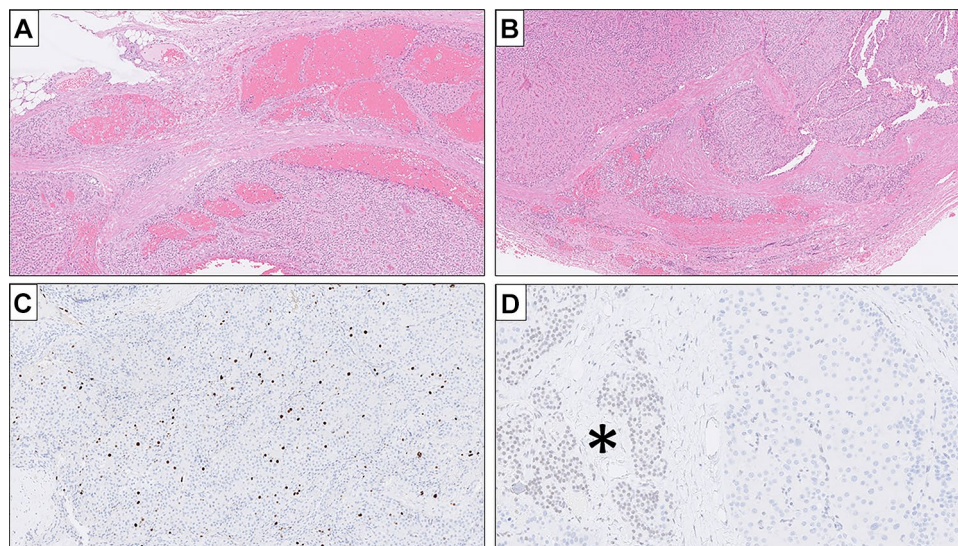


Fig. 3 Diagnostic predicaments in atypical parathyroid tumors. **A** Parathyroid tumors often exhibit peliosis (erythrocyte-filled cavitations without an endothelial layer mimicking a true blood vessel), and tumor cells may falsely appear to invade a vascular space. CD34 (endothelial marker) and CD61 (thrombus identification) would both be negative in this case. **B** Same tumor exhibiting fibrous bands,

mimicking capsular invasion. **C** Atypical parathyroid tumors often display an increased Ki-67 labeling index similarly to parathyroid carcinoma. **D** Atypical parathyroid tumor displaying absent parafibrin immunoreactivity. Note the retained expression in the normal rim (marked with asterisk). This tumor was found to carry a germline *CDC73* gene mutation, and the risk of recurrence is not neglectable

normal ranges should be considered pathological. A cellular parenchyma alone is not always a sign of pathological disease in the absence of increased glandular weight. For instance, it is not uncommon to identify vitamin D deficiency-related slightly cellular and slightly enlarged parathyroid glands. Moreover, in the case of parathyroid lipoadenoma, the cellularity may be sparse in a parathyroid gland that is enlarged and increased in glandular weight [26, 27]. Therefore, it is crucial to accurately document the weight of a parathyroid gland [1]. Nevertheless, most abnormal parathyroids are enlarged and cellular, and they are typically reflected in either parathyroid hyperplasia or parathyroid neoplasia.

Traditionally, the term “parathyroid hyperplasia” has been applied to multiglandular parathyroid disease; however, the concept of hyperplasia is generally no longer supported in the context of primary hyperparathyroidism since affected glands are usually composed of multiple “clonal” neoplastic proliferations [1–3]. In light of these findings and management implications for patient care, the 2022 WHO classification endorses primary hyperparathyroidism-related multiglandular parathyroid disease as a germline susceptibility-driven multiglandular parathyroid neoplasia. Therefore, germline screening of affected individuals is recommended, taking into consideration the overall morphological and molecular immunohistochemistry findings as well as clinical and laboratory findings. While there are some morphological and immunohistochemical harbingers of inherited hyperparathyroidism, pathologists must be given the appropriate clinical information on whether the patient clinically has a primary or secondary/tertiary hyperparathyroidism.

In the 2022 WHO classification, the spectrum of parathyroid neoplasms encompasses parathyroid adenoma (benign parathyroid tumor), atypical parathyroid tumor (parathyroid tumor of uncertain malignant potential), and parathyroid carcinoma. This morphological spectrum is mainly reflected in all patients with primary hyperparathyroidism. Epidemiology data from several countries show an increase in the frequency of parathyroid carcinoma diagnoses [28–32]; however, parathyroid adenomas are still the most common neoplastic correlates of autonomous PTH secretion. Patients with tertiary hyperparathyroidism may, although extremely rare, develop parathyroid carcinoma [33, 34].

The 2022 WHO bluebook reviews many aspects of parathyroid disorders. Pathologists must be provided relevant clinical and laboratory findings when assessing parathyroidectomy specimens. In addition to precise weight and measurements of a parathyroid gland, the clinical type of hyperparathyroidism (primary vs secondary vs tertiary), the number of involved parathyroid glands, the family history of hyperparathyroidism or genetic susceptibility in the family, the history of a former manipulation to parathyroid glands (e.g., fine-needle aspiration biopsy, PTH washout, ethanol injection), the preoperative imaging results, the preoperative serum calcium, PTH and vitamin D levels, and the results of intraoperative PTH assays along with postoperative calcium and PTH levels are all critical parameters in the accurate pathological characterization of hyperparathyroidism [1, 35]. The identification of significant drop of intraoperative PTH (usually defined as greater than 50%) indicates the removal of the source of hyperparathyroidism and often correlates with a uniglandular parathyroid disease [1]. This information provides a guidance to surgeons on the extent of parathyroid exploration especially when dealing with a Sestamibi and CT-scan non-localizing parathyroid disease.

There are also clinical and laboratory findings that should alert the diagnosticians to the possibility of a parathyroid carcinoma. These include (i) palpable neck mass, (ii) parathyroid gland measuring > 3 cm, (iii) severe hypercalcemia characterized one of the following: > 3 mmol/L, > 12 mg/dL, 3–4 mg over the upper limit of normal, (iv) significantly elevated PTH levels (> 3 times the upper limit of normal, usually 10 times the upper limit of normal), (v) 3rd to 2nd generation PTH ratio > 1, and (vi) intraoperative observation of adhesion to adjacent structures [35–44].

In the absence of metastatic tumor spread, these worrisome clinical and laboratory findings alone do not warrant a definitive diagnosis of parathyroid carcinoma. The final diagnosis of malignancy should be based on histopathological examination of an entirely submitted parathyroidectomy specimen. The new WHO classification outlines diagnostic criteria applied to atypical parathyroid tumors and parathyroid carcinomas, and provides information on the role of ancillary techniques. The details of various clinicopathological manifestations are discussed in other questions of this review article.

Fig. 4 Chief cell parathyroid adenoma. **A–B** Parathyroid adenoma composed predominantly of chief cells with well-defined cytoplasmic membranes. A rim of hypocellular/normal parathyroid tissue is also present (A)

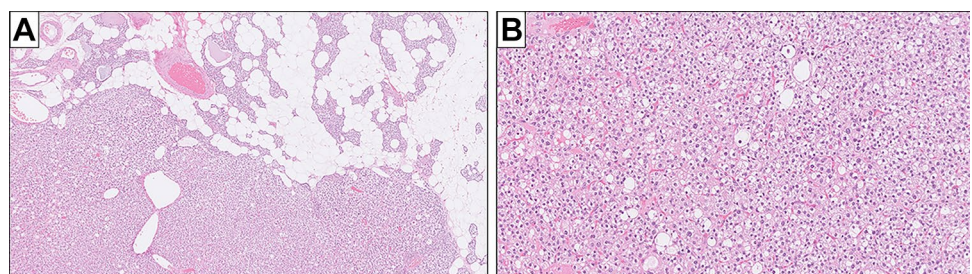
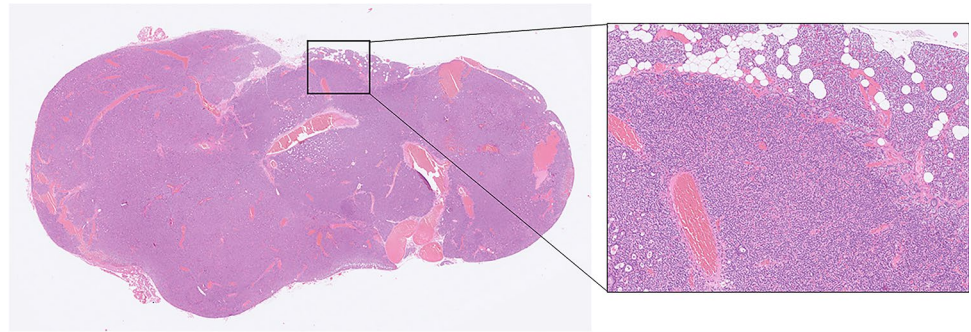


Fig. 5 Chief cell parathyroid adenoma. Chief cell parathyroid adenoma which is well-circumscribed and in the inset shows a rim of normal appearing parathyroid tissue



Question 3. What Are the Clinicopathological Correlates of Parathyroid Adenomas in the 2022 WHO Classification? What Does Multiglandular Parathyroid Adenomas Mean?

Clinically, parathyroid adenoma has evolved from a rare disease presenting with renal stones, bone pain due to bone loss, and osteitis fibrosa cystica [45] to a frequently diagnosed often asymptomatic disease identified on elevated serum calcium levels. The extent of screening of serum calcium levels in the general population correlates with the rate of asymptomatic hyperparathyroidism [46]. Moreover, increasing measurements of PTH in normocalcemic patients leads to an increased detection rate of “normocalcemic primary hyperparathyroidism”, with a prevalence of up to 3.4% in postmenopausal women as reported in a Swedish study [47]. These individuals may benefit from surgery for preservation of bone density and reduction of renal stones, but an individual approach is still suggested until further data are available [48]. The size of the parathyroid adenoma correlates with calcium/PTH level. For instance, smaller adenomas (40–100 mg) are associated with lower to normal serum levels of calcium and mild to asymptomatic hyperparathyroidism [49], whereas parathyroid adenomas > 1 cm are more frequently associated with higher levels of serum calcium and PTH and increased possibility of bone disease or hypercalcemic crisis [50]. Although rare, “giant parathyroid adenomas” can occur and measure several centimeters [51].

Histologically, parathyroid adenoma is a well-delineated neoplasm composed of chief, transitional, oncocytic, and water clear cells (Fig. 4). A rim of normal appearing parathyroid tissue is often observed in over half the cases (Fig. 5) [52]. A rim is identified more often when the gland is transected horizontal to the vascular pole. Stromal fat is usually markedly decreased or absent in adenomas. Chief cells show small regular nuclei without nucleoli and scant cytoplasm, and nuclear pleomorphism is usually mild, when present. There is a delicate network of capillaries, and chief cells often show a palisading along these capillaries at the border

of the nests. Follicular structures are frequently formed, and if follicular structures are prominent can be mistaken for thyroid tissue (Fig. 6). While immunopositivity for PTH can confirm tissue as parathyroid, this distinction may be difficult during intraoperative consultation. However, the following features may assist pathologists in the distinction of parathyroid origin: distinct cell membranes, the lack of bi-refringent calcium-oxalate crystals in the material within follicular structures, and/or the presence of intracellular lipid. In addition, parathyroid cells are also generally more vacuolated and smaller than thyrocytes, often have empty follicles, and may have rounder nuclei with denser chromatin than thyrocytes.

Focal oncocytic change can occur in the background of a chief-cell predominant adenoma. However, the term “oncocytic parathyroid adenoma” is applied when the tumor consists of > 75% of oncocytic cells (Fig. 7). These adenomas are larger [53, 54], with possibly higher preoperative serum calcium levels (10.8 vs 10.4 mg/dL) [53] and are more frequently localized with Sestamibi scan [55].

Water clear cell parathyroid adenomas are composed entirely of “water clear” cells and represent 1.5 of 1000 parathyroid adenomas (Fig. 8) [56]. The cytoplasm is distended and vacuolated, with hyperchromatic nuclei often allocated to the basal aspect of the cell. The cell membranes

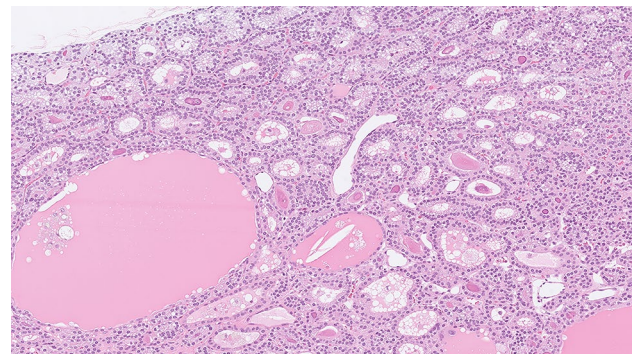
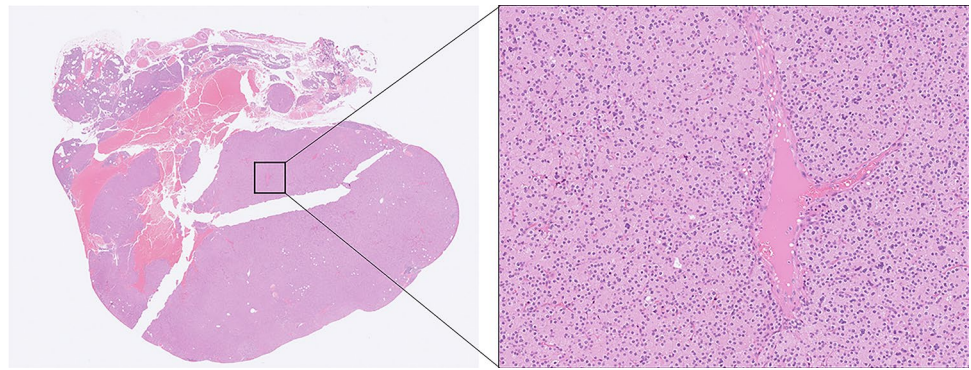


Fig. 6 Parathyroid adenoma with follicle formation. Follicle formation, if prominent, in parathyroid tissue can be mistaken for thyroid tissue

Fig. 7 Oncocytic parathyroid adenoma. Oncocytic parathyroid adenoma consists of greater than 75% oncocytic cells. The inset shows oncocytic cells with well-defined cell membranes



are clearly visible. The granular vacuolization is most likely attributed to an accumulation of glycogen, as positive periodic acid-Schiff (PAS) and negative diastase-PAS reactions usually are reported in water clear cell adenoma [57, 58]. Ultrastructurally, the glycogen-rich vacuoles observed in parathyroid water clear cell lesions appear to be derived from dilated cisterns of the granular endoplasmic reticulum, alternatively from secretory Golgi-derived granules [59]. As in chief cell adenomas, the stromal fat content is reduced or absent, and the cells stain positive for chromogranin A, PTH (Fig. 9) and GATA3 [60]. These lesions are often larger than chief cell adenomas, but may be difficult to locate by scintigraphy. The molecular pathogenesis of these tumors is largely unknown; however, their peculiar features warrant further investigations.

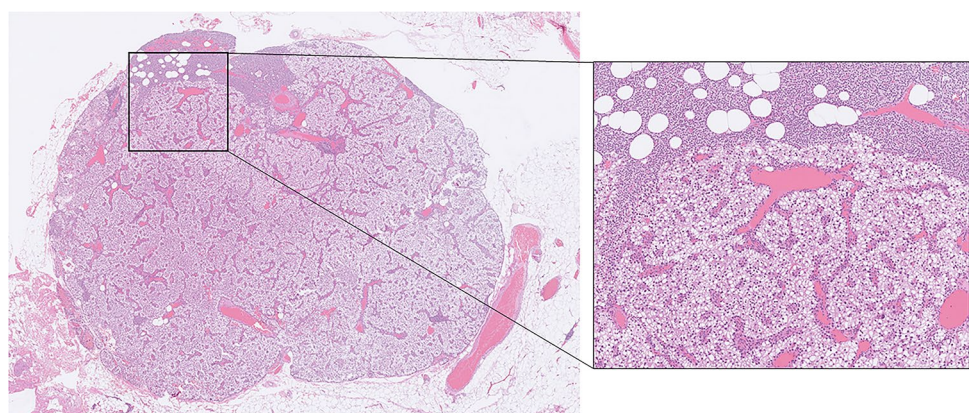
Degenerative changes, including cyst formation and hemorrhage, may occur in larger adenomas [56]. If these cystic changes comprise > 50% of the tumor, the terminology cystic parathyroid adenoma may be used. These tumors can be misinterpreted as thyroid cysts in scintigraphy due to lack of MIBI accumulation. These cystic changes can be associated with areas of fibrosis and focal calcifications [61], not to be misinterpreted as signs of malignancy.

Parathyroid lipoadenomas are tumors with an increase in both parathyroid parenchymal and adipose tissue, with

more than 50% of the glandular volume consisting of adipose tissue (Fig. 10) [26, 27, 62]. As a result, these tumors may not be localized on Sestamibi scan [27]. The abnormal weight of the gland (> 40–60 mg) is an important finding that should alert the diagnostician to the possibility of a parathyroid lipoadenoma in the appropriate morphological context and clinical setting [26, 27]. A significant drop of intraoperative PTH and evidence of postoperative biochemical cure also supports the diagnosis [26, 27]. Middle-aged female patients are slightly overrepresented among individuals with lipoma adenomas. The molecular etiology is unknown.

Up to 15% of individual with primary hyperparathyroidism (PHPT) show multiglandular disease with two or more parathyroid adenomas. This affects more frequently the superior parathyroid glands than the inferior glands [63]. Multiglandular disease, as well as male sex, age of occurrence under 45 years, and malignancy are factors associated with inherited hyperparathyroidism and should prompt consideration of genetic testing in light of morphological and molecular immunohistochemistry findings [64]. Multiglandular parathyroid disease was formerly regarded as a type of “hyperplasia”, but this nomenclature is a misnomer as multiglandular disease in the primary hyperparathyroidism is now generally thought to be composed of several synchronous or metachronous monoclonal proliferations.

Fig. 8 Water clear cell parathyroid adenoma. This water clear cell parathyroid adenoma is composed entirely of water clear cells with distended in vacuolated cytoplasm and hyperchromatic nuclei. A rim of normal appearing parathyroid tissue is present in this water clear cell parathyroid adenoma



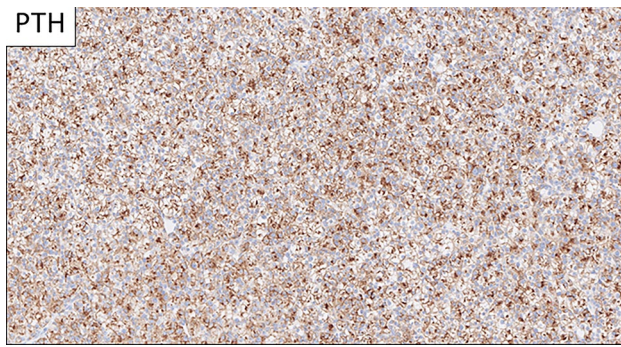


Fig. 9 Water clear cell parathyroid adenoma and parathyroid hormone staining. Similar to chief cell adenomas, these tumors are positive for parathyroid hormone as shown in the figure as well as chromogranin-A and GATA3

From an immunohistochemical standpoint, parathyroid adenomas show diffuse immunopositivity for PTH and GATA3, as well as chromogranin-A and may show staining for synaptophysin [10]. Thyroid follicular cell markers TTF1, thyroglobulin, and monoclonal PAX8 are negative. The use of some polyclonal PAX8 antibodies may show weak to moderate reactivity in parathyroid proliferations as well as in other neuroendocrine neoplasms. Therefore, pathologists should be aware of the clone of PAX8 antibodies used in their laboratory. Nuclear parafibrin staining is almost always present (Fig. 11), except for rare adenomas arising in individuals with pathogenic germline *CDC73* variants [13, 65].

Question 4. What Are the Clinicopathological Correlates of Parathyroid Carcinoma in the 2022 WHO Classification?

Histological criteria for the pathological diagnosis of parathyroid carcinoma have not changed under the 2022 WHO classification. However, the definition of what constitutes

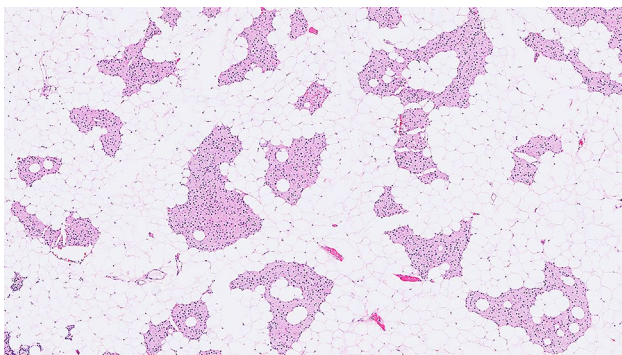


Fig. 10 Parathyroid lipoadenoma. This parathyroid lipoadenoma shows an increase in both parathyroid parenchymal and adipose tissue with more than 50% of the volume of the gland being composed of adipose tissue

an invasive growth was clearly discussed in this classification. Therefore, the histological diagnosis of parathyroid carcinoma is restricted to a parathyroid neoplasm that shows one of the followings: (i) angioinvasion (vascular invasion), (ii) lymphatic invasion, (iii) perineural (or intraneural) invasion, (iv) local malignant invasion into adjacent anatomic structures, or (v) histologically/cytologically documented metastatic disease (Fig. 12).

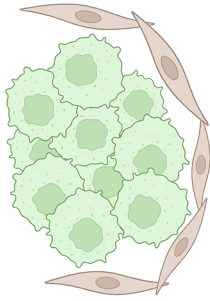
The complete submission of a parathyroid neoplasm is essential to assess the status of invasive growth. Moreover, multiple levels can also assist the assessment of invasive growth. Angioinvasion is defined as tumor invading through a vessel wall and associated thrombus, or intravascular tumor cells admixed with thrombus (Fig. 12) [35, 38, 66]. Given the fenestrated endothelium of endocrine tissues, angioinvasion is assessed at the intersection of the tumor and non-tumorous parenchyma, but not within the tumor substance. When a fibrin/thrombus complex is subtle, intravascular tumor cells (positive for GATA3 and PTH) admixed with platelet–fibrin thrombus complex can be highlighted using the Martius-Scarlet blue histochemistry or CD61 immunohistochemistry (Fig. 12). Lymphatic invasion can be confirmed using D2-40 immunohistochemistry. Perineural invasion requires at least the involvement of the epineurium.

Some parathyroid neoplasms may be associated with complete or incomplete encapsulation, the invasion into the lesion capsule does not qualify for a diagnosis of carcinoma since invasion requires local invasion into adjacent structures. An intrathyroidal parathyroid neoplasm or superior glands that are often embedded in the thyroid pseudocapsule, these features should be revisited when assessing invasive growth in such manifestations.

The differential diagnoses of parathyroid carcinoma include atypical parathyroid tumor, parathyromatosis (microscopic foci of parathyroid tissue that usually grows at the site of a former surgery or biopsy due to an implantation of lesional parathyroid tissue), a benign parathyroid proliferation (adenoma or hyperplasia) with worrisome histologic alterations following fine needle aspiration or ethanol injection, and contour irregularities seen in parathyroids from patients with longstanding secondary/tertiary hyperparathyroidism [67–74]. The clinical information and biomarker studies can narrow down the differential diagnoses.

Longstanding secondary or tertiary hyperparathyroidism may be associated with enlarged parathyroid glands with some atypical features including contour irregularity that can mimic invasive growth. Thus, caution is urged before the diagnosis of parathyroid carcinoma is made in the setting of advanced chronic renal failure. A malignant diagnosis should only be offered when there is unequivocal angioinvasion, spread outside the neck, or very marked invasive growth — for example unequivocal invasion into esophagus,

Parathyroid adenoma



PFIB +

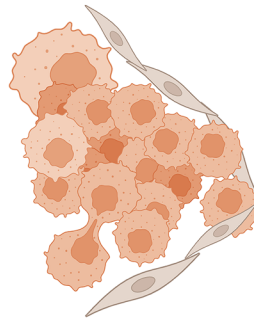
PGP9.5 -

APC +

GAL-3 -

Ki-67 ↓

Atypical parathyroid tumor



PFIB + or -

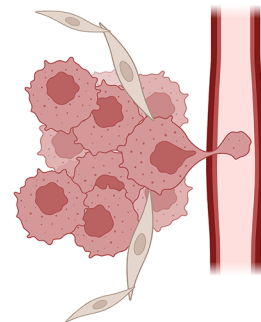
PGP9.5 + or -

APC + or -

GAL-3 + or -

Ki-67 ↓ ↑

Parathyroid carcinoma



PFIB -

PGP9.5 +

APC -

GAL-3 +

Ki-67 ↑

Fig. 11 Ancillary immunohistochemical markers in parathyroid tumors. Whereas parathyroid adenomas (left) recurrently display positivity for parafibromin (PFIB) and APC, they often stain negative for galectin-3 (GAL-3) and PGP9.5. The Ki-67 index is often below 1%.

Atypical parathyroid tumors (center) may exhibit a variable immunohistochemical phenotype, in which cases with risk of recurrences often are parafibromin-deficient and display immunophenotypes mimicking that of parathyroid carcinomas (right)

or skeletal muscle rather than just microscopic extension into the immediate adipose tissue.

Another important aspect of the new WHO classification is related to an increased emphasis on parathyroid carcinoma as a potentially hereditary disease associated with HPT-JT or isolated familial hyperparathyroidism syndrome due to pathogenic germline *CDC73* variants which encodes parafibromin [9, 14, 19, 20, 75–77]. Thus, the possibility of germline susceptibility should be considered in all patients with parafibromin-deficient parathyroid carcinoma even in the absence of a suggestive personal history [19, 21, 78]. Sporadic and what was clinically thought to be apparently sporadic-looking parathyroid carcinomas are often associated with biallelic *CDC73* mutation/inactivation [21, 22, 79–82]. The *CDC73* mutations are frequently germline, indicative of de novo mutations or undetected germline alterations [21, 22, 83]. Given the role of parafibromin in malignancy, it has been

proposed that a lower threshold for invasive growth may be needed to support the diagnosis of parathyroid carcinoma in parafibromin deficient parathyroid tumors [9].

The new classification has also brought an emphasis on the judicious use of immunohistochemical biomarkers to support a diagnosis of malignancy in the differential diagnosis with parathyroid carcinoma. Among these, loss of expression of parafibromin, APC, RB, E-cadherin, p27, Bcl-2a, mdm-2, and 5-hmC and positivity for PGP9.5, galectin-3, hTERT, and p53 overexpression along with increased Ki67 labeling index (often > 5%) [10, 11, 13, 15, 17, 35, 38, 66, 84–91] are considered biomarker correlates of parathyroid carcinoma. Although no individual marker is definitive, combinations of these markers are particularly useful in difficult to classify cases [10, 17, 85–87, 89, 92, 93]. The details of these biomarkers are discussed in the question that deals with ancillary tools.

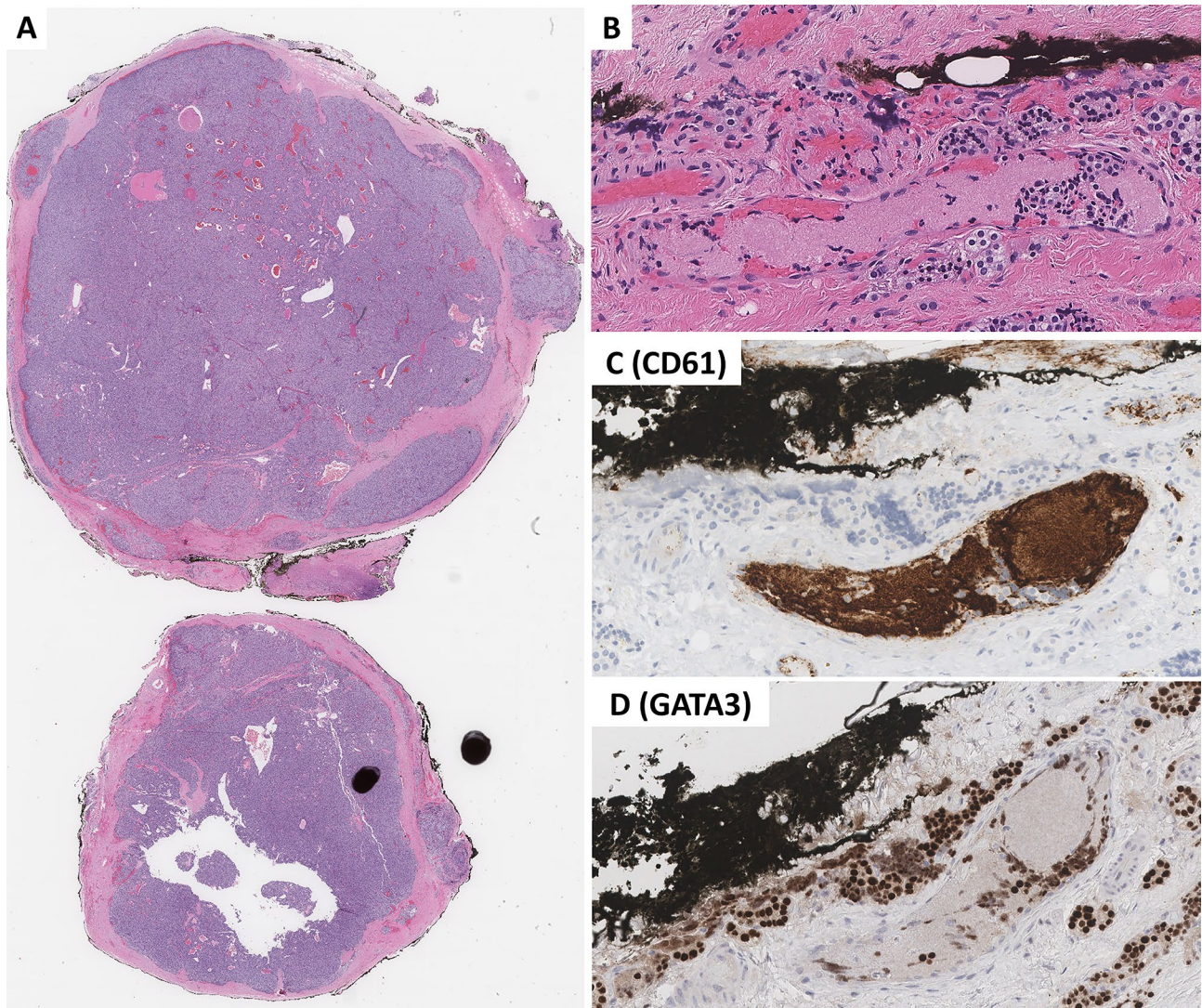


Fig. 12 Parathyroid carcinoma. **A** This is an example of a parathyroid carcinoma that is angioinvasive. Although parathyroid adenomas and atypical parathyroid tumors can have some atypical features such as mitotic activity in fibrosis, they do not show unequivocal capsular,

vascular, or perineural invasion. **B** Angioinvasion with tumor within a vessel associated with thrombus. **C** CD61 immunohistochemistry highlighting the platelet fibrin thrombus complex. **D** GATA3 immunostain highlighting the intravascular tumor cells

Question 5. How Are Atypical Parathyroid Tumors Defined in the 2022 WHO Classification?

Atypical parathyroid tumor is defined as a parathyroid neoplasm that demonstrates atypical cytological and architectural features (Figs. 1 and 13), but lacks unequivocal capsular, vascular, or perineural invasion or invasion into adjacent structures or metastases. Atypical parathyroid tumors usually occur sporadically but can occur in the setting of inherited hyperparathyroidism [94]. The serum calcium levels are often intermediate between that of a parathyroid adenoma and a parathyroid carcinoma.

Atypical parathyroid tumors show some worrisome histologic features often seen in parathyroid carcinoma including adherence to adjacent structures, monotonous sheet-like or trabecular growth, cytologic atypia, fibrosis (which can be band-like, tumor cells extending into but not through the tumor capsule), and mitotic activity (> 5 per 50 high-power fields/ $\sim 10\text{mm}^2$), but they do not show unequivocal invasion as would be required for diagnosis of parathyroid carcinoma (Figs. 2 and 3). Atypical parathyroid tumors do not show full-thickness capsular invasion, vascular invasion, perineural invasion, or invasion into adjacent structures as would be diagnostic for parathyroid carcinoma.

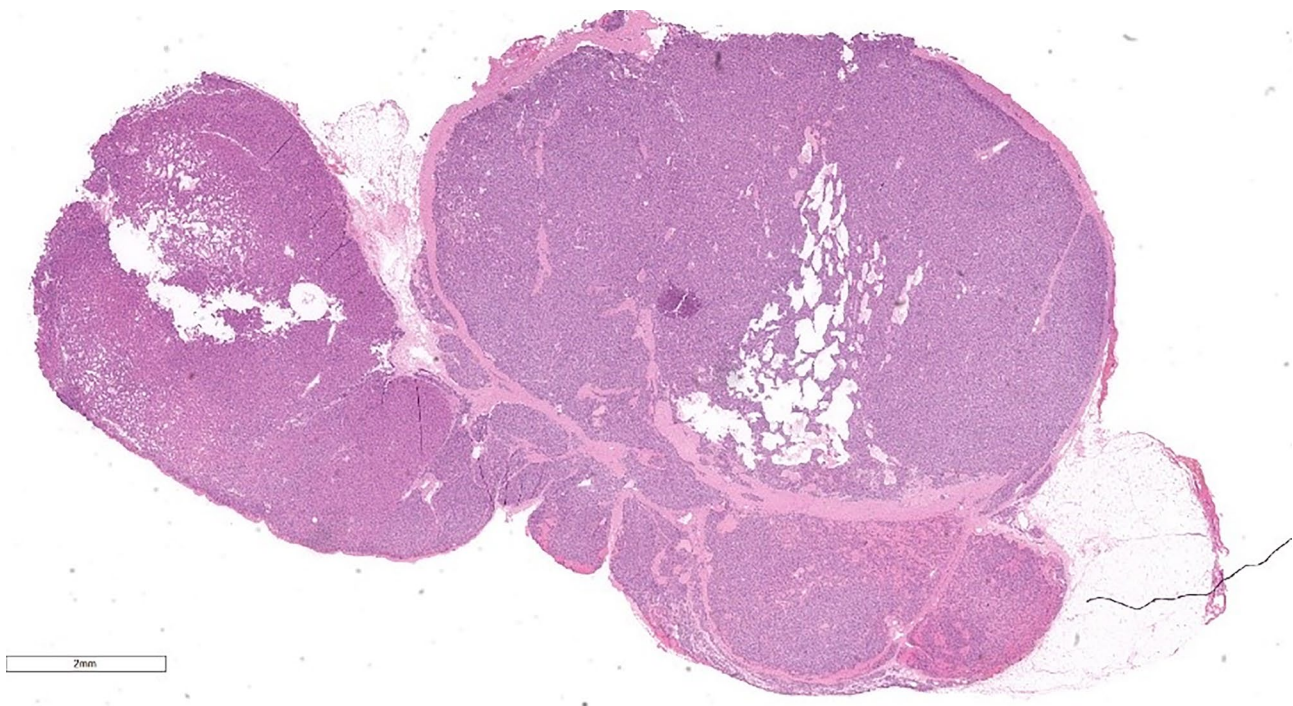


Fig. 13 Atypical parathyroid tumor. Atypical parathyroid tumor showing fibrous bands and fibrosis but lacking definitive invasion

Atypical parathyroid tumors are composed predominantly of chief cells with variable amounts of oxyphil and transitional cells and may show monotonous, solid growth of sheets of cells in closely packed nests similar to parathyroid carcinoma (Fig. 2). Mitotic activity can be seen in benign and malignant parathyroid disease, but a mitotic activity that exceeds 5 mitoses per 50 high-power fields/ $\sim 10\text{mm}^2$ in association with coagulative tumor necrosis, macronucleoli, atypical mitoses, or Ki67 proliferation index $> 5\%$ are highly worrisome features for malignancy (Figs. 1 and 3) [94, 95]. Parathyroid carcinoma is often have a Ki-67 labeling index of greater than 5%, parathyroid adenomas typically have a much lower labeling index whilst the proliferative index of atypical parathyroid tumors may be intermediate (Fig. 14). However, a definitive diagnosis of parathyroid carcinoma requires invasive growth or metastasis as discussed in the former question (Fig. 1).

Conventional parathyroid adenomas can show areas of fibrosis particularly if they are large. Fibrosis can also be seen with prior manipulation such as fine-needle aspiration biopsy or cystic parathyroid neoplasm in which there may be hemosiderin deposition or irregularity in the contour to suggest prior trauma [67, 72]. Fibrosis may also be seen in the setting of secondary or tertiary hyperparathyroidism. Conventional parathyroid adenomas can also have occasional mitotic figures. The presence of focal fibrosis or occasional mitotic figures (< 5 per 50 high-power fields/ $\sim 10\text{mm}^2$) is

insufficient to qualify a parathyroid neoplasm as an atypical parathyroid tumor. The term atypical parathyroid tumor is reserved for a parathyroid neoplasm with atypical features for which the differential diagnosis is parathyroid carcinoma. These features have been reflected in the algorithm provided (Fig. 1).

Immunohistochemically, atypical parathyroid tumors show positivity for PTH, chromogranin-A, and GATA3. Many biomarkers, including Ki67, PGP9.5, cyclin D1, p27, P53, galectin 3, RB, Bcl-2a, mdm2, and E-cadherin, have been studied in parathyroid neoplasms (Fig. 11) [10, 17, 54, 84, 85, 93, 96–98]. However, these are not able to consistently differentiate parathyroid carcinomas from adenomas, but may provide help in the diagnostic workup of parathyroid tumors showing atypical features.

Parafibromin is encoded by *CDC73*, and complete absence of nuclear parafibromin immunoreactivity and parathyroid neoplasm is regarded as “parafibromin deficiency” indicating an underlying biallelic somatic or germline *CDC73* inactivation [10–12, 91]. However, point mutations of *CDC73* may result in preserved parafibromin expression, thus the presence of parafibromin does not rule out the possibility of an underlying germline pathogenic variants [13, 14]. Parathyroid adenomas generally retain nuclear immunoreactivity for parafibromin, however those in the setting of underlying *CDC73* mutations (such as hyperparathyroidism jaw tumor syndrome) will also show loss of parafibromin [13]. Somatic *CDC73* mutations are also associated

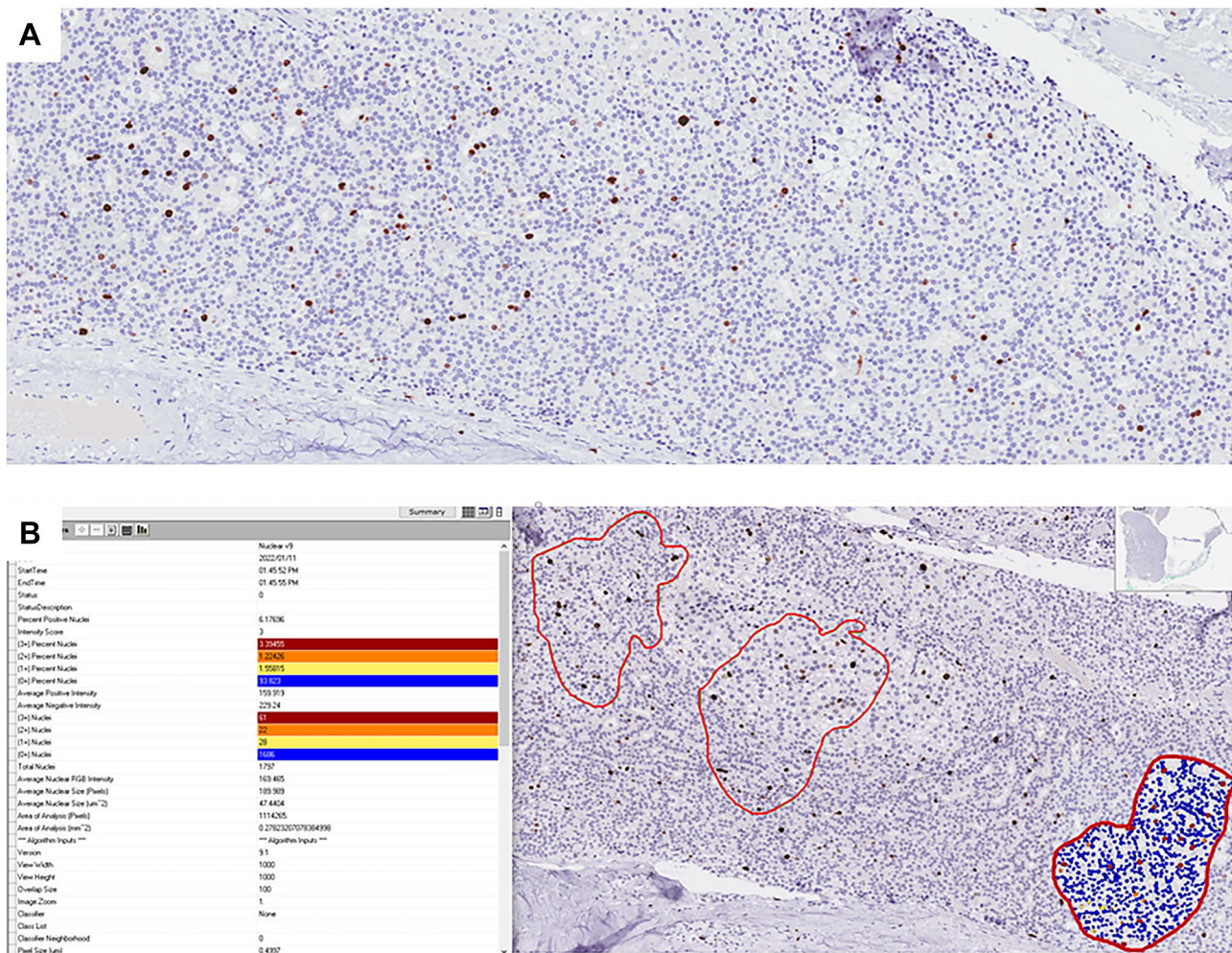


Fig. 14 Ki-67 labeling in an atypical parathyroid tumor. **A** Immunostaining showing proliferating cells in an atypical parathyroid tumor. **B** Imaging showing labeling cells in an atypical parathyroid tumor

with sporadic parathyroid carcinoma [10, 13, 17, 18]. Evidence suggests that parafibromin may be helpful in predicting outcome in atypical parathyroid tumors [15, 16]. For instance, parafibromin deficient atypical parathyroid tumors have been shown to develop metachronous disease in other glands, and also as true recurrence [11, 15, 16, 91]. Thus, long-term follow-up is indicated for parafibromin deficient parathyroid neoplasms [11, 15, 16, 91]. The new classification also encourages a routine germline *CDC73* mutation testing in the setting of a parafibromin deficient parathyroid neoplasm, as well as in all parathyroid carcinomas (Fig. 1) [11, 12, 91].

In summary, the 2022 WHO classification categorizes atypical parathyroid tumor as a neoplasm of uncertain malignant potential. Most atypical parathyroid tumors behave similarly to parathyroid adenomas and do not recur after excision, however recurrent disease, often metachronous, and metastasis have been reported especially in the

context of parafibromin deficiency [9, 11, 15, 16]. Therefore, a clinical and biochemical follow-up only is recommended for the management of this tumor type.

Question 6. Which Ancillary Tools Should Pathologists Consider Utilizing in the Accurate Assessment of Parathyroid Tumors?

While the diagnosis of an atypical parathyroid tumor or parathyroid carcinoma is strictly based on histopathological criteria, there are several ancillary markers that the pathologist could use in the diagnostic work-up and/or to identify underlying genetic aberrations of value in the clinical approach to of the individual patient. Although the vast majority of parathyroid tumors are benign and often straight-forward to diagnose with routine morphology alone, there are several

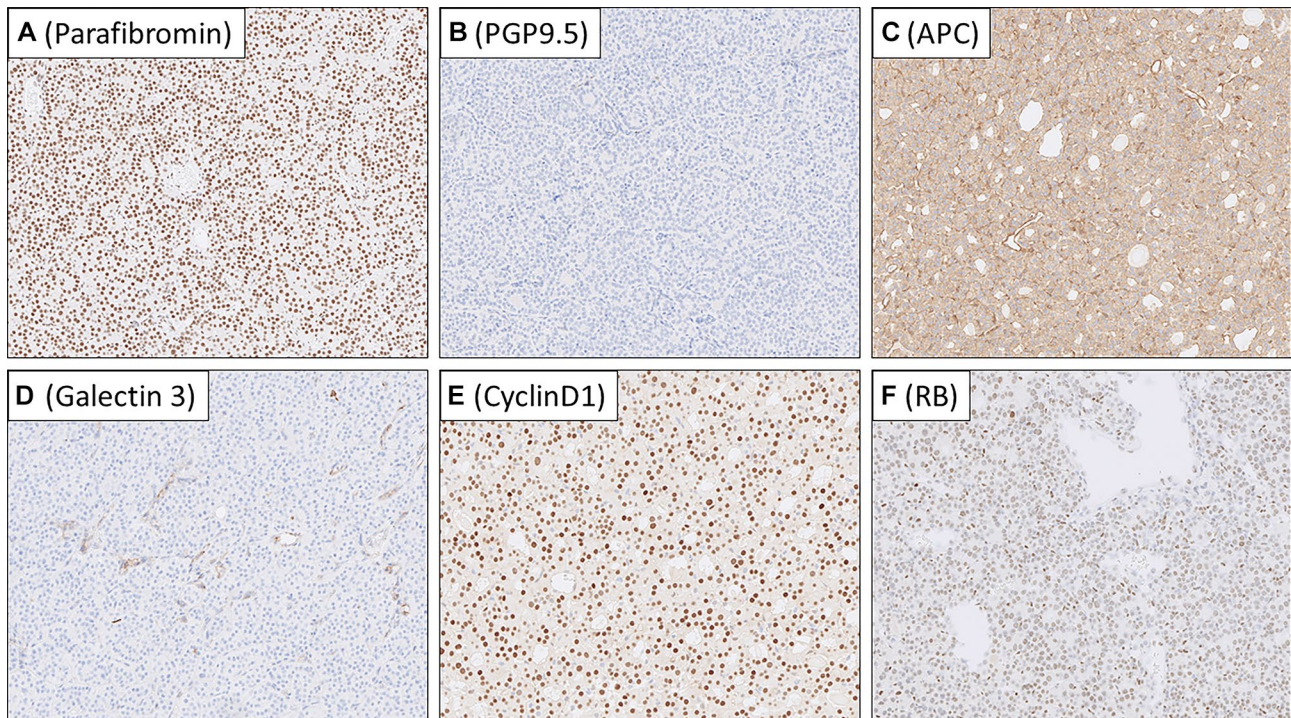


Fig. 15 Immunohistochemical features of parathyroid adenoma. **A** Retained nuclear parafibromin expression is noted in virtually all parathyroid adenomas without *CDC73* gene aberrancies. **B** PGP9.5 expression is generally absent. **C** Cytoplasmic APC immunoreactivity is observed in the majority of parathyroid adenomas, while lost in

subsets of parathyroid carcinoma. **D** Galectin-3 expression may occasionally be seen in atypical parathyroid tumors and parathyroid carcinoma but is usually absent in adenoma. **E** CyclinD1 overexpression is recurrently seen in parathyroid adenoma. **F** RB expression may be heterogenous in parathyroid adenoma, but there is no global loss

instances in which immunohistochemistry may be of direct value to the pathologist.

Parathyroid cells show neuroendocrine differentiation, and therefore, parathyroid tumors exhibit immunoreactivity with chromogranin A and occasionally also to synaptophysin, whereas expression of second-generation neuroendocrine markers such INSM1 seem to be absent [99–101]. Moreover, normal parathyroid cells and tumors almost always stain positive for PTH and GATA3 and are consistently negative for TTF1 — thereby setting them apart from thyroid tissue. While monoclonal PAX8 antibodies are negative in parathyroid tissues, other polyclonal PAX8 antibodies may show staining in parathyroid tumors and should therefore not be used to distinguish between parathyroid and thyroid [102].

After correctly identifying a parathyroid tumor, the pathologist must assess the risk of malignancy. While the gold standard of this procedure is based upon histologic assessment of malignant features discussed elsewhere in this review, there are several immunohistochemical markers available to aid in the evaluation of potentially troublesome parathyroid tumors. Most of this research has been focused on identifying markers that distinguish parathyroid carcinoma from adenoma, and studies have found significant differences

in expression for many proteins — of which some of the most important are listed in Fig. 11. Most notably, parathyroid adenomas often retain their immunoreactivity for parafibromin, APC, E-cadherin, p27, RB, Bcl-2a, mdm2, and 5-hmC, while one or several of these markers usually exhibit negative staining in parathyroid carcinoma (Fig. 15) [10, 11, 17, 84, 90, 91, 103]. On the contrary, while parathyroid carcinomas often show PGP9.5, Galectin-3, and p53 expression, these markers are not typically expressed in parathyroid adenomas (Figs. 11, 15, and 16) [13, 66, 86, 87, 89, 104]. The increased proliferative indices of parathyroid carcinoma can be identified through a Ki-67 labeling index, which is usually above 5%, while parathyroid adenomas typically display much lower proliferative indices (Fig. 11) [84, 105, 106]. Even so, the overlap between parathyroid adenomas, hyperplasias, and carcinomas in terms of the proliferation labeling index is considerable, limiting the utility of this marker particularly if it not used in combination with others [107]. There is no formal grading system in parathyroid carcinomas, but the 2022 WHO classification encourages the use of Ki-67 immunohistochemistry in parathyroid carcinomas (Fig. 17).

Other biomarkers such as the bona fide parathyroid oncogene CyclinD1 is up-regulated in parathyroid adenoma and carcinoma alike, and thus displays reduced specificity

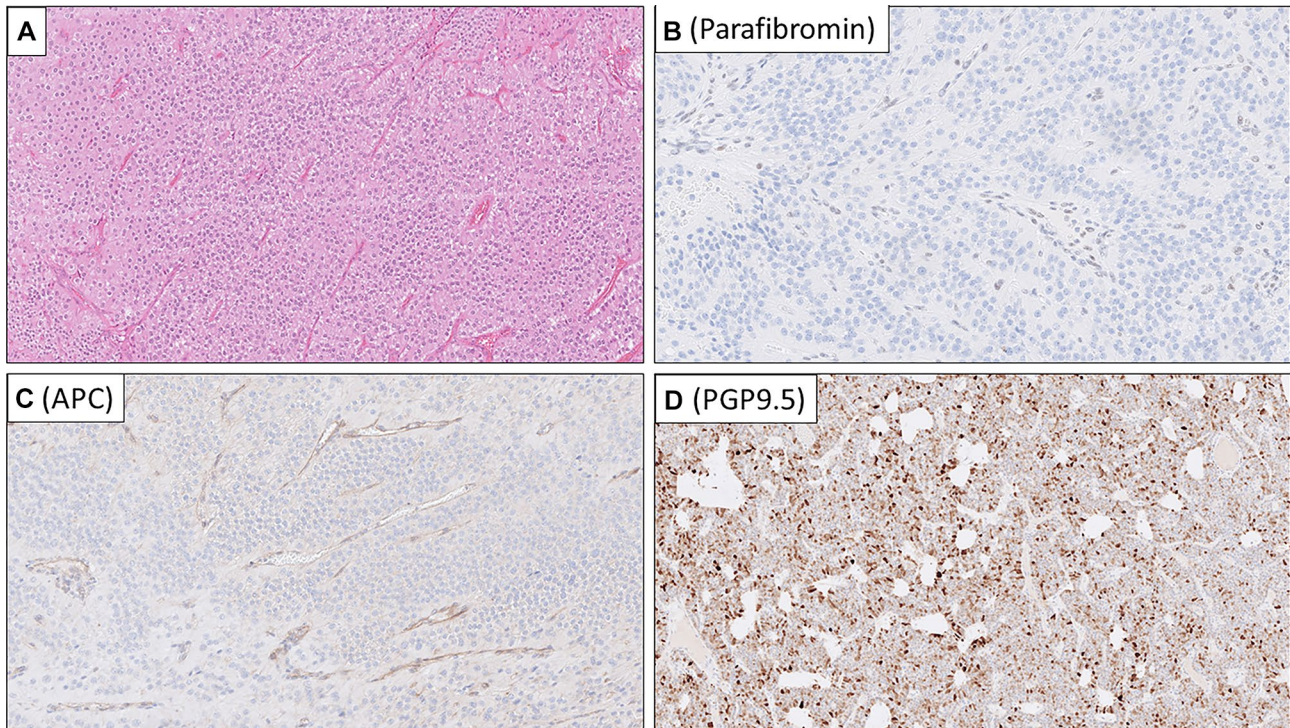


Fig. 16 Histological and immunohistochemical features of parafibromin-deficient parathyroid tumors. **A** Parathyroid carcinoma displaying sheet-like growth, eosinophilic cytoplasm, nuclear enlargement and perinuclear cytoplasmic clearing. **B** This tumor stained diffusely negative for parafibromin, while internal controls (fibroblasts, endothelial cells) are weakly

positive. **C** The same cases showed absence of APC immunoreactivity, which has also been coupled to parathyroid malignancy. **D** PGP9.5 expression was diffusely positive, also supporting the diagnosis

towards malignant disease (Fig. 15) [65, 108, 109]. Similarly, loss of Rb protein expression was initially suggested to be highly specific for loss of heterozygosity of the *RB1* gene locus, a feature seen in some parathyroid carcinomas [110], but ensuing studies found this genetic aberrancy also to be

present in subsets of parathyroid adenomas [106, 111]. Also, the Rb stain may be heterogenous even in cases with retained *RB1* alleles, making the interpretation of this marker difficult (Fig. 15). Nevertheless, the global loss of Rb expression is considered as a concerning finding.

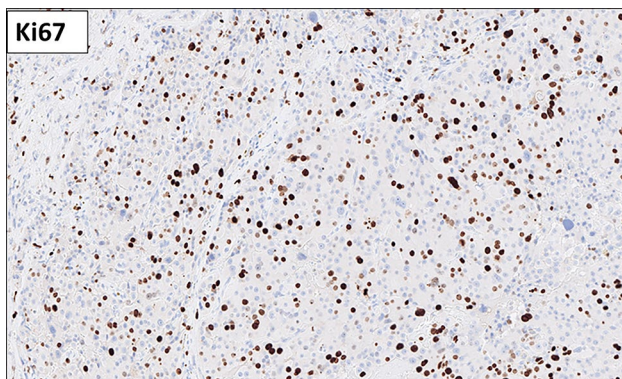


Fig. 17 Ki-67 proliferation index in parathyroid carcinomas. Most parathyroid carcinomas show a labeling index that exceeds 5%. Currently, there is no formal grading based on tumor proliferation; however, Ki-67 is considered as a continuous variable in the risk stratification

Given the association between parafibromin (protein encoded by *CDC73*) expression and *CDC73*-mutations that are enriched in parathyroid carcinomas, the 2022 WHO classification puts emphasis on the use of parafibromin immunohistochemistry as a clinically useful marker. Complete loss of nuclear parafibromin immunoreactivity in all tumor cells identifies a parathyroid tumor as “parafibromin-deficient”, a phenomenon highly correlated to underlying *CDC73* gene mutations, irrespective of whether the variant is found in somatic or germline tissues [9]. Mutations are often deleterious and accompanied with deletions of the wild-type allele in somatic tissues [21, 22, 79]. Alternate modes of parafibromin dysregulation apart from *CDC73* gene mutations, such as aberrant *CDC73* promoter methylation, are not recurrent themes in parathyroid tumors [112, 113]. While some authors report diffuse loss of nuclear expression in the presence of internal positive controls (fibroblasts, endothelial cells) in *CDC73* gene-mutated cases (Fig. 16),

other groups have observed a reduction of the proportion of parafibromin positive cell nuclei rather than a complete absence — possibly due to methodological differences in the staining protocol or technical issues or difficulties (Fig. 18) [9, 11, 13, 82, 114]. Thus, additional genetic testing may be suggested in both situations. However, in the 2022 WHO classification, complete nuclear loss of expression of parafibromin is considered as parafibromin-deficiency. Subsets of cases may show loss of nucleolar parafibromin expression and concurrent retained nuclear staining, possibly signifying underlying *CDC73* gene mutations in the nucleolar localization signal (NoS) sequence (Fig. 18) [11, 115, 116]. As the disruption of nucleolar parafibromin also may drive parathyroid tumorigenesis, this finding may be considered as indicating the possibility of underlying pathogenic mutation [117]. Therefore, the loss of nucleolar reactivity requires molecular testing to confirm its biological significance. A genotype–phenotype correlation has been suggested, in which the authors noted that parafibromin-deficient tumors have distinct features as discussed in the last question of this review.

A parafibromin-deficient phenotype can occasionally be seen in parathyroid adenomas (particularly in the setting of underlying pathogenic germline *CDC73* variants), but parafibromin deficiency may be more frequently observed in atypical parathyroid tumors and is highly prevalent among parathyroid carcinomas. The possibility of a seemingly sporadic looking parathyroid adenoma that arises in the background of germline pathogenic *CDC73* variants is not an uncommon event in the absence of family history or other manifestations of HPT-JT syndrome [19, 22, 65]. In the absence of germline *CDC73* variants, a parafibromin-deficient parathyroid adenoma diagnosis may be due to a somatic *CDC73* gene mutation leading to sporadic parathyroid adenomas often with cystic features. Therefore, individuals with parafibromin-deficient parathyroid neoplasms should undergo *CDC73* gene sequencing to avoid missing a potential germline alteration (that can present as a de novo event in the proband), but with the notion that the genetic aberrancy may be somatic if germline (constitutional) *CDC73* screening is negative [16]. In some series, the proportion of parafibromin-deficient atypical parathyroid tumors is equal to that of parathyroid carcinoma, a finding that further supports a shared molecular phenotype between these groups [118]. Parafibromin deficiency in an atypical parathyroid tumor is a particularly worrisome for the possibility of aggressive behavior as discussed in the former question.

Apart from immunohistochemistry alone, various combinations of immunohistochemical and in situ hybridization approaches have also been evaluated, but these observations need confirmatory studies before being recommended in the clinical setting [100].

Question 7. What Are the Molecular Correlates of Parathyroid Tumors? Are There any Diagnostic Molecular Studies in the Workup of Parathyroid Tumors?

Deleterious germline mutations of the *MEN1* gene on chromosome 11q13 underlie the MEN1 syndrome in which multiglandular primary hyperparathyroidism is the cardinal disease, and it is therefore not surprising to find somatic *MEN1* mutations and deletions as the main genetic abnormality in sporadic parathyroid adenomas as well [119–122]. The *MEN1* mutations are often accompanied by loss of heterozygosity (LOH) of the wild-type allele, thereby fulfilling the two-hit hypothesis for a bona fide tumor suppressor gene. *MEN1* encodes menin, a ubiquitously expressed protein with a variety of cellular functions, affecting specific transcription factors as well as epigenetic programs through various histone modifications [18]. Specifically, menin inhibits the JunD transcription factor, which in turn is known to activate gene programs coupled to proliferation [123]. Moreover, menin mediates increased tri-methylation of H3K4, allowing an opening of the chromatin which in turn facilitate gene transcription (Fig. 19) [124]. Beyond *MEN1* aberrancies, somatic mutations in other genes propelling the formation of parathyroid adenomas are rarely described [125–127]. Interestingly, the *CDC73* gene encoding parafibromin is recurrently mutated in parathyroid tumors with unknown or established malignant potential (atypical parathyroid tumors and parathyroid carcinoma, respectively). But it should be noted that small subsets of cystic parathyroid adenomas also may harbor somatic mutations in this gene [65]. Other recurrent mechanisms of interest in parathyroid adenoma include overexpression of the proto-oncogene cyclin D1 (encoded by the *CCND1* gene) is found in large subsets of parathyroid adenomas [108, 109]. In rare instances, this phenomenon is due to a chromosome 11 inversion, bringing the *CCND1* gene under regulation from the PTH gene promoter [128], but aberrant promoter methylation of so-called cyclin dependent kinase inhibitors (CDKIs) may also cause cyclin D1 up-regulation [129]. Cyclin D1 associates with the CDK4 complex and promotes passage through the G1 phase of the cell cycle by inhibiting the retinoblastoma protein (Rb), so up-regulation of cyclin D1 will promote proliferation (Fig. 19) [130]. Other genes with altered expression levels in parathyroid adenoma compared to normal tissues include the observed down-regulation of *CASR* and *VDR* mRNA, two main parathyroid “brake pedal function” genes that normally inhibit the proliferative activity of the parathyroid glands when binding to their ligands calcium and vitamin D, respectively (Fig. 19) [131].

The association between inactivating *CDC73* gene mutations and parathyroid carcinoma is best exemplified by the

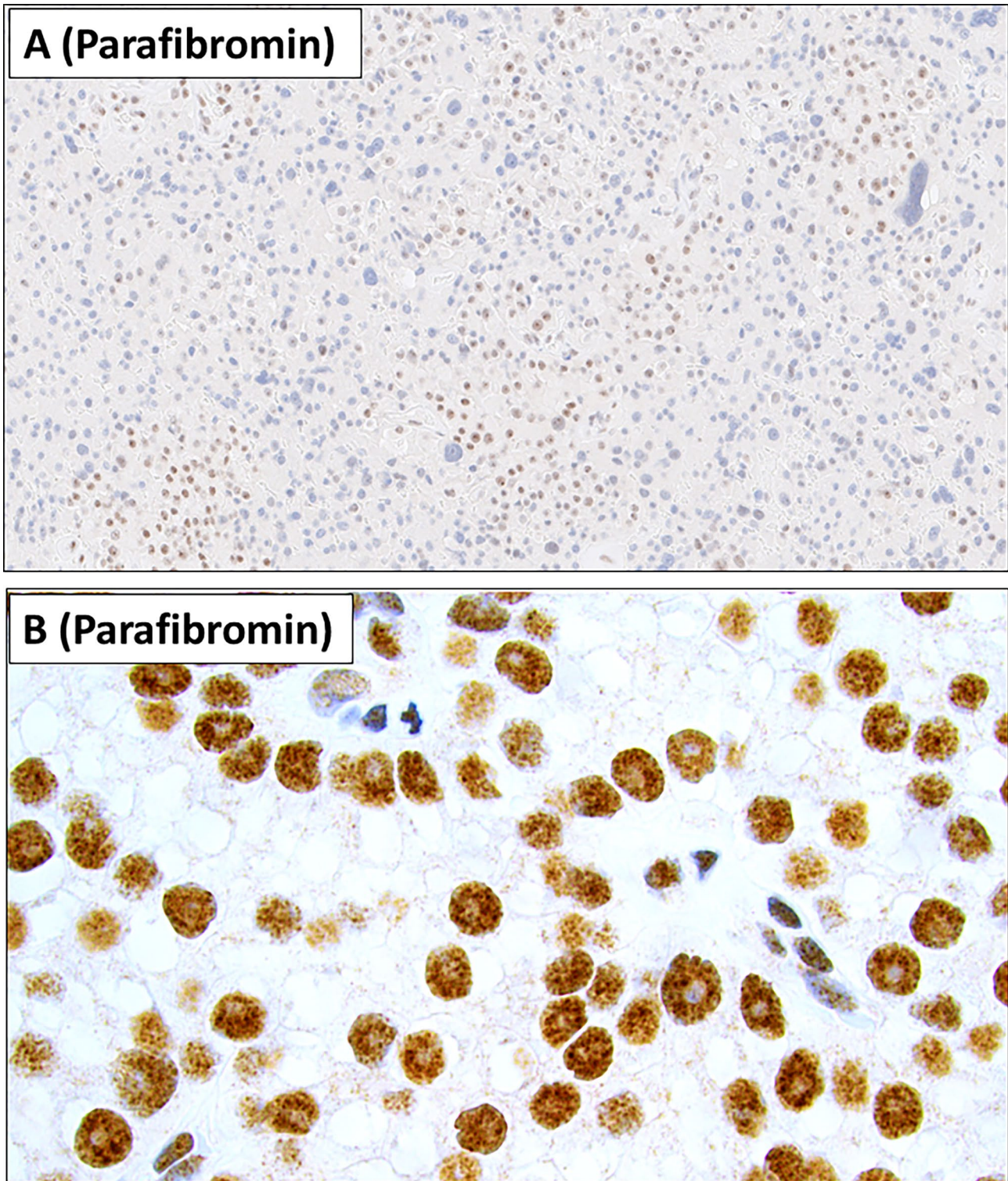


Fig. 18 Aberrant parafibromin immunostaining in parathyroid carcinoma. **A** Parathyroid carcinoma exhibiting partial loss of nuclear parafibromin immunoreactivity. This pattern has been reported in *CDC73* mutated parathyroid tumors and has been interpreted as

parafibromin-deficient by some institutions. Increased Ki-67 labeling index in the same case. **B** Nucleolar loss of parafibromin immunoreactivity may be coupled to *CDC73* missense mutations perturbing the nucleolar localization signals of this protein

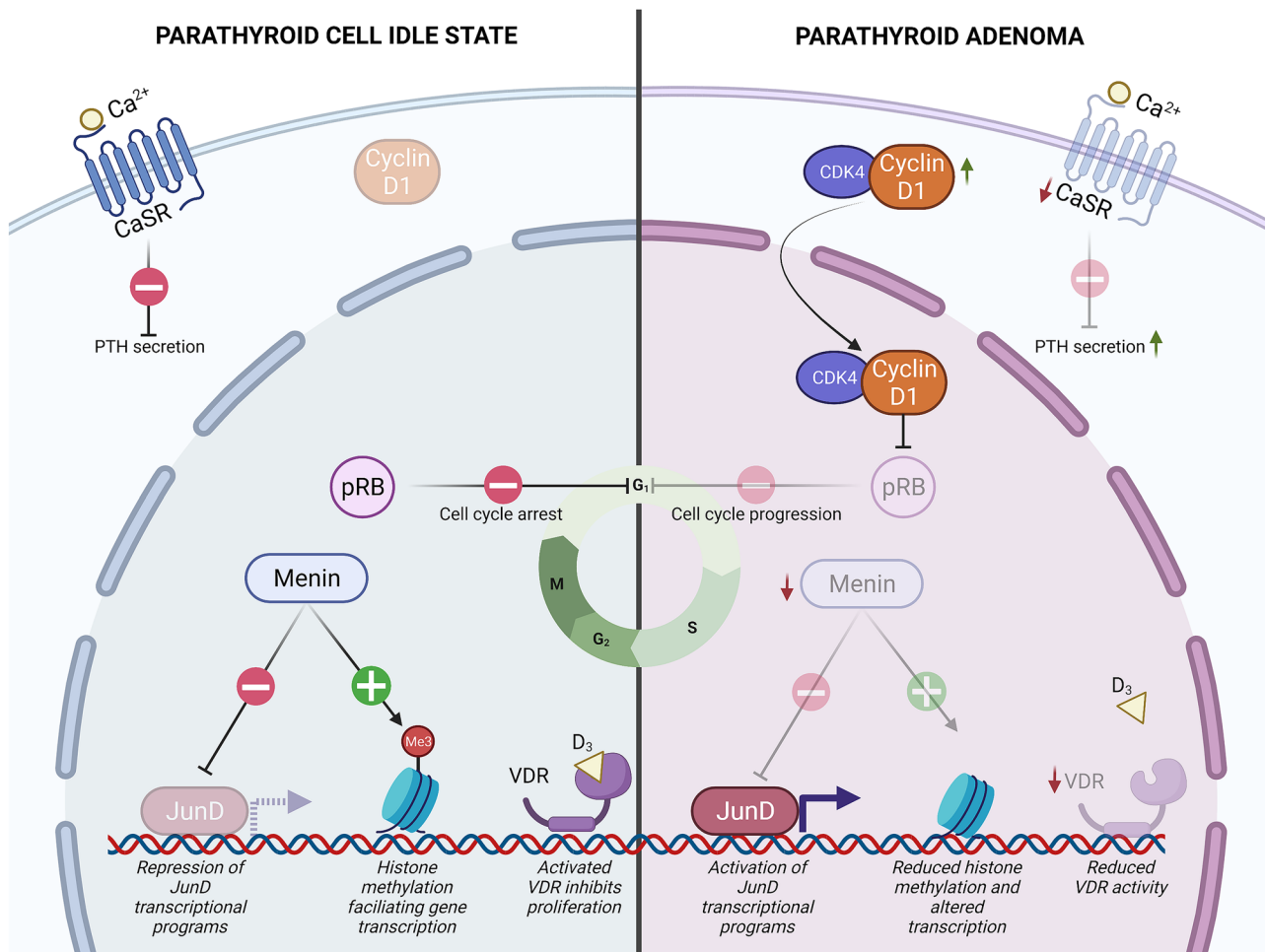


Fig. 19 Parathyroid idle state vs adenoma. Schematic overview of normal parathyroid cell regulation (left) compared to somatic molecular alterations underlying the development of parathyroid adenoma (right). Under normal physiological conditions, the normal parathyroid cell is idle. Extracellular calcium binds to the G-protein coupled transmembrane calcium sensing receptor (CaSR), causing a reduction of PTH secretion. Likewise, activated vitamin D (D_3) will bind the nuclear vitamin D receptor (VDR), also inhibiting parathyroid cell proliferation. The proliferative state is also regulated by the retinoblastoma protein (pRb), a nuclear tumor suppressor protein that promotes cell cycle arrest. Menin (encoded by the *MEN1* tumor suppressor gene) is abundantly expressed in the nuclear compartment, which

in turn represses the JunD transcription factor from activating gene programs leading to proliferation, while simultaneously promoting methylation of various histones which enables transcription of specific gene sequences, including various cell cycle regulators essential for growth control. In parathyroid adenomas, *CaSR* and *VDR* mRNA levels are often downregulated, causing an increase in PTH release and proliferative activity. Moreover, *MEN1* is recurrently mutated and/or deleted, causing activation of JunD transcriptional programs as well as a reduction in histone methylation. Also, cyclin D1 levels are frequently increased in parathyroid adenoma, leading to the nuclear accumulation of the CDK4-cyclin D1 complex, in turn inhibiting pRb, leading to cell cycle progression

HPT-JT syndrome, in which parathyroid malignant tumors are markedly overrepresented compared to the incidence in the general population [19, 21, 78]. Similarly, between 40 and 80% of sporadic parathyroid carcinomas display inactivating *CDC73* gene mutations, often in combination with loss of the remaining allele [21, 22, 79, 81]. Strikingly, these mutations are often found to be present also on the germline level, suggesting a high rate of previously undetected syndromic cases, or representing de novo alterations [21, 22, 83]. The *CDC73* mutations are often deleterious, causing

disruption of the nuclear localization signals, leading to aborted nuclear translocation [13, 132, 133].

CDC73 encodes parafibromin, a predominantly nuclear protein with multiple tumor suppressive functions. Parafibromin has been established as a member of the human RNA polymerase II-associated factor (hPAF) complex involved in facilitating DNA transcription [134, 135], and has also been shown to regulate the expression of the proto-oncogenes *CCND1* (encoding cyclin D1) and *c-Myc*, as well as interacting with the Wnt pathway effector beta-catenin [136–138].

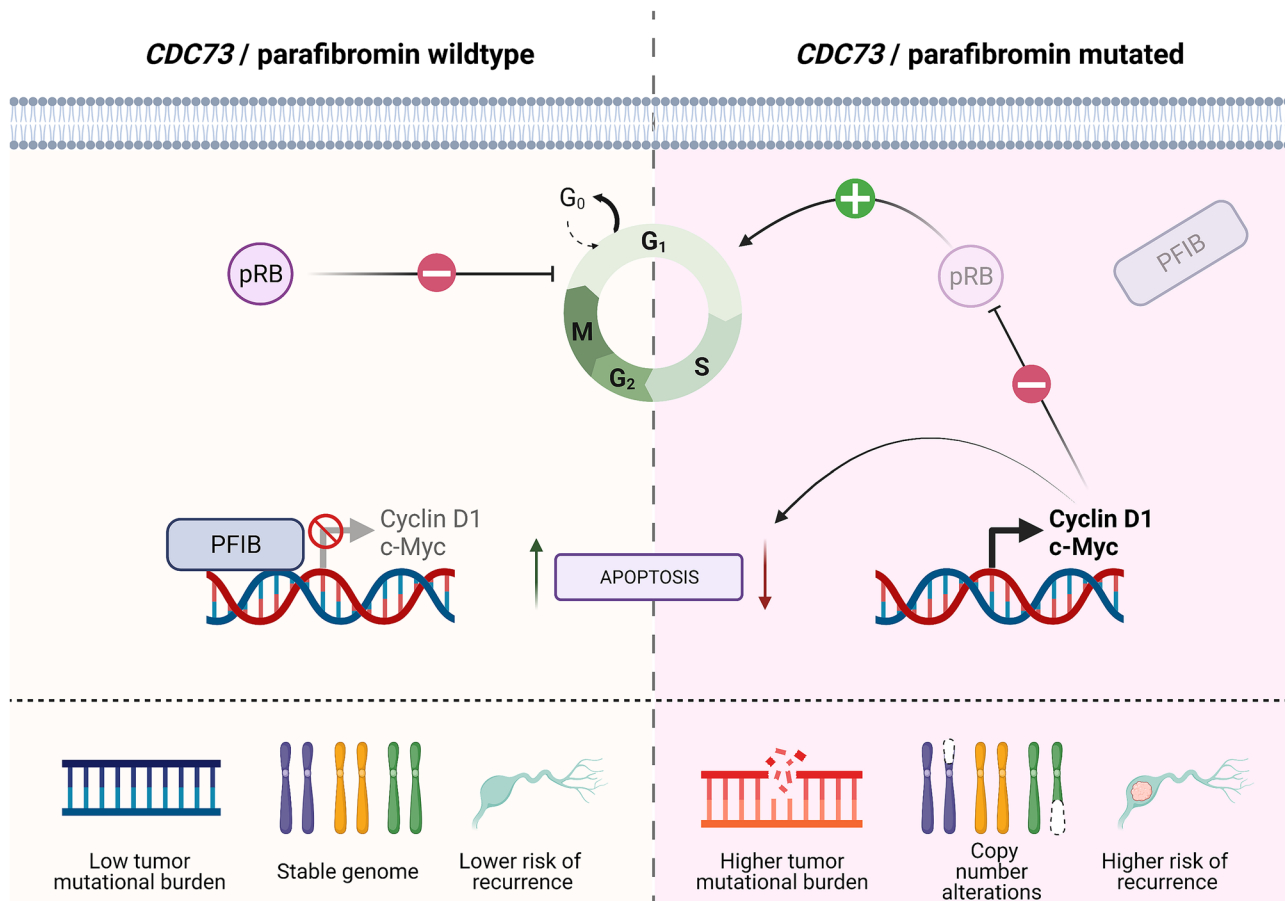


Fig. 20 Parafibromin functions. Comparison of cellular mechanisms and effects in *CDC73*/parafibromin (PFIB) wild-type (left) vs. mutated parathyroid tumors (right). In *CDC73* wild-type parathyroid tumors, nuclear parafibromin is down-regulating the expression of proto-oncogenes *CCND1* (encoding cyclin D1) and *c-Myc*, while retinoblastoma protein (pRb) levels inhibit cell cycle progression. Moreover, parafibromin may also stimulate apoptosis. In the event of a *CDC73* mutation, repression of *CCND1*/cyclin D1 and *c-Myc* is

lifted, leading to cell cycle progression and reduced/inhibited apoptosis. The bottom panel depicts genomic differences in *CDC73* wild-type vs. *CDC73* mutated carcinomas. *CDC73* gene wild-type cases usually exhibit low tumor mutational burden (TMB) and a stable genome, while *CDC73*-mutated tumors display increased TMB and copy number alterations. Moreover, the risk of disease recurrence (as exemplified here by a regional lymph node metastasis) is higher in *CDC73*-mutated/PFIB deficient carcinomas

Inactivating *CDC73* gene mutations most often lead to the reduction of parafibromin nuclear levels, thus leading to up-regulation of *CCND1*/cyclin D1 and *c-Myc* mRNA, leading to cell cycle progression and inhibition of apoptosis (Fig. 20).

In addition to *CDC73* mutations, somatic mutations in parathyroid carcinoma seem to amass in PI3K- and TP53-related pathways [139]. Moreover, mutations in the other cancer-related gene elements have been reported for small subsets of cases, including the *TERT* promoter and the *DICER1* tumor suppressor gene [139].

Moreover, epigenetic dysregulation is commonly observed in parathyroid carcinomas, with aberrant promoter methylation levels reported for the adenomatous polyposis coli (APC) tumor suppressor gene, the cell cycle regulators cyclin-dependent kinase inhibitor 2A and 2B (CDKN2A

and CDKN2B) [140, 141]. On a global level, reduction of 5-hydroxymethylcytosine (5hmC) expression was recently reported, indicative of decreased de-methylation events across the genome [142]. Via methylation array analyses, parathyroid adenoma and carcinoma seem to exhibit distinct epigenetic profiles with 263 genes found significantly different in methylation patterns between these two groups [140]. Interestingly, hypermethylation of the Wnt pathway associated genes *SFRP1*, *SFRP2*, and *SFRP4* was noted in parathyroid tumors as compared to normal parathyroid tissues, further establishing the role of a dysregulated Wnt pathway in parathyroid tumorigenesis [140].

Molecular data suggest that parathyroid adenoma and carcinoma to a large extent harbor different genetic aberrancies. For example, while parathyroid adenomas recurrently

display deleterious and somatic *MEN1* gene mutations, this gene is rarely mutated in atypical parathyroid tumors and parathyroid carcinomas. Similarly, while somatic *CDC73* gene mutations are found in large proportions of atypical parathyroid tumors and parathyroid carcinomas, these events are very seldomly identified in sporadic adenomas. Indeed, atypical parathyroid tumors in general display a genetic profile that more closely resembles parathyroid carcinoma than adenoma, suggesting a genetic relationship between these entities [143]. However, the exact mechanisms driving the possible transformation into full-blown malignancy are not fully understood.

On the expression level, parathyroid lesions fall into three main clusters, a hyperplasia cluster (mostly hyperplastic parathyroid lesions), a *CDC73* driven cluster (including both sporadic and hereditary parathyroid adenoma and carcinoma with *CDC73* gene mutations) and a *MEN1*-related cluster (including mostly adenomas) [144]. This clustering proposes that parathyroid tumors with *CDC73* gene mutations follow pathways separate from *MEN1*-mutated parathyroid tumors, and suggests additional molecular alterations may influence the malignant behavior of parathyroid tumors apart from *CDC73*, given the similar expressional profiles of *CDC73* mutated parathyroid adenoma and carcinoma. Studies have indicated that *CDC73*-mutated adenomas may harbor a distinct cytogenetic profile compared to carcinomas with the same mutation, making it likely that additional factors are at play [145].

In terms of molecular work-up in the clinical setting, there are no clear-cut recommendations in the new 2022 WHO classification. However, parafibromin deficient tumors and parathyroid carcinomas are indications for genetic testing. We know that *CDC73*-mutated parathyroid carcinomas display increased tumor mutational burden, an overall genomic instability and poorer patient outcomes compared to *CDC73* wild-type parathyroid carcinomas (Fig. 16) [146]. Although most pathology laboratories do not perform *CDC73* gene sequencing on the somatic level as part of their routine diagnostic work-up, parafibromin immunohistochemistry is of value in this context, as a negative stain may identify cases in which an underlying *CDC73* gene event is probable. Moreover, parathyroid carcinomas often display mutations in actionable genes suitable for targeted treatment, such as neurofibromatosis type 1 (NF1), phosphatase and tensin homolog (PTEN), and phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA), to name a few [139, 147]. Thus, comprehensive sequencing of selected cases may be of potential clinical value if conventional therapy is deemed unsuccessful. These findings also expand the molecular correlates of *CDC73* wild-type disease and may help us better understand the heterogeneous spectrum of sporadic parathyroid carcinomas.

Question 8. What Are Correlates of Genetic Predisposition in Parathyroid Tumors? What Are Morphologic and Immunohistochemical Harbingers of Germline Susceptibility in Parathyroid Tumors?

The distinction of inherited hyperparathyroidism from sporadic manifestations is an evolved clinical responsibility of endocrine pathologists. The recently defined MEN5 syndrome (due to germline pathogenic *MAX* variants) [8] has expanded the well-established inherited hyperparathyroidism syndromes which include HPT-JT, MEN1, MEN2, MEN4, and familial-isolated hyperparathyroidism syndromes [7]. In addition, there are rare germline pathogenic variants including but not limited to *GCM2* that can cause inherited familial hyperparathyroidism.

As a general practice rule, any of the following conditions require exclusion of underlying germline susceptibility: (a) young age (< 45 years old), (b) primary hyperparathyroidism with multiglandular parathyroid disease, (c) family history for primary hyperparathyroidism or components of well-defined inherited hyperparathyroidism syndromes, (d) personal history of tumors that can be a component of inherited hyperparathyroidism syndromes, (e) histological findings suggestive of *CDC73*-related disease, (f) parafibromin deficiency, (g) global loss of p27, menin, or *MAX* in the setting of multiple nodular proliferations in a single gland or in the background of multiglandular parathyroid disease, and (h) recurrence of primary hyperparathyroidism due to asynchronous parathyroid involvement.

The ideal approach would be to perform either a whole exome or whole-genome sequencing in any patients with

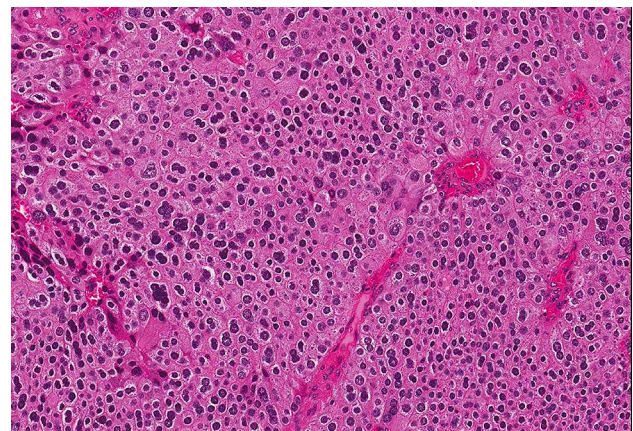


Fig. 21 Parafibromin deficient parathyroid tumor in HPT-JT syndrome. A parafibromin deficient parathyroid tumor arising in the setting of HPT-JT syndrome. In this case, there are several morphological clues, including eosinophilic cytoplasm with perinuclear cytoplasmic clearing imparting an almost “koilocytic like” appearance, occasional binucleate cells and a sheet-like growth pattern

a suspected inherited hyperparathyroidism based on the former criteria. However, problems related to access and cost are major limitations. Therefore, a select panel or targeted gene testing is typically performed in most practices. Pathologists may help multidisciplinary team by performing biomarkers and recognizing characteristic features of a subset of germline manifestations.

Genotype-morphological phenotype correlations have thought us that *CDC73*-related parathyroid neoplasms are often reflected in sheet like (rather than acinar) growth pattern, nuclear enlargement but with relatively preserved N:C ratios, eosinophilic cytoplasm but with perinuclear cytoplasmic clearing and scattered binuclear or multinucleate cells (Fig. 21). Such manifestations should be further assessed with parafibromin immunohistochemistry. However, it is emphasized that parafibromin may be a difficult stain to perform and interpret and that not all tumors associated with biallelic mutation/inactivation of *CDC73* will show loss of

immunohistochemical expression of parafibromin [9, 11, 13, 23, 87]. Thus, molecular testing and genetic counseling may still be appropriate in suggestive clinical or morphological expression even if parafibromin demonstrates apparently preserved expression.

MEN1-related manifestations have a distinct multinodular growth reflecting multiple small adenomas arising in the background of multiglandular parathyroid disease (Fig. 22). *MEN4* and the recently defined *MEN5* syndrome also show *MEN1*-like findings. Therefore, the demonstration of the global loss of *menin*, *p27*, and *MAX* expression in multinodular proliferations may facilitate the screening for *MEN1*-, *MEN4*-, and *MEN5*-related pathogenesis. While the overall performance of *MAX* immunohistochemistry has been questioned by some experts, molecular immunohistochemistry findings alone only serve as an ancillary screening tool. Therefore, germline testing is required to distinguish somatic/epigenetic inactivation from an underlying genetic susceptibility.

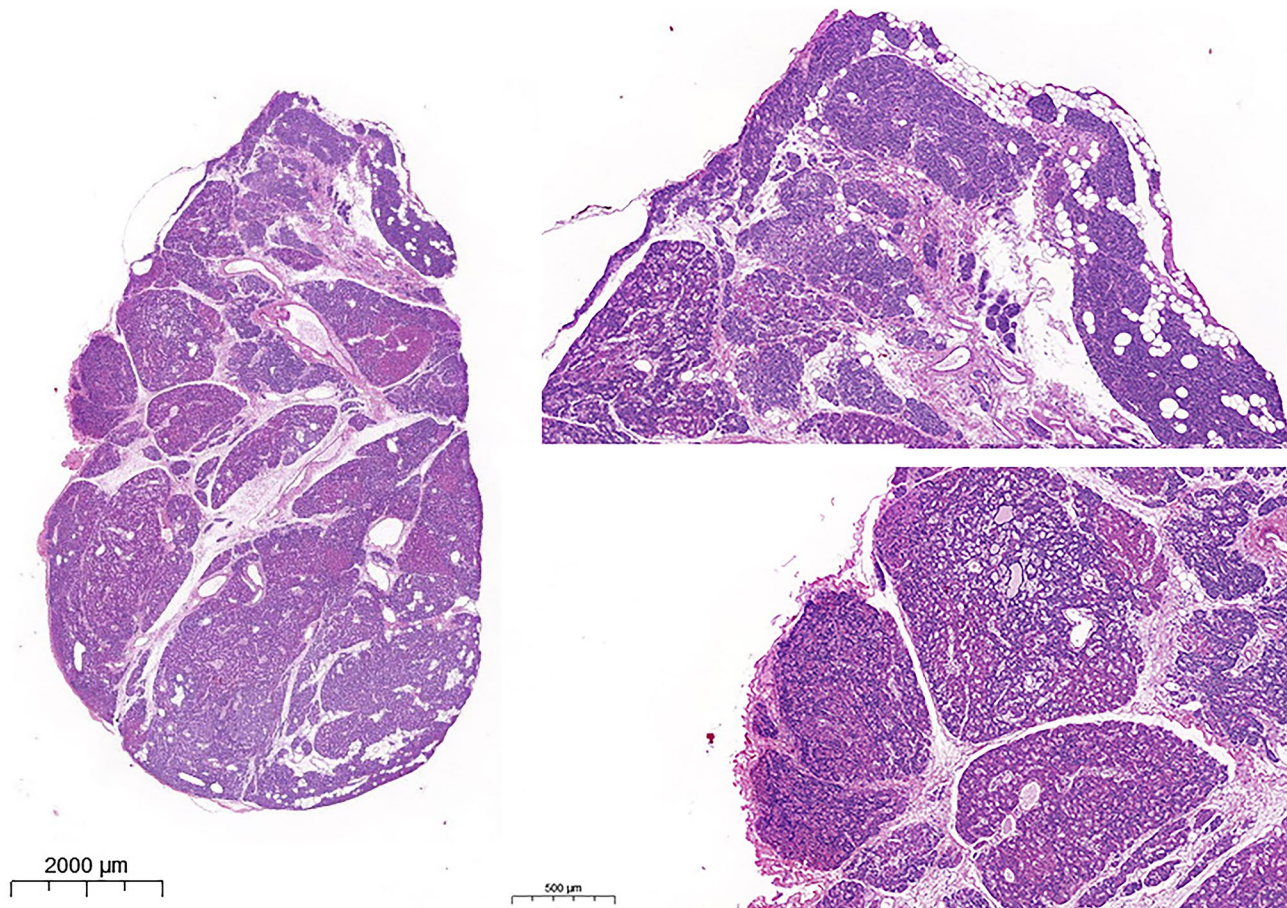


Fig. 22 Parathyroid gland of a *MEN1* patient. In the upper part of the gland, a hypercellular rim of “normal” parathyroid is recognizable (upper right). The gland is composed of many individual nodules/microadenomas (lower right)

Declarations

Disclosures This review article was handled by an independent editor and peer-reviewed as per the journal standards.

Ethics Approval Not applicable.

Consent for Publication All authors consent to publication.

Competing Interests Dr. Ozgur Mete is the Editor-in-Chief of *Endocrine Pathology*. Dr. Lori Erickson is the Senior Associate Editor of *Endocrine Pathology*. The remaining authors declare that they have no competing interest.

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