Genomics and Epigenomics in Parathyroid Neoplasia: from Bench to Surgical Pathology Practice

C. Christofer Juhlin1,2 · Lori A. Erickson3

Accepted: 24 November 2020 / Published online: 2 December 2020 © The Author(s) 2020

Abstract
The majority of parathyroid disease encountered in routine practice is due to single parathyroid adenoma, of which the majority arise as sporadic tumors. This is usually a straightforward diagnosis in endocrine pathology when in the appropriate clinical setting, although subsets of cases will exhibit atypical histological features that may warrant additional immunohistochemical and genetic analyses to estimate the malignant potential. Parathyroid carcinomas on the other hand, are bona fide malignant tumors characterized by their unequivocal invasion demonstrated through routine histology or metastasis. The ultimate endpoint for any molecular marker discovered through laboratory investigations is to be introduced in clinical routine practice and guide the surgical pathologist in terms of diagnostics and prognostication. For parathyroid tumors, the two main diagnostic challenges include the distinction between parathyroid adenoma and parathyroid carcinoma, as well as the pinpointing of hereditable disease for familial screening purposes. While numerous markers on genetic, epigenetic, and protein levels have been proposed as discriminative in these aspects, this review aims to condense the scientific coverage of these enigmatic topics and to propose a focused surgical pathology approach to the subject.

Keywords Parathyroid tumors · Primary hyperparathyroidism · Pathology · Genetics · Review

Introduction

General Background
In the clinical setting, the tumor responsible for primary hyperparathyroidism (PHPT) is usually a parathyroid adenoma. The vast majority of parathyroid adenomas are functioning due to an altered set point in terms of calcium sensing mechanisms, and the ensuing parathyroid hormone (PTH) secretion leads to hypercalcemia that may cause diverse symptoms in the afflicted patient [1]. However, the majority of parathyroid adenomas are identified through serum calcium screening. The peak incidence is among 50–60-year-old individuals, and the female to male ratio increases with increased age at diagnosis, reaching 5:1 among patients > 75 years of age [2]. The treatment is surgical, and cure rates at tertiary centers are usually high [3]. Most cases are preoperatively localized using combinations of various imaging techniques, such as neck ultrasound, single-photon emission computed tomography (SPECT/CT), and/or technetium (99mTc) sestamibi scintigraphy, and the endocrine surgeon can thus plan for a focused parathyroidectomy [4, 5]. Although the bulk of PHPT cases are sporadic tumors arising through the somatic acquisition of genetic aberrancies in driver genes, approximately 5% of cases are associated with familial disease. If a familial syndrome is suspected, a four-gland exploration with subtotal parathyroidectomy or total parathyroidectomy with intramuscular reimplantation is often the preferred strategy, as these patients may develop multiglandular, metachronous disease—and some also carry a risk of developing parathyroid carcinoma [6, 7].

From a histopathological perspective, parathyroid adenomas are usually well-circumscribed tumors composed of chief cells arranged in micro-acinar or palisading formations (Fig. 1a) [8]. Subsets of cells can exhibit hyperchromatic nuclei with nuclear atypia, and multinucleated tumor
cells are sometimes observed. Mitotic figures can be seen in parathyroid adenoma and parathyroid carcinoma. The stromal fat content is reduced compared to normal gland histology, and a rim of normal appearing (although suppressed) parathyroid tissue can usually be seen, particularly in smaller parathyroid adenomas and less commonly seen in larger adenomas (Fig. 1a). What appears to be a rim of normal-appearing parathyroid tissue cannot be used to differentiate parathyroid adenoma from multiglandular disease, as up to 10% of parathyroid “hyperplasia” (multiglandular disease) may have what appear to be rims of normal parathyroid. Moreover, as intraoperative frozen section analyses not always are effective in distinguishing single parathyroid adenomas from multiglandular involvement, intraoperative PTH assays are probably more reliable in this context [9].

In order to diagnose a parathyroid adenoma, there must be no signs of malignant behavior, such as vascular or perineural invasion. Regarding their immunophenotype, most parathyroid adenomas are positive for chromogranin A, PTH, and GATA3. Numerous histological variants have been described, of which oncocytic parathyroid adenomas are the most common, followed by unusual variants such as parathyroid lipoadenomas and water-clear cell adenomas [8, 10–12]. From a clinical perspective, oncocytic features associate to larger tumor size. Oncocytic parathyroid tumors are functional but may not have as elevated serum calcium or parathyroid hormone levels as comparable conventional parathyroid adenomas. Lipoadenomas might possibly correlate to the presence of arterial hypertension—suggesting that these subclassifications might disclose clinical considerations of importance [11, 13].

Subsets of parathyroid tumors exhibit atypical features usually observed in carcinomas, but yet lacking unequivocal criteria of malignancy such as invasive growth into periparathyroidal tissues (thyroid gland or soft tissues), lymphovascular or perineural invasion, regional and/or distant metastases. These features may include a trabecular growth pattern, fibrotic bands, tumor cells within the capsule, increased mitotic/proliferative activity, and nuclear atypia with macro nucleoli (Fig. 1b, c) [8, 14–16]. Parathyroid lesions with these features are termed “atypical parathyroid tumors” and could be considered tumors with unknown malignant potential—even though the majority of these cases will behave benign in a clinical context, with few recurrences following parathyroidectomy [17, 18]. Due to the fact that small subsets of atypical tumors will recur as metastatic parathyroid carcinomas, numerous studies have tried to highlight the malignant potential of this tumor subgroup using various
combinations of histology and molecular markers, which are discussed in detail below.

Parathyroid carcinoma is the malignant form of PHPT. Quite rare, accounting for < 1% of PHPT, parathyroid carcinoma is an entity which from an endocrine pathology perspective always is feared, often discussed, but rarely diagnosed (personal observations) [8, 19]. Unlike parathyroid adenomas, which are more common in women than in men, parathyroid carcinomas affect woman and men equally. Clues to a parathyroid carcinoma diagnosis can be obtained preoperatively, as these tumors often are larger than adenomas, may be clinically palpable, and usually are associated with high serum calcium levels (often > 13.5 mg/dl) with patients exhibiting various symptoms related to hypercalcemia, such as nephrolithiasis and bone disease [20, 21]. Perioperative findings of the tumor being adherent to adjacent structures can also be information of value when assessing these rare lesions, and for this reason, surgeons usually opt in for an en bloc resection of the afflicted ipsilateral thyroid lobe when suspecting parathyroid carcinoma (Fig. 1d) [21]. However, one must be cautious in evaluating a parathyroid that may be adhered to adjacent structures such as the thyroid gland, as parathyroid adenomas may also be firmly adhered to this organ. The difference of course is that parathyroid adenomas are not invasive of the thyroid while parathyroid carcinomas may be invasive of the adjacent thyroid. Parathyroid carcinomas exhibit local invasive behavior and may spread locally to adjacent structures and later on to distant sites (Fig. 1e, f) [8]. Chemotherapy and/or radiotherapy are largely ineffective, and the 10-year survival rate is around 50–70% [22], with death often due to hypercalcemia. Morbidity due to complications following repetitive neck surgery (hypoparathyroidism, recurrent nerve palsy) is also high [23].

The differentiation of parathyroid carcinoma from other carcinomas in the neck is usually straightforward. Parathyroid carcinomas are neuroendocrine tumors and generally positive for chromogranin A. Other neuroendocrine tumors in the neck such as medullary thyroid carcinoma will also show staining for chromogranin A, but medullary thyroid carcinomas are usually positive for thyroid transcription factor 1 while negative for parathyroid hormone. Calcitonin is usually positive in medullary thyroid carcinoma and usually negative in parathyroid carcinoma; however, unusual staining patterns can be seen such an exceptional case of parathyroid carcinoma positive for calcitonin and calcitonin gene related peptide [24]. Moreover, practicing pathologist should also be aware that medullary thyroid carcinomas can express PAX8 in a clone-dependent manner, in which absence of immunoreactivity is noted when monoclonal PAX8 antibodies are applied, as opposed to positive staining using polyclonal antibodies [25, 26]. As parathyroid tissue might stain positive for polyclonal PAX8 antibodies, we recommend a distinguishing panel of PTH and TTF1 when assessing these differentials [27].

Although a plethora of epigenetic and genetic aberrancies have been identified in parathyroid tumors, few markers have paved their way into clinical routine practice. In this review, we focus on two main clinical predicaments concerning parathyroid tumors, namely, (1) the identification of tumors associated to various underlying syndromes of importance for genetic counseling, and (2) the distinction between benign and malignant tumors to triage each patient to correct follow-up and treatment algorithms. In the following sections, we highlight molecular markers of importance that facilitate these diagnostic quandaries, and discuss their potential as discriminative clinical markers of value to the surgical pathologist.

Familial PHPT: Underlying Causes

Much of what we today know about mutational driver events in parathyroid tumorigenesis stems from earlier work in kindreds with familial PHPT, long before the appearance of next-generation sequencing techniques. By genetic linkage analyses of family members with autosomal dominant PHPT, candidate gene loci segregating with disease-afflicted individuals were identified, followed by identification of mutational events by cumbersome Sanger sequencing of a large number of candidate genes within these regions. By this methodology, germline mutations of the MEN1 (multiple endocrine neoplasia type 1), RET (rearranged during transfection), and CDC73 (cell division cycle protein 73) (originally entitled hyperparathyroidism type 2, HRPT2) genes as events underlying the development of multiple endocrine neoplasia type 1 (MEN1), multiple endocrine neoplasia type 2A (MEN2A), and hyperparathyroidism-jaw tumor (HPT-JT) syndromes, respectively (Table 1) [28–36]. These three conditions exhibit high prevalence of PHPT, occurring in approximately 90% of MEN1 kindred, 20–30% of MEN2A kindred, and in 80% of HPT-JT kindred [8]. In MEN1 patients, PHPT is the most common disease manifestation, followed by pituitary tumors (30–40% of patients), duodenal and pancreatic neuroendocrine tumors (most often gastrinoma, insulinoma and/or glucagonoma, 40% of patients), and adrenocortical lesions (20–45% of patients) [8, 37]. In MEN2A, most patients (> 90%) develop medullary thyroid carcinoma and pheochromocytoma (50%), whereas PHPT is more uncommon, occurring in 15–30% of patients [8, 38]. For HPT-JT kindred, 70–80% develop PHPT, whereas approximately 10% of patients also develop ossifying fibromas of the mandible or maxilla [8, 39]. PHPT in MEN1 usually presents as multiglandular disease that may develop in synchronous or metachronous settings and may be asymmetric. For the MEN2A syndrome, the PHPT may present as multiglandular or single gland disease. There
Table 1 Overview of general constitutional and somatic genetic aberrations in parathyroid adenomas and carcinomas

| Familial forms | MEN1 mut. | RET mut. | CDC73 mut. | CaSR mut. | GCM2 mut. | CDKi family mut. | PTH-CCND1 inversion | CTNNB1 mut. | EZH2 mut. | mtDNA mut. | TP53 mut. | TERTp mut. | RIZ1 meth. | RASSF1A meth. | APC meth. |
|----------------|-----------|----------|-------------|-----------|-----------|------------------|---------------------|-------------|-----------|------------|-----------|-----------|------------|---------------|----------|
| MEN1           | 1         | -        | -           | -         | -         | -                | -                   | -           | -         | -          | -         | -         | -          | -             | -        |
| MEN2a          | -         | 1        | -           | -         | -         | -                | -                   | -           | -         | -          | -         | -         | -          | -             | -        |
| HPT-JT         | -         | -        | 1           | -         | -         | -                | -                   | -           | -         | -          | -         | -         | -          | -             | -        |
| FHH1           | -         | -        | 1           | -         | -         | -                | -                   | -           | -         | -          | -         | -         | -          | -             | -        |
| FIHP           | 2         | 3        | 3           | 3         | -         | -                | -                   | -           | -         | -          | -         | -         | -          | -             | -        |
| MEN1 variants  | -         | -        | -           | -         | -         | 1                | -                   | -           | -         | -          | -         | -         | -          | -             | -        |

Sporadic PHPT

| PA | 3 | - | 4 | - | - | - | 4 | 4 | 4 | 4 | 2 | - | - | 3 | 1 | 2 |
| PC | 4 | - | 2 | - | - | - | - | - | - | - | 4 | 4 | - | - | 2 |

*mut* mutation, *meth* aberrant promoter methylation, *CDKi* cyclin-dependent kinase inhibitor, *mtDNA* mitochondrial DNA, *TERTp* TERT promoter, *PA* parathyroid adenoma, *PC* parathyroid carcinoma

**Germline**

**Somatic**

(1) Almost always present (81–100%)
(2) Frequently present (41–80%)
(3) Sometimes present (11–40%)
(4) Rarely present (≤10%)
- - not reported
are exceedingly few reports of unequivocal parathyroid carcinomas arising in MEN1 or MEN2A kindred [40–42]. The hyperparathyroidism in HPT-JT syndrome is usually associated with a parathyroid adenoma; however, 15–40% of patients carrying CDC73 mutations or gene deletions will develop parathyroid carcinoma [8, 43, 44].

Apart from these three syndromes, hyperparathyroidism is also seen as the sole feature in familial isolated hyperparathyroidism (FIHP). These families often, but not always, present with germline mutations in either MEN1, CDC73, or the calcium sensing receptor (CaSR) gene, of which the latter is also associated to the development of familial hypocalciuric hypercalcemia type 1 (FHH1) [45–50]. The reason why some patients with germline mutations develop full-blown MEN1, HPT-JT, and FHH1 syndromes, while others develop FIHP, is not clearly understood—as there is no apparent genotype to phenotype correlation in terms of mutation types and exon localization. Recently, germline activating glial cells missing transcription factor 2 (GCM2) gene mutations were also coupled to FIHP, adding yet another candidate to the growing palette of genes underlying the development of familial hyperparathyroidism [51]. Moreover, germline inactivating mutations in cyclin-dependent kinase inhibitor (CDKI) genes have been found in rare families with MEN1-like syndromes (with mutations in either CDKN1A, CDKN2B, or CDKN2C) or the MEN4 syndrome, a phenotypic MEN1 syndrome characterized by mutations in CDKN1B [50, 52, 53].

**Somatic Genetics in Parathyroid Adenomas**

Given the identification of the MEN1, RET, and CDC73 gene aberrancies as main responsible for the development of familial PHPT, numerous studies followed in which the involvement of these genes were assessed in sporadic parathyroid tumors. Approximately 25–40% of all sporadic parathyroid adenomas harbor LOH of the MEN1 gene locus at 11q13, and half of these cases also exhibit an inactivating MEN1 mutation of the remaining allele (Table 1, Fig. 2) [54, 55]. Interestingly, an association between somatic MEN1 mutations and mild PHPT symptoms has been observed, possibly arguing for early-stage events in parathyroid tumorigenesis [55]. Moreover, while somatic CDC73 gene mutations have been reported in small subsets of sporadic parathyroid adenomas, no reports on somatic RET gene mutations in parathyroid adenoma have been noted [35, 56, 57].

Moreover, mutational and/or epigenetic silencing of other genes predisposing for FIHP and MEN1-like syndromes have also been detected in small subsets of apparent sporadic parathyroid adenomas, including CDKN1A, CDKN1B, CDKN2A, CDKN2B, CDKN2C, and GCM2 [53, 58–60]. Of particular interest, CDKN1B encodes p27 and down-regulation of p27 has been described in PAs, both on the RNA- and protein level [61–63]. Moreover, CDKN1B mutations have also been functionally linked to the development of parathyroid tumors, thereby solidifying the role of aberrant cell cycle regulation in the development of parathyroid adenomas [64]. Continuing on the cell cycle topic, a recurrent chromosomal inversion involving the peri-centromeric portion of chromosome 11 has been observed in exceedingly small subsets of sporadic parathyroid adenomas. This rearrangement causes the juxtaposition of the PTH 5’ regulatory region to the CCND1 oncogene coding region, causing a constitutively expression of the CCND1 corresponding protein cyclin D1. Although rare in sporadic parathyroid adenoma, overexpression of cyclin D1 is a common event, and therefore, other mechanisms apart from rare chromosomal inversions involving CCND1 or mutations in cyclin D1-regulating CDKIs are expected to play a role. Instead, promoter hypermethylation and down-regulation of various CDKIs could probably explain the commonly observed cyclin D1 upregulation in parathyroid adenomas (Fig. 2) [65].

Apart from the discovery of somatic alterations in established parathyroid adenoma susceptibility genes discussed above, the advent of next-generation sequencing techniques has led to the discovery of additional gene mutations of possible importance to parathyroid adenoma development. By whole-exome sequencing of parathyroid adenomas, an activating missense mutation in the methyltransferase gene enhancer of zeste homolog 2 (EZH2) was detected in one out of 8 adenomas interrogated, and additional targeted sequencing of 185 adenomas revealed one additional case with the same mutation [66]. The EZH2 gene is an epigenetic regulator of chromatin accessibility with an association to tumorigenesis in general, thereby providing additional validity of this gene constituting a possible contributor of parathyroid adenoma development—which was also verified using functional experiments in a parathyroid cell line [67, 68]. Since then, additional whole-exome sequencing studies have corroborated low frequencies of EZH2 mutations in sporadic adenomas [69].

Additional mutational events occurring in low frequencies of parathyroid adenomas include activating CTNNB1 mutations, of which some have been reported as homozygous in small numbers [70], although not reproduced by others [71–73]. CTNNB1 encodes beta-catenin, a central onco-protein regulating the Wingless type (Wnt) pathway, and data suggests that nuclear accumulation of beta-catenin could be an important player in the development of parathyroid adenomas—either through activating mutations or through aberrantly expressed Wnt co-receptors (Fig. 2) [74].

On the epigenetic level, apart from hypermethylation of CDKIs mentioned above, aberrant methylation has also been reported for numerous tumor-related genes, including WT1, SFRP1, SFRP2, SFRP4, RIZ1, APC, and RASSF1A.
[59, 65, 75–77]. Most notably, RASSF1A hypermethylation was strongly associated to down-regulation on the mRNA level in virtually all parathyroid adenomas, thereby constituting one of the most commonly known genetic aberrancies in this disease (Table 1) [75]. Moreover, on a global level, adenomas seem to exhibit similar levels of methylation as non-tumorous parathyroid tissues, suggesting that epigenetic dysregulations are driven by gene-specific events and not due to a general hypo- or hypermethylation pattern [75].

Regarding specific parathyroid adenoma subtypes, there is also an established correlation between oncocytic parathyroid adenomas and somatic mutations in genes encoded by mitochondrial DNA (mtDNA), especially mitochondrial respiratory chain complex genes NADH dehydrogenase 1, 4, and 5 (ND1, ND4, and ND5) (Table 1, Fig. 2). As oncocytic tumors in general exhibit prominent amounts of mitochondria, the association is intriguing [78]. However, as no recurrent mutations were observed, these findings mandate functional verification before a true driver status could be assigned to any of these alterations.

Somatic Genetics in Parathyroid Carcinomas

Somatic CDC73 gene mutations are the most frequent somatic alteration in parathyroid carcinoma (Table 1, Fig. 2) [79–82]. These mutations are in general disruptive due to
premature truncations or frameshift alterations, alternatively the mutations are of missense nature in conserved regions encoding the nuclear localization signals (NLSs) or the human polymerase-associated factor 1 (hPAF1) complex of the corresponding protein product, termed parafibromin. Apart from mutations, LOH encompassing the CDC73 gene locus and aberrant CDC73 promoter methylation have also been reported as somatic events in parathyroid carcinoma [83–85].

Parafibromin is a member of the hPAF regulatory complex, a key transcriptional unit that interacts with RNA polymerase II and facilitates transcriptional activity due to histone-modifying and chromatin remodeling processes [86]. Parafibromin is associated with tumor-suppressive properties, as (a) the majority of CDC73 germline mutations in HPT-JT and FIHP kindred as well as the bulk of somatic mutations in sporadic parathyroid carcinomas are disruptive [35, 43, 79–81, 87–89], (b) the majority of tumors with CDC73 mutations exhibit loss of parafibromin expression [57, 89–95], and (c) functional experiments with CDC73 plasmids support an anti-proliferative effect of the wild-type protein [96]. Indeed, parafibromin has been found to regulate cyclin D1 levels, exhibit pro-apoptotic effects, regulate the Wnt pathway through interactions with beta-catenin, as well as the c-Myc oncogene through direct binding to the promoter region of this gene [96–100]. Intriguingly, parafibromin also seems to exhibit oncogenic features in the presence of certain molecular partners, suggesting a yin-yang modus operandi of this protein [101].

In contrast to parathyroid adenomas, somatic MEN1 mutations are very infrequent in parathyroid carcinomas, but nonetheless reported [102]. Given the exceedingly low rate of malignant PHPT in MEN1 kindred, other genetic events apart from this aberrancy are expected to drive the invasive behavior in parathyroid carcinoma.

TP53 gene mutations are among the most common genetic aberrations in malignant epithelial tumors, although these genetic events seem to be unusual in parathyroid carcinomas [103–105]. Even so, LOH of one TP53 allele seems to be more common [103]. Similarly, loss of the retinoblastoma 1 (RB1) gene, encoding pRB, is observed in the majority of parathyroid carcinomas, however inactivating mutations have not been reported [106–108]. The P53 and pRB proteins are regulators of cell cycle progression and two bona fide tumor suppressors usually required to be silenced on both alleles in order to promote neoplasia, and given the fact that parathyroid carcinomas recurrently exhibit absent pRB expression, additional inactivating mechanisms apart from mutations are most likely operational [106].

Next-generation sequencing studies on parathyroid carcinomas are rare, not surprising given the low prevalence of this disease in general. In a recently published whole-genome sequencing study of 23 parathyroid carcinomas, the authors conclude that CDC73 gene mutations were the most common sequence aberration, occurring in almost 40% of cases. Increased copy number variants were seen in parathyroid carcinomas with CDC73 mutations, and these cases also carried an increased tumor mutational burden and poorer patient outcome. In unrelated, exome-based studies, recurrent mutations in AarF domain containing kinase 1 (ADCK1) and prune homolog 2 with BCH domain (PRUNE2) have been reported in parathyroid carcinomas; however, their functional roles has not been elucidated [109, 110]. Moreover, a general overrepresentation of mutations in genes associated with DNA repair and cell cycle regulation seems evident [111, 112], and rare mutations in established cancer-associated genes have also been identified, for example succinate dehydrogenase complex flavoprotein subunit A (SDHA) and DICER1. From a therapeutic perspective, the majority of parathyroid carcinomas might carry alterations suitable for molecular targeted therapies, thus highlighting the potential role for next-generation sequencing as a tool to identify cases with potential for targeted therapeutic interventions [113].

Promoter mutations of the telomerase reverse transcriptase (TERT) gene are heavily implicated in human cancers, as they convey increased TERT expression, which in turn promotes immortalization. However, these mutations seem fairly rare in parathyroid carcinomas, although these tumors in general express TERT protein [112, 114, 115]. As of this, alternative mechanisms leading to increased TERT expression in parathyroid carcinomas are suspected.

Aberrant epigenetic mechanisms are also at play in parathyroid carcinomas. Via global methylome analyses, parathyroid carcinomas and adenomas seem to exhibit hypermethylation of CDKN2B, CDKN2A, WTI, SFRP1, SFRP2, and SFRP4 [39]. Moreover, beside the recurrent CDC73 promoter hypermethylation discussed above, altered methylation levels of the adenomatosus polyposis coli (APC) promoter 1A region is recurrently seen in parathyroid tumors, although the APC mRNA expression seem to be retained by an unmethylated 1B promoter region [75, 116]. In parathyroid carcinomas specifically, loss of APC protein expression is a frequent event, and this could in part be due to aberrant methylation patterns rather than gene mutations [116–118]. The APC protein is a tumor suppressor that regulates the Wnt pathway, and loss of APC expression is therefore thought to stimulate proliferation in parathyroid cells. In more recent years, the regulation of epigenetic demethylation has been highlighted in cancer, especially the discovery of the TET1/TET2 enzymatic activity catalyzing oxidation of 5-methylcytosine (5mC) to generate 5-hydroxymethylcytosine (5hmC). In parathyroid carcinomas, 5hmC levels and TET1 expression have been found extensively reduced, suggesting a general reduction in de-methylation events across the genome [119, 120]. As this phenomenon
is tightly linked to the presence of TERT promoter mutations in unrelated cancer types, the general absence of these mutations in parathyroid carcinoma would suggest that other molecular mechanisms influence the observed lack of global de-methylation—a subject worthy of follow-up studies [121].

The Diagnostic Challenges

**MEN1-Related PHPT: Could the Pathologist be of any Help?**

The MEN1 syndrome is by far the most common among the hereditary conditions detailed in this review, with one case per 40,000 as opposed to MEN2A (1 case per 2 million) and the HPT-JT syndrome (unknown prevalence, but expected to be exceedingly low) [8]. Therefore, surgical pathologists will most likely diagnose MEN1 related parathyroid adenomas to a much larger extent than MEN2A, HPT-JT, FIHP, or MEN1-like related cases. Although the MEN1 syndrome often is diagnosed long before the patient is subjected to parathyroidectomy, the non-total penetrance in younger years as well as the occurrence of de novo MEN1 mutations in subsets of patients with healthy parents allow for subsets of patients being misclassified as sporadic PHPT patients. As up to 10% of individuals with primary parathyroid “hyperplasia” (multiglandular disease, adenomatosis, multiple adenomas) have MEN1, genetic screening for familial disease has been considered for all patients with primary parathyroid “hyperplasia.” From a diagnostic pathology standpoint, is there a way for the attentive pathologist to aid in the detection of syndromic PHPT in cases where the syndromic association was not evident preoperatively?

**MEN1** was early on assigned a tumor-suppressor gene status, as the bulk of mutational events in MEN1 kindred reported represent inactivating events leading to premature truncations of the menin protein [122]. Moreover, most parathyroid adenomas arising in MEN1 patients harbor loss of heterozygosity (LOH) of the **MEN1** wild-type allele on the somatic level, thereby arguing in favor for the Knudson’s “two hit” theory in which bi-allelic inactivation of a tumor suppressor gene is needed in order for a tumor to develop [123–125]. Therefore, the question arises if clinical screening using expressionanalyses targeting menin could be a cheap and efficient way for the surgical pathologist to triage menin-negative PHPT cases for genetic screening—as loss of menin expression would be indicative of **MEN1** gene aberrancies.

Menin is a predominantly nuclear protein with a highly conserved sequence among species, and the protein is universally expressed in most human tissues [122]. The protein harbors nuclear localization signals and leucine zipper motifs, both of which are features needed to regulate gene expression though direct interaction with DNA elements. For example, menin has been shown to interact with JunD, a proto-oncogene encoding a transcription factor regulating apoptosis and TP53 gene activation (Fig. 2) [126]. Moreover, menin has been proposed as an important player in the regulation of various signaling networks associated to tumor development, such as the TGF-beta, RAS, Wingless type (Wnt) pathways—as well as influencing the expression of the telomerase reverse transcriptase (TERT) gene [127–130].

From a histological context, the MEN1-related parathyroid disease is often multiglandular, although not always synchronous in presentation [8]. Unlike sporadic parathyroid adenomas, MEN1-related tumors are often devoid of a rim of normal parathyroid tissue, and they may be composed of chief cells arranged in compact, sheet-like formations [8, 131]. The tumors may exhibit fibrosis and have similar appearance as parathyroid hyperplastic lesions arising in the setting of chronic renal failure, and almost always lack invasive features. Overall, findings of multiglandular disease and absence of a normal parathyroid rim in a PHPT patient should raise the suspicion of the MEN1 syndrome; however, a “rim” can also be seen in up to 10% of multiglandular disease. In all, there is no histological feature that sets MEN1-related adenomas apart from sporadic ones.

Expression studies regarding menin and associations to underlying MEN1 mutational status in parathyroid tumors have been promising, as menin immunohistochemistry seem to exhibit high sensitivity to detect underlying **MEN1** mutations and/or gene deletions [132, 133]. Even so, the interpretation of menin immunohistochemistry is not straightforward, and the lack of comprehensive studies on the subject makes menin immunohistochemistry inappropriate for clinical routine screening purposes. Moreover, as the **MEN1** gene is frequently deleted and mutated also on the somatic level in sporadic parathyroid adenomas, additional factors are needed to bring into consideration before suspecting MEN1 syndrome–related PHPT from expressionanalyses in the pathology laboratory. For example, the penetrance of PHPT in MEN1 patients reaches 95% at 50 years of age, and therefore, the diagnosis of MEN1 in older patients with multiglandular disease should not be completely overlooked—although the bar for considering an MEN1 diagnosis should be even lower in adolescent patients [37]. In all, a concerted teamwork between endocrine surgeons and pathologists covering the patient’s medical history, disease presentation, radiology, and histopathology is probably needed to properly identify all MEN1-related PHPT in the clinical setting.

**HPT-JT-Associated PHPT: Clues from the Histopathological Workup?**

As HPT-JT patients carry germline CDC73 gene mutations or deletions, it comes as no surprise that the prevalence of parathyroid carcinoma in this patient category is much
higher (15–30%) than in unselected PHPT patient cohorts (<1%). As parathyroid carcinoma is so uncommon, it has been suggested that individuals diagnosed with parathyroid carcinoma should be promptly evaluated for HPT-JT. Additionally, if the pathologist raises the suspicion that the patient is indeed an HPT-JT kindred, a raised awareness of the increased parathyroid carcinoma risk is mandated when diagnosing the tumor. Easily recognizable features such as a tumor being cystic may in itself raise the possibility of HPT-JT syndrome. In the most comprehensive study yet regarding morphological features of CDC73 mutated parathyroid tumors, the authors conclude that these lesions are characterized by sheet-like, compact growth rather than the usual acinar patterns visualized in the bulk of parathyroid tumors, as well as a typical eosinophilic cytoplasm distinct from the granular oxyphilic cell type often observed in areas of parathyroid adenomas [89]. Many tumors also exhibited enlarged nuclei and a perinuclear cytoplasmic clearing. In all, these findings suggest that the occurrence of these histological parameters might suggest the occurrence of an underlying CDC73 gene mutation, and these cases should preferably be investigated more thoroughly with parafibromin immunohistochemistry and/or CDC73 gene sequencing.

**Pinpointing Parathyroid Carcinoma in the Diagnostic Setting**

Parathyroid carcinoma is the malignant form of PHPT. It constitutes only 0.5–3% of PHPT cases. As many parathyroid tumors present with various degrees of atypia, although not fulfilling the current WHO criteria for parathyroid carcinoma, there is a great need to identify malignant potential in these lesions by other means than histology alone. Moreover, any clinical marker of value in this context would need to exhibit very high specificity, in order to avoid falsely diagnosing benign tumors as carcinoma. The bulk of studies on this subject have attempted to address this dilemma via immunohistochemistry and/or CDC73 gene sequencing.

One of the earliest immunohistochemical markers proposed to identify parathyroid carcinoma was Ki-67, an extensively used proliferation marker used to identify cells in active (non-G0) phases of the cell cycle. Although there seem to be an overrepresentation of carcinomas among high-proliferative parathyroid tumors, the overlap between parathyroid adenomas and carcinomas is considerable [134–139]. In a similar fashion, investigations of the cell cycle protein cyclin D1 was initially proven of value, as it was found upregulated in most parathyroid carcinoma compared to adenomas [140]. However, subsequent studies have identified overlap in expression between benign and malignant groups [117, 141].

Detection of widespread LOH of the RB1 locus in parathyroid carcinoma with loss of its corresponding protein pRB prompted the investigation of pRB expression in parathyroid tumors—but with divergent results both concerning staining outcomes as well as LOH events [108, 134, 135, 142, 143]. In short, while LOH of the RB1 locus seem to confer high sensitivity for the detection of parathyroid carcinoma, a significant amount of adenomas harbor the same genetic aberrancy, and the reduced specificity renders this marker of less value when screening for rare carcinomas in PHPT. Moreover, a positive pRB staining seems to be variable in terms of the proportion tumor cells stained, making the interpretation somewhat challenging [108].

Following the advent of tissue microarrays, a study concluded that the combined positivity of p27, B-cell lymphoma 2 (bcl-2), and mouse double minute 2 homolog (mdm2) adjoined by a low Ki-67 proliferation index indicated a benign clinical behavior of any given parathyroid tumor, as this profile was not evident in any parathyroid carcinoma but found in the vast majority of adenomas [136]. Both bcl-2 and mdm2 are members of the P53 pathway, thereby furthermore solidifying the relationship between aberrant P53 signaling and parathyroid carcinoma. However, follow-up studies have demonstrated overlaps between parathyroid adenomas and carcinomas, not least the finding of down-regulation of p27 in large subsets of parathyroid adenomas [63, 144–146]. P53 immunoreactivity shows variable staining in benign and malignant parathyroid tumors and does not appear to be useful as a single marker in separating these tumors [144, 144, 147]. Other diagnostic nomograms have been evaluated to differentiate malignant from benign parathyroid neoplasms, including among others protein gene product 9.5 (PGP 9.5), Ki-67, galectin-3, E-cadherin, and pRb markers [148]. More novel combinations of immunohistochemical and in situ hybridization approaches have also been evaluated, such as long noncoding RNA expression [149].

The most well-studied and reproduced marker in the context of distinguishing parathyroid carcinoma from adenoma is parafibromin, the 531 amino acid protein product of the CDC73 main transcript [35]. Given the increased parathyroid carcinoma risk in HPT-JT kindred as well as the association between somatic CDC73 mutations and sporadic parathyroid carcinoma, subsequent immunohistochemical analyses regarding parafibromin early-on proved of value to detect the majority of CDC73 mutated cases, including most parathyroid carcinomas [90, 91, 93–95, 118]. Moreover, the vast majority of parathyroid adenomas analyzed have been shown to retain parafibromin immunoreactivity, apart from subsets of sporadic cases with a predominant cystic growth pattern, HPT-JT-associated parathyroid adenomas, and
atypical parathyroid tumors [17, 57, 93, 150]. Thus, with the exceptions of the previously mentioned exceptions, parafibromin is useful for screening purposes in the PHPT population and can be a highly useful marker of malignant potential in many cases. The value of this marker in the clinical setting has been assessed both by comprehensive meta-analyses and by recent reports from high-volume centers [89, 92, 151, 151].

As parafibromin is a predominant nuclear protein, loss of nuclear immunoreactivity is considered pathognomonic for an underlying inactivating CDC73 mutation. As most CDC73 mutations, be it germline or somatic, are either deleterious, located in early exons (causing premature termination) or missense alterations in important regulatory regions (such as the nuclear localization regions), mutated parafibromin is usually unable to reach its nuclear localization [97, 151, 152]. Most studies therefore seem to agree that almost all parathyroid tumors with wild-type CDC73 sequence display a diffuse positive parafibromin stain (Fig. 3a), while reduced or absent nuclear parafibromin expression is an aberrant staining pattern and might signal the presence of a mutation (Fig. 3b, d, e). While diffuse loss of parafibromin expression is usually seen, reduced (but not complete) nuclear parafibromin expression can also be noted in CDC73 mutated cases, occurring in subsets of tumor nuclei in a chessboard type of pattern (Fig. 3b) [92, 94]. Additionally, rare cases with parafibromin-negative nucleolar compartments have also been reported, which could be of importance given the nucleolar roles of wild-type parafibromin [88, 153–157]. Specifically, subsets of parathyroid tumors with CDC73 mutations thought to disrupt the nucleolar localization signals might exhibit retained nuclear parafibromin immunoreactivity while evidently displaying negative nucleolar staining—and practicing pathologist should therefore be aware of this staining pattern as well (Fig. 3c) [156]. As most studies focus on the 2H1 monoclonal parafibromin antibody targeting the N-terminal, the usage of different antibodies should not explain these different staining patterns. Indeed, the “partial loss” pattern described above has been reported in cases assessed with four different parafibromin antibodies, suggesting that these observations are not biased by the selection of a specific target epitope [94]. The interpretation of parafibromin immunohistochemistry is therefore not completely straightforward, and additionally, the laboratory processing (including different antigen retrieval techniques and primary antibody incubation time) might affect the overall staining

![Fig. 3 Examples of parafibromin and APC immunohistochemical staining patterns.](image-url)
outcome [94, 131]. Moreover, parafibromin expression is often found stronger in the tumor periphery than in central aspects of the lesion, and therefore internal controls (such as endothelial cells) should be assessed and noted as positive before a negative parafibromin stain is called out (Fig. 3d, e) [93, 131].

Most parathyroid tumors will be assessed for parafibromin immunoreactivity if they are atypical, i.e., lack undisputable evidence of malignancy but display worrisome features (be it clinical or histological) often seen in parathyroid carcinomas. The preponderance of these tumors stain positive, although large subsets can be parafibromin deficient [17, 91, 94, 150]. How should these cases be interpreted, and what should be reported to the surgeon? Consulting the literature, it seems evident the vast majority parafibromin-negative atypical tumors will behave benign, even after long-term follow-up [17, 91]. Even so, the recurrence rate is not entirely negligible, and the finding of an aberrant parafibromin stain should also highlight the need to exclude an underlying germline CDC73 gene mutation—especially if there is a positive family history or if the proband itself exhibits multiglandular disease (Fig. 4) [17, 89, 91].

Given the non-perfect parafibromin sensitivity and specificity, researchers have been looking for additional markers to complement parafibromin immunohistochemistry. Shortly after parafibromin was coupled to the Wnt pathway, investigations of Wnt regulators detected widespread loss of APC immunoreactivity in most parathyroid carcinomas—which has later been coupled to promoter hypermethylation in PCs (Fig. 3f) [116, 117, 150]. As parathyroid adenomas are APC positive in general, the marker has gained ground as a clinical adjunct to parafibromin [117, 118]. However, as panels with two negative markers have its limitations in terms of interpretation associated to tissue fixation, researchers have looked for markers upregulated in parathyroid carcinomas. In this aspect, galectin-3 and PGP9.5 have both been shown to stain positive in the majority of parathyroid carcinomas while only expressed in few adenomas, and different panel combinations using galectin-3 and/or PGP9.5 with parafibromin have proven more reliable than using parafibromin alone [158-161].

Overall, the parathyroid carcinoma diagnosis is reserved for cases exhibiting unequivocal histological signs of invasive behavior, and no single molecular marker has yet been able to safely predict the malignant potential in atypical

**Histopathological triaging of parathyroid tumors**

Fig. 4 A proposed histopathological triaging scheme of a parathyroid tumor (PT) using immunohistochemistry (IHC). Parathyroid adenoma (PA) are rarely subjected to parafibromin (PFIB) immunohistochemistry, as these tumors almost always stain positive (+). However, subsets of cases with a cystic phenotype or with a known coupling to the hyperparathyroidism-jaw tumor (HPT-JT) or familial isolated hyperparathyroidism (FIHP) syndromes might be observed with reduced or negative immunoreactivity (−), and PFIB IHC might help triage cases for CDC73 gene sequencing. An atypical parathyroid tumor (atypical PT) displays some histological features of PC without fulfilling the WHO criteria for PC (invasive growth). If positive for PFIB, these tumors have a low risk of recurrence, while PFIB negative cases have a non-negligible risk worthy of follow-up and should be interrogated for CDC73 gene mutations (somatic and germline). PCs should probably be CDC73 gene sequenced irrespectively of PFIB IHC outcome, as the sensitivity to detect CDC73 gene mutations with PFIB IHC is non-perfect. Created with BioRender.com.
cases—which is most likely influenced by the general abundance of atypia in benign tumors and the rarity of truly metastatic cases. Moreover, there is also a potential sampling bias, as malignant tumors are not primarily resected if advanced disease is present at the time of diagnosis, and the fact that tumors with a molecular potential for spread might be resected well before their dissemination.

Discussion

The molecular background of parathyroid neoplasia is well characterized from a driver gene perspective, not least due to the identification of mutations in genes responsible for the development of hereditary PHPT. These genes were subsequently found aberrant also in sporadic cases, and still—some 20 years after original identification—enjoy their position as the top recurrently mutated genes in parathyroid tumors. Modern molecular analyses have since then expanded our knowledge regarding common dysregulations in parathyroid tumors, and highlighted both genetic and epigenetic changes involved in the development of PHPT. Next-generation sequencing analyses of both parathyroid adenoma and carcinoma cohorts have helped identify additional genes of potential impact for both groups, although it is clearly demonstrated that the “low-hanging fruit” in terms of highly recurrent events already have been picked.

From a clinical perspective, CDC73 mutations and loss of parafibromin expression have been firmly established as events coupled to parathyroid malignancy. Additionally, comprehensive sequencing of parathyroid carcinoma has identified mutations suitable for molecular targeted therapy as well as overall mutational burden as a prognostic tool of potential value, thereby highlighting the usage of modern genetic analyses where histology and immunohistochemistry might be insufficient. Given the rapid evolution of molecular techniques used for complimentary analyses in the routine clinical setting, the combination of histology, immunohistochemistry and next-generation sequencing might constitute standard work-up for parathyroid tumors displaying significant atypia in the near future.

To summarize the field for the surgical pathologist, parathyroid adenoma is usually a straightforward diagnosis, but there are histological patterns coupled to underlying syndromes worth remembering—as they could help identify hereditary disease of importance for clinical follow-up. When present, histologically atypical features in a parathyroid lesion could signify malignant potential, but invasive growth is still the only accepted criteria to diagnose parathyroid carcinoma. A plethora of immunohistochemical markers could potentially aid in the identification of parathyroid carcinoma, but many display subpar specificity and should be interpreted with caution. Loss of parafibromin nuclear expression correlates with the presence of an underlying CDC73 gene mutation, which in turn is coupled to an increased risk of developing parathyroid carcinoma. The mutation might also be present in germline tissues and thus predispose to familial disease.

Even though much of the genetic landscape of parathyroid tumors has been studied, two key queries remain to be deciphered, namely (1) the identification of additional driver genes responsible for the development of parathyroid adenomas, and (2) the quest for additional molecular events driving the malignant potential of parathyroid tumors besides parafibromin. As approximately half of parathyroid adenomas lack mutations in the most common driver genes, additional genetic events are expected to be found. Moreover, as the majority of HPT-JT kindred develop adenomas, other genetic aberrations apart from the inactivation of CDC73 are likely to be required to propel the invasive behavior in parathyroid carcinomas. Hopefully, these questions can be solved in the future by comprehensive, next-generation sequencing studies using multi-center tumor cohorts.

Acknowledgements The authors are thankful to Dr. Anders Höög, Karolinska Institutet, Stockholm, Sweden, for providing macroscopic and microscopic images of parathyroid tumor specimen.

Funding Open Access funding provided by Karolinska Institute This work was supported by the Swedish Cancer Society.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit http://creativecommons.org/licenses/by/4.0/.

References

1. Brown EM (1983) Four-parameter model of the sigmoidal relationship between parathyroid hormone release and extracellular calcium concentration in normal and abnormal parathyroid tissue. J Clin Endocrinol Metab 56:572–581. https://doi.org/10.1210/jcem-56-3-572
2. Fraser WD (2009) Hyperparathyroidism. Lancet 374:145-158. https://doi.org/10.1016/S0140-6736(09)60507-9
3. Donatini G, Marciniak C, Lenne X, Clément G, Brundet A, Sebag F et al, on the behalf of AFCE Study Group (2020) Risk
Factors of Redo Surgery After Unilateral Focused Parathyroidectomy: Conclusions From a Comprehensive Nationwide Database of 13,247 Interventions Over 6 Years. Ann Surg. 272:801-806. https://doi.org/10.1097/SLA.0000000000002469

4. Patel SG, Saunders ND, Jamshed S, Weber CJ, Sharma J (2019) Multimodal Preoperative Localization improves Outcomes in Reoperative Parathyroidectomy: A 25-Year Surgical Experience. Am Surg 85:939–943. https://doi.org/10.1177/000313481908500932

5. Borumandi F, Daultrey H, Laji K, Bosman D, Ashford N, Alam P (2019) Detectability and surgical outcomes in parathyroid surgery - a single-centre experience. Br J Oral Maxillofac Surg 57:330–335. https://doi.org/10.1016/j.bjoms.2019.03.007

6. Nastos C, Papaconstantinou D, Kofopoulos-Lymperis E, Peppa M, Pikoulis A, Lykoudis P et al (2020) Optimal extent of initial parathyroid resection in patients with multiple endocrine neoplasia syndrome type 1: A meta-analysis. Surgery. S0039-6060(20)30553-5. https://doi.org/10.1016/j.surg.2020.08.021

7. Iacobone M, Camozzi V, Mian C, Pennelli G, Pagetta C, Casalide E et al (2020) Long-Term Outcomes of Parathyroidectomy in Hyperparathyroidism-Jaw Tumor Syndrome: Analysis of Five Families with CDC73 Mutations. World J Surg 44:508–516. https://doi.org/10.1007/s00268-019-05156-y

8. Lloyd RV, Osamura RY, Klöppel G, Rosai J (2017) International Agency for Research on Cancer WHO classification of tumours of endocrine organs, 4th edition. International Agency for Research on Cancer, Lyon

9. Duan K, Gomez Hernandez K, Mete O (2018) Clinicopathological correlates of hyperparathyroidism. J Clin Pathol 68:771–787. https://doi.org/10.1136/jclinpath-2015-203186

10. Ezzat T, Maclean GM, Parameswaran R, Phillips B, Komar V, Mihai R et al (2013) Primary hyperparathyroidism with water clear cell content: the impact of histological diagnosis on clinical management and outcome. Ann R Coll Surg Engl 95:e60-62. https://doi.org/10.1308/003588413X13511609957533

11. Juhlin CC, Falhammar H, Zedenius J, Nilsson I-L, Höög A (2020) Lipoadenoma of the Parathyroid Gland: characterization of an Institutional Series Spanning 28 Years. Endocr Pathol 31:156–165. https://doi.org/10.1007/s12022-020-09616-3

12. Hyrcza MD, Sargin P, Mete O (2016) Parathyroid Lipoadenoma: a Clinicopathological Diagnosis and Possible Trap for the Unaware Pathologist. Endocr Pathol 27:34–41. https://doi.org/10.1007/s12022-015-9404-5

13. Howson P, Kruijff S, Aniss A, Pennington T, Gill AJ, Dodds T et al (2015) Oxyphil Cell Parathyroid Adenomas Causing Primary Hyperparathyroidism: a Clinico-Pathological Correlation. Endocr Pathol 26:250–254. https://doi.org/10.1007/s12022-015-9378-3

14. Sandelin K, Auer G, Bondeson L, Grimmelius L, Farnebo LO (1992) Prognostic factors in parathyroid cancer: a review of 95 cases. World J Surg 16:724–731. https://doi.org/10.1007/BF02067369

15. Bondeson L, Sandelin K, Grimmelius L (1993) Histopathological variables and DNA cytometry in parathyroid carcinoma. Am J Surg Pathol 17:820–829. https://doi.org/10.1097/00000478-199308000-00007

16. Delellis RA (2008) Challenging lesions in the differential diagnosis of endocrine tumors: parathyroid carcinoma. Endocr Pathol 17:820–829. https://doi.org/10.1097/00000478-199308000-00007

17. McCoy KL, Seethala RR, Armstrong MJ, Nikiforova MN, Stang MT, Carty SE et al (2015) The clinical importance of parathyroid atypia: is long-term surveillance necessary? Surgery 158:929–935; discussion 935–936. https://doi.org/10.1016/j.surg.2015.06.022

18. Lee PK, Jarosek SL, Virnig BA, Evasovich M, Tuttle TM (2007) Trends in the incidence and treatment of parathyroid cancer in the United States. Cancer 109:1736–1741. https://doi.org/10.1002/cncr.22599

19. Shane E (2001) Clinical review 122: parathyroid carcinoma. J Clin Endocrinol Metab 86:485–493. https://doi.org/10.1210/jcem.86.2.7207

20. Lenschow C, Schrägle S, Kircher S, Lorenz K, Machens A, Drale H et al, on behalf of the NEKAR study group (2020) Clinical Presentation, Treatment, and Outcome of Parathyroid Carcinoma: results of the NEKAR Retrospective International Multicenter Study. Ann Surg. https://doi.org/10.1097/SLA.0000000000001444

21. Limberg J, Stefanova D, Ullmann TM, Thiesmeyer JW, Bains S, Beninato T et al (2020) The Use and Benefit of Adjuvant Radiotherapy in Parathyroid Carcinoma: a National Cancer Database Analysis. Ann Surg Oncol. https://doi.org/10.1245/s10434-020-08825-8

22. Harari A, Waring A, Fernandez-Ranvier G, Hwang J, Suh I, Mitmaker E et al (2011) Parathyroid carcinoma: a 43-year outcome and survival analysis. J Clin Endocrinol Metab 96:3679–3686. https://doi.org/10.1210/jc.2011-1571

23. Hodgson A, Pabkar S, Tayyari F, Young JEM, Mete O (2019) Diagnostic Pitfall: parathyroid Carcinoma Expands the Spectrum of Calcitonin and Calcitonin Gene-Related Peptide Expressing Neuroendocrine Neoplasms. Endocr Pathol 30:168–172. https://doi.org/10.1007/s12022-019-9572-9

24. Baloch Z, Mete O, Asa SL (2018) Immunohistochemical Biomarkers in Thyroid Pathology. Endocr Pathol 29:91–112. https://doi.org/10.1007/s12022-018-9532-9

25. Gucer H, Caliskan S, Kefeli M, Mete O (2020) Do You Know the Details of Your PAX8 Antibody? Monoclonal PAX8 (MRQ-50) Is Not Expressed in a Series of 45 Medullary Thyroid Carcinomas. Endocr Pathol 31:33–38, 2020. https://doi.org/10.1007/s12022-019-09603-3

26. Erickson LA, Mete O (2018) Immunohistochemistry in Diagnostic Parathyroid Pathology. Endocr Pathol 29:113–129. https://doi.org/10.1007/s12022-018-9527-6

27. Thakker RV, Bouloux P, Wooding C, Chotai K, Broad PM, Spurr NK et al (1989) Association of parathyroid tumors in multiple endocrine neoplasia type 1 with loss of alleles on chromosome 11. The New England Journal of Medicine 321:218–224. https://doi.org/10.1056/NEJM198907273210403

28. Nakamura Y, Larsson C, Julier C, Byström C, Skogseid B, Wells S et al (1989) Localization of the genetic defect in multiple endocrine neoplasia type 1 within a small region of chromosome 11. American Journal of Human Genetics 44:751–755

29. Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjöld M (1988) Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 332:85–87. https://doi.org/10.1038/332085a0

30. Mulligan LM, Eng C, Healey CS, Clayton D, Kwok JB, Gardner E et al (1994) Specific mutations of the RET proto-oncogene are related to disease phenotype in MEN 2A and FMTC. Nature Genetics 6:70–74. https://doi.org/10.1038/ng0194-70

31. Eng C, Smith DP, Mulligan LM, Nagai MA, Healey CS, Pond MA et al (1994) Point mutation within the tyrosine kinase domain of the RET proto-oncogene in multiple endocrine neoplasia type 2B and related sporadic tumours. Human Molecular Genetics 3:237–241, 1994. https://doi.org/10.1038/hmg.3.2.237

32. Hofstra RM, Landsvater RM, Catto VG, Ceccherini I, Stulp RP, Stelwagen T, Luo Y et al (1994) A mutation in the RET proto-oncogene
associated with multiple endocrine neoplasia type 2B and sporadic medullary thyroid carcinoma. Nature 367:375–376. https://doi.org/10.1038/367375a

34. Szabó J, Heath B, Hill VM, Jackson CE, Zarbo RJ, Mallette LE et al (1995) Hereditary hyperparathyroidism-jaw tumor syndrome: The endocrine tumor gene HRPT2 maps to chromosome 1q21-q31. American Journal of Human Genetics 56:944–950

35. Carpten JD, Robbins CM, Villalanca A, Forsberg L, Presciuttini S, Bailey-Wilson J et al (2002) HRPT2, encoding parafibromin, is mutated in hyperparathyroidism-jaw tumor syndrome. Nat Genet 32:676–680. https://doi.org/10.1038/ng1048

36. Teh BT, Farnebo F, Kristofferson U, Sundelin B, Cardinal J, Axelson R et al (1996) Autosomal dominant primary hyperparathyroidism and jaw tumor syndrome associated with renal hamartomas and cystic kidney disease: linkage to 1q21-q32 and loss of the wild type allele in renal hamartomas. The Journal of Clinical Endocrinology and Metabolism 81:4204–4211. https://doi.org/10.1212/jcem.81.12.8954016

37. Thakk er RV (2014) Multiple endocrine neoplasia type 1 (MEN1) and type 4 (MEN4). Molecular and Cellular Endocrinology 386:2–15. https://doi.org/10.1016/j.mce.2013.08.002

38. Eng C (2019) Multiple Endocrine Neoplasia Type 2. GeneReviews®. https://www.ncbi.nlm.nih.gov/books/NBK1257. Accessed 9 October 2020.

39. Torresan F, Iacobone M (2019) Clinical Features, Treatment, and Surveillance of Hyperparathyroidism-Jaw Tumor Syndrome: An Up-to-Date and Review of the Literature. Interna- tional Journal of Endocrinology 2019:1761030. https://doi.org/10.1155/2019/1761030

40. Jenkins PJ, Satta MA, Simmgen M, Drake WM, Williamson C, Lowe DG et al (1997) Metastatic parathyroid carcinoma in the MEN2A syndrome. Clinical Endocrinology 47:747–751. https://doi.org/10.1046/j.1365-2265.1997.3421147.x

41. del Pozo C, García-Pascual L, Balsells M, Barahona M-J, Veloso E, González C et al (2011) Parathyroid carcinoma in multiple endocrine neoplasia type 1. Case report and review of the literature. Hormones (Athens) 10:326–331. https://doi.org/10.14310/horm.2010.1325

42. Singh Ospina N, Sebo TJ, Thompson GB, Clarke BL, Young WF (2016) Prevalence of parathyroid carcinoma in 348 patients with multiple endocrine neoplasia type 1 - case report and review of the literature. Clinical Endocrinology 84:244–249. https://doi.org/10.1111/cen.12714

43. Bricaire L, Oudou M-F, Cardot-Bauters C, Delemere B, North M-O, Salenave S et al, on behalf of the GTE Group (2013) Frequent large germline HRPT2 deletions in a French National cohort of patients with primary hyperparathyroidism. J Clin Endocrinol Metab 98:E403–408. https://doi.org/10.1210/jc.2012-2789

44. Iacobone M, Masi G, Barzon L, Porzianato A, Macchi V, Iacobone E et al (2009) Hyperparathyroidism-jaw tumor syndrome: a report of three large kindred. Langenbeck’s Archives of Surgery 394:817–825. https://doi.org/10.1007/s00423-009-0511-y

45. Huang SM, Duh QY, Shaver J, Siperstein AE, Clark LR et al (1997) Familial isolated hyperparathyroidism: familial expression of the hyperparathyroidism-jaw tumor locus in 1q21-q32 in a subset of families. J Clin Endocrinol Metab 83:2114–2120. https://doi.org/10.1210/jcem.83.6.4896

46. Kassem M, Kruse TA, Wong FK, Larsson C, Teh BT (2000) Familial isolated hyperparathyroidism as a variant of multiple endocrine neoplasia type 1 in a large Danish pedigree. J Clin Endocrinol Metab 85:165–167. https://doi.org/10.1210/jcem.85.1.6299

47. Aida K, Koishi S, Tawata M, Onaya T (1995) Molecular cloning of a putative Ca(2+)-sensing receptor cDNA from human kidney. Biochem Biophys Res Commun 214:524–529. https://doi.org/10.1006/bbrc.1995.2318

48. Brown EM (1997) Mutations in the calcium-sensing receptor and their clinical implications. Horm Res 48:199–208. https://doi.org/10.1159/000185516

49. Marx SJ, Goitzman D (2019) Evolution of Our Understanding of the Hyperparathyroid Syndromes: a Historical Perspective. J Bone Miner Res 34:22–37. https://doi.org/10.1002/jbmr.3650

50. Guan B, Welch JM, Sapp JC, Ling H, Li Y, Johnston JJ et al (2016) GCM2-Activating Mutations in Familial Isolated Hyperparathyroidism. Am J Hum Genet 99:1034–1044. https://doi.org/10.1016/j.ajhg.2016.08.018

51. Agarwal SK, Mateo CM, Marx SJ et al (2009) Rare germline mutations in cyclin-dependent kinase inhibitor genes in multiple endocrine neoplasia type 1 and related states. J Clin Endocrinol Metab 94:1826–1834. https://doi.org/10.1210/jc.2008-2083

52. Costa-Guda J, Soong C-P, Parekh VI, Agarwal SK, Arnold A et al (2013) Germline and somatic mutations in cyclin-dependent kinase inhibitor genes CDKN1A, CDKN2B, and CDKN2C in sporadic parathyroid adenomas. Horm Cancer 4:301–307. https://doi.org/10.1007/s12672-013-0147-9

53. Heppner C, Kester MB, Agarwal SK, Debeelenko LV, Emmert-Buck MR, Guru SC et al (1997) Somatic mutation of the MEN1 gene in parathyroid tumours. Nat Genet 16:375–378. https://doi.org/10.1038/ng0897-375

54. Carling T, Correa P, Hessman O, Hedberg J, Skogseid B, Lindberg D et al (1998) Parathyroid MEN1 gene mutations in relation to clinical characteristics of nonfamilial primary hyperparathyroidism. J Clin Endocrinol Metab 83:2960–2963. https://doi.org/10.1210/jcem.83.8.4977

55. Uchino S, Naguchi S, Nagamoto M, Sato M, Yamashita H, Yamashita H et al (2000) Absence of somatic RET gene mutation in sporadic parathyroid tumors and hyperplasia secondary to uremia, and absence of somatic Men1 gene mutation in MEN2A-associated hyperplasia. Biomed Pharmacother 54 Suppl 1:100s–103s. https://doi.org/10.1016/s0753-3322(00)80023-2

56. Juhlin C, Larsson C, Yakoleva T, Leibiger I, Leibiger B, Alimov A et al (2006) Loss of parafibromin expression in a subset of parathyroid adenomas. Endocr Relat Cancer 13:509–523. https://doi.org/10.1677/erc.1.01058

57. Costa-Guda J, Marinoni I, Mota, Pellegrina, L. and Arnaud A (2011) Somatic mutation and germine sequence abnormalities in CDKN1B, encoding p27Kip1, in sporadic parathyroid adenomas. J Clin Endocrinol Metab 96:E701–706. https://doi.org/10.1210/jc.2010-1338

58. Starker LF, Svedlund J, Udelman S, Dralle H, Akerström G, Starkschall G et al (2004) Reduced CDKN1B, encoding p27Kip1, in sporadic parathyroid carcinoma. J Clin Endocrinol Metab 89:2265–2271. https://doi.org/10.1210/jc.2004-01995.x

59. Eckersall LA, Lin J, Wollan P, Thompson GB, van Heerden JA, Lloyd RV (1999) Parathyroid hyperplasia, adenomas, and carcinomas: differential expression of p27Kip1 protein. Am J Pathol 153:1159–1165. https://doi.org/10.1016/S0002-9440(10)66976-8
92. Juul CC, Nilsson I-L, Lagerstedt-Robinson K, Stenman A, Brännström R, Tham E et al (2019) Parafibromin immunostainings of parathyroid tumors in clinical routine: A near-decade experience from a tertiary center. Mod Pathol 32:1082–1094. https://doi.org/10.1038/s41379-019-0252-6

93. Gill AJ, Clarkson A, Gimn O, Keil J, Drahle H, Howell VM et al (2006) Loss of nuclear expression of parafibromin distinguishes parathyroid carcinomas and hyperparathyroidism-jaw tumor (HPT-JT) syndrome-related adenomas from sporadic parathyroid adenomas and hyperplasias. Am J Surg Pathol 30:1140–1149. https://doi.org/10.1097/01.pas.0000209827.39477.4f

94. Juul CC, Villablanca A, Sandelin K, Haglund F, Nordenström J, Forsberg L et al (2007) Parafibromin immunoreactivity: its use as an additional diagnostic marker for parathyroid tumor classification. Endocr Relat Cancer 14:501–512. https://doi.org/10.1677/ERC-07-0001

95. Kim HK, Oh YL, Kim S-H, Lee DY, Kang H-C, Lee JI et al (2012) Parafibromin immunohistochemical staining to differentiate parathyroid carcinoma from parathyroid adenoma. Head Neck 34:201–206. https://doi.org/10.1002/hed.21716

96. Woodard GE, Lin L, Zhang J-H, Agarwal SK, Marx SJ, Simonds WF (2005) Parafibromin, product of the hyperparathyroidism-jaw tumor syndrome gene HRPT2, regulates cyclin D1/PRAD1 expression. Oncogene 24:1272–1276. https://doi.org/10.1038/sj. onc.1208274

97. Lin L, Czapiga M, Nini L, Zhang J-H, Simonds WF (2007) Nuclear localization of the parafibromin tumor suppressor protein implicated in the hyperparathyroidism-jaw tumor syndrome enhances its proapoptotic function. Mol Cancer Res 5:183–193. https://doi.org/10.1158/1541-7786.MCR-06-0129

98. Lin L, Zhang J-H, Panicker LM, Simonds WF (2008) The Parafibromin tumor suppressor protein inhibits cell proliferation by repression of the c-myc proto-oncogene. Proc Natl Acad Sci U S A 105:17420–17425. https://doi.org/10.1073/pnas.0710725105

99. Kikuchi I, Takahashi-Kanemitsu A, Sakiyama N, Tang C, Tang P-J, Noda S et al (2016) Dephosphorylated parafibromin is a transcriptional coactivator of the Wnt/Hedgehog/Notch pathways. Nat Commun 7:12887. https://doi.org/10.1038/ncomms12887

100. Mosimann C, Hausmann G, Basler K (2006) Parafibromin/Hyrax activates Wnt/Wg target gene transcription by direct association with beta-catenin/Armadillo. Cell 125:327–341. https://doi.org/10.1016/j.cell.2006.01.053

101. Takahashi A, Tatsugami K, Kikuchi I, Obuse C, Saito Y, Seidi A et al (2011) SHP2 tyrosine phosphatase converts parafibromin/Cdc73 from a tumor suppressor to an oncogenic driver. Mol Cell 43:45–56. https://doi.org/10.1016/j.molcel.2011.05.014

102. Haven CJ, van Puijenbroek M, Tan MH, Teh BT, Fleuren GJ, van Wezel T et al (2007) Identification of MEN1 and HRPT2 Cdc73 from a tumor suppressor to an oncogenic driver. Mol Cell 34:201–206. https://doi.org/10.1016/j.molcel.2006.01.053

103. Cetani F, Pardi E, Viacava P, Pollina GD, Fanelli G, Picone A et al (1994) Loss of the retinoblastoma tumor-suppressor gene in parathyroid adenoma and carcinoma. J Clin Endocrinol Metab 78:201–210. https://doi.org/10.1210/jcem.78.6.8200932

104. Kim HK, Oh YL, Kim S-H, Lee DY, Kang H-C, Lee JI et al (2019) Comprehensive Genomic Characterization of Parathyroid Cancer Identifies Novel Candidate Driver Mutations and Core Pathways. J Endocr Soc 3:544–559. https://doi.org/10.1210/jc.2018-00043

105. Haglund F, Juhlin CC, Villablanca A, Forsberg L, Sandelin K, Brännström R et al (2009) Loss of expression for the Wnt pathway components adenomatous polyposis coli and glycogen synthase kinase 3-beta in parathyroid carcinomas. Int J Oncol 34:481–492

106. Hosny Mohammed K, Siddiqui MT, Willis BC, Zaharieva Tsvetкова D, Mohamed A, Patel S et al (2017) Parafibromin, APC, and MIB-1 Are Useful Markers for Distinguishing Parathyroid Carcinomas From Adenomas. Appl Immunohistochem Mol Morphol 25:731–735. https://doi.org/10.1097/PAI.0000000000000378

107. Barazeghi E, Gill AJ, Sidhu S, Norlén O, Dina R, Palazzo FF et al (2016) 5-Hydroxymethylcytosine discriminates between parathyroid adenomas and hyperplasias. Appl Immunohistochem Mol Morphol 25:731–735. https://doi.org/10.1097/PAI.0000000000000378

108. Cetani F, Pardi E, Viacava P, Pollina GD, Fanelli G, Picone A et al (2004) A reappraisal of the Rb1 gene abnormalities in the diagnosis of parathyroid cancer. Clin Endocrinol (Oxf) 60:999–1006. https://doi.org/10.1111/j.1365-2265.2004.01954.x

109. Yu W, McPherson JR, Stevenson M, van Eijk R, Heng HL, Newey P et al (2015) Whole-exome sequencing studies of parathyroid carcinomas reveal novel PRUNE2 mutations, distinctive mutational spectra related to APOBEC-catalyzed DNA mutagenesis and mutational enrichment in kinases associated with cell migration and invasion. J Clin Endocrinol Metab 100:E360-364. https://doi.org/10.1210/jc.2014-3238

110. Pandya C, Uzilov AV, Bellizzi J, Lau CY, Moe AS, Strahl M et al (2007) Genomic profiling reveals mutational landscape in parathyroid carcinomas. JCI Insight 2:e92061. https://doi.org/10.1172/jci.insight.92061

111. Clarke CN, Katsonis P, Hsu T-K, Koire AM, Silva-Figueroa A, Christakis I et al (2019) Nuclear localization of the parafibromin tumor suppressor protein implicated in the hyperparathyroidism-jaw tumor syndrome enhances its proapoptotic function. Mol Cancer Res 5:183–193. https://doi.org/10.1158/1541-7786.MCR-06-0129

112. Juhlin CC, Haglund F, Villablanca A, Forsberg L, Sandelin K, Brännström R et al (2009) Loss of nuclear expression of parafibromin distinguishes parathyroid adenomas and hyperplasias. Am J Surg Pathol 30:1140–1149. https://doi.org/10.1097/01.pas.0000209827.39477.4f

113. Kang H, Pettinga D, Schubert AD, Ladenson PW, Ball DW, Chung JH et al: Genomic Profiling of Parathyroid Carcinoma Reveals Genomic Alterations Suggesting Benefit from Therapy. Oncologist 24:791–797. https://doi.org/10.1634/theoncologist.2018-0334

114. Bränström R, Tham E et al (2019) Parafibromin immunohistochemical staining against hTERT. Int J Mol Med 43:45–56. https://doi.org/10.1016/j.ijmm.2011.05.014

115. Tayyebi N, Brännström R et al: Driver Mutations and Core Pathways. J Endocr Soc 3:544–559. https://doi.org/10.1210/js.2018-00043
152. Zhu R, Wang Z, Hu Y (2020) Prognostic role of parafibromin staining and CDC73 mutation in patients with parathyroid carcinoma: a systematic review and meta-analysis based on individual patient data. Clin Endocrinol (Oxf) 92:295–302. https://doi.org/10.1111/cen.14161

153. Bradley KJ, Bowl MR, Williams SE, Ahmad BN, Partridge CJ, Patmanidi AL et al (2007) Parafibromin is a nuclear protein with a functional monopartite nuclear localization signal. Oncogene 26:1213–1221. https://doi.org/10.1038/sj.onc.1209893

154. Hahn MA, Marsh DJ (2005) Identification of a functional bipartite nuclear localization signal in the tumor suppressor parafibromin. Oncogene 24:6241–6248. https://doi.org/10.1038/sj.onc.1208778

155. Panicker LM, Zhang J-H, Dagur PK, Gastinger MJ, Simonds WF (2010) Defective nucleolar localization and dominant interfering properties of a parafibromin L95P missense mutant causing the hyperparathyroidism-jaw tumor syndrome. Endocr Relat Cancer 17:513–524. https://doi.org/10.1677/ERC-09-0272

156. Juhlin CC, Haglund F, Obara T, Arnold A, Larsson C, Höög A (2011) Absence of nucleolar parafibromin immunoreactivity in subsets of parathyroid malignant tumours. Virchows Arch 459:47–53. https://doi.org/10.1007/s00428-010-1032-3

157. Hahn MA, Marsh DJ (2007) Nucleolar localization of parafibromin is mediated by three nucleolar localization signals. FEBS Lett 581:5070–5074. https://doi.org/10.1016/j.febslet.2007.09.050

158. Bergero N, De Pompa R, Sacerdote C, Gasparri G, Volante M, Bussolati G et al (2005) Galectin-3 expression in parathyroid carcinoma: immunohistochemical study of 26 cases. Hum Pathol 36:908–914. https://doi.org/10.1016/j.humpath.2005.06.020

159. Truran PP, Johnson SJ, Bliss RD, Lennard TWJ, Aspinall SR (2014) Parafibromin, galectin-3, PGP9.5, Ki67, and cyclin D1: using an immunohistochemical panel to aid in the diagnosis of parathyroid cancer. World J Surg 38:2845–2854. https://doi.org/10.1007/s00268-014-2700-2

160. Fernandez-Ranvier GG, Khanafshar E, Tacha D, Wong M, Kebebew E, Duh Q-Y et al (2009) Defining a molecular phenotype for benign and malignant parathyroid tumors. Cancer 115:334–344. https://doi.org/10.1002/cncr.24037

161. Howell VM, Gill A, Clarkson A, Nelson AE, Dunne R, Delbridge LW et al (2009) Accuracy of combined protein gene product 9.5 and parafibromin markers for immunohistochemical diagnosis of parathyroid carcinoma. J Clin Endocrinol Metab 94:434–441. https://doi.org/10.1210/jc.2008-1740

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.