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Immunohistochemistry in Diagnostic Parathyroid Pathology

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Abstract
Pathologists are usually readily able to diagnose parathyroid tissues and diseases, particularly when they have knowledge of the clinical information, laboratory findings, and radiographic imaging studies. However, the identification of parathyroid tissue or lesions can be difficult in small biopsies, ectopic locations, supranumerary glands, and in some oxyphil/oncocytic lesions. Widely available immunohistochemical studies such as chromogranin-A, synaptophysin, keratin, parathyroid hormone, thyroglobulin, and thyroid transcription factor-1 can help in difficult cases. One of the most difficult diagnostic aspects faced by the pathologist in evaluating parathyroid is distinguishing between parathyroid adenoma, particularly atypical adenoma, and parathyroid carcinoma. Many markers have and continue to be evaluated for diagnostic utility, and are even beginning to be studied for prognostic utility. Single immunohistochemical markers such as parafibromin and Ki-67 are among the most studied and most utilized, but many additional markers have and continue to be evaluated such as galectin-3, PGP9.5, Rb, bcl2, p27, hTERT, mdm2, and APC. Although not widely available in many laboratories, a panel of immunohistochemical markers may prove most useful as an adjunct in the evaluation of challenging parathyroid tumors.

Keywords Parathyroid · Parafibromin · Parathyroid carcinoma · Atypical parathyroid adenoma · Parathyroid hormone · Immunohistochemistry

Introduction

Immunohistochemistry can be useful in the diagnosis and classification of parathyroid tissue and tumors. In difficult cases, immunohistochemical studies can be used in distinguishing parathyroid from other tissues and tumors occurring in the neck such as thyroid folliculogenic and medullary tumors as well as unusual tumors occurring in the neck. With an understanding of the clinical, laboratory, and radiographic features, most parathyroid lesions can be readily classified. However, the diagnosis of parathyroid carcinoma can, at times, be difficult. Immunoperoxidase studies can be useful in this setting.

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Identification of Tissue/Tumor as Parathyroid

Parathyroid tissue is usually readily identifiable, but in difficult cases, ectopic locations, or in small biopsies immunohistochemical studies may be useful in differentiating parathyroid tissues and tumors from other tissues and tumors, such as thyroid. Parathyroid tissue is usually composed of chief cells and may have oxyphilic cells, transitional cells, and clear cells. The parenchymal cells are usually intermixed with adipocytes of varying amounts, with adipose comprising 10 to 30% of the glandular volume [1]. The cellularity of a parathyroid gland varies within and among individuals. Generally, a parathyroid gland weighing > 40 mg is abnormal [1]. One of the most common issues facing diagnostic pathologists is differentiating parathyroid tissue from thyroid tissue, which can be particularly difficult in small biopsies, frozen section specimens, and ectopic locations or supernumerary parathyroid glands. Differentiating intrathyroidal parthyroid lesions from thyroid lesions or oxyphilic parathyroid lesions from Hurthle cell tumors of the thyroid can also be challenging. In addition, parathyroid proliferations can sometimes exhibit areas of papillary and/or acinar growth with variable intraluminal secretory material that can be mistaken for primary thyroid follicular

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epithelial proliferations. Features useful in differentiating parathyroid cells from thyroid cells include well-defined cell membranes and intracellular lipid droplets in parathyroid cells, while crystals may be seen in the thyroglobulin in thyroid parenchyma.

In difficult cases, immunoperoxidase studies may be helpful in identifying a tumor as parathyroid rather than thyroid or other tumor type. In modern times, the type of lesion is strongly suspected by patient's clinical history, laboratory studies, and often imaging studies. In unusual or problematic cases such as an intrathyroidal parathyroid neoplasm, immunohistochemical studies may be helpful in differentiating a parathyroid tumor from a thyroid tumor. In endocrine pathology, a panel of immunohistochemical markers is usually utilized (Table 1).

Neuroendocrine Markers

Parathyroid tissues are neuroendocrine tissues and as positive for neuroendocrine markers—the most useful of which are chromogranin-A and synaptophysin. Chromogranin-A is the most specific neuroendocrine marker used in common clinical practice. Chief cells may stain more intensely for chromogranin-A than oxyphil cells and hyperfuntioning parathyroids may stain less intensely than normal parathyroid or rims of parathyroid in adenomas [2]. Synaptophysin is not a specific neuroendocrine marker. Synaptophysin is positive in adrenal cortical tissues and tumors. Although not specific, it is the second most useful neuroendocrine marker in common practice. Other markers (neuron specific enolase, NCAM/CD56, etc.…) that may stain neuroendocrine tissues are nonspecific and often of little practical utility as they often cause more confusion than clarification. Although chromogranin-A and synaptophysin are the most useful neuroendocrine markers, they are used as part of a panel of immunohistochemical stains in identifying tissue or a tumor as parathyroid as other neuroendocrine tumors may also occur in the thyroid, neck and other sites. Thus, the demonstration of neuroendocrine differentiation alone is insufficient to confirm parathyroid origin. The application of a panel of immunohistochemical markers including keratin, transcription factors, and PTH is generally necessary to confirm parathyroid origin.

Keratins

Parathyroid tissue and tumors are positive for keratins. Cam5.2 is one of the most useful keratins in evaluating neuroendocrine tumors. Parathyroid tissue is also positive for keratins 7, 8, 18, and 19 [1]. Keratin 14 expression is expressed in most oxyphil adenomas and one third of chief cells, but may be absent in oxyphil parathyroid carcinomas [3]. While other neuroendocrine epithelial tumors are positive for keratins (medullary thyroid carcinoma, neuroendocrine tumors of the lung, pancreas, and gastrointestinal tract, among others), keratin immunopositivity is helpful in differentinating parathyroid tissue from other neuroendocrine tumors such as paragangliomas which can occur in the neck and intrathyroidally. With the exception of paragangliomas of the cauda-equina and gangliocytic paragangliomas, paragangliomas (and pheochromocytomas) usually do not show keratin immunopositivity [4].

Hormones (Parathyroid Hormone, Thyroglobulin, Calcitonin)

Parathyroid tissues and tumors are positive for parathyroid hormone (Fig. 1). This stain is not always highly robust, and a panel of immunostains is often used. Also, parathyroid hormone may stain chief cells more intensely than oxyphil cells and hyperfuntioning parathyroid may stain less than normal parathyroid or rims of parathyroid in adenomas [2]. Parathyroid tissues and tumors are usually positive for parathyroid hormone and negative for thyroglobulin—helping in separating parathyroid from thyroid folliculogenic tissues and tumors. Like most hormones, parathyroid hormone can occassionally be aberrantly expressed in other tumors. Like parathyroid tissues and tumors, medullary thyroid carcinomas are positive for chromogranin-A and synaptophysin and negative for thyroglobulin. Medullary thyroid carcinomas are usually positive for calcitonin, and

Table 1 Immunostains in differentiatial of parathyroid vs other tissues/tumors

	Chromogranin-A/ synaptophysin	Parathyroid hormone*	Thyroid transcription factor-1	Thyroglobulin	Keratin
Parathyroid	+	+	–	–	+
Thyroid - folliculogenic	–	–	+	+	+
Thyroid - medullary	+	–	+	–	+
Paraganglioma	+	–	–	–	–
Neuroendocrine lung tumors	+	–	+	–	+
Neuroendocrine GI Tumors	+	–	–	–	+

*PTH-related peptide (PTH-rp) can be expressed by medullary thyroid, paragangliomas, neuroendocrine lung tumors, and neuroendocrine GI tumors

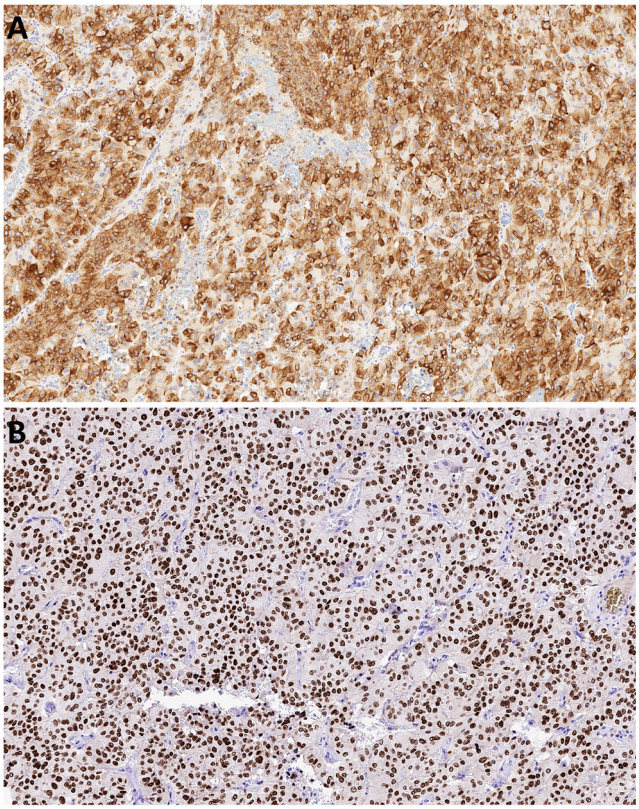

Fig. 1 The role of PTH and GATA3 immunohistochemistry in parathyroid pathology. Diffuse positivity for PTH (**a**) and GATA-3 (**b**) immunopositivity in a parathyroid neoplasm

patients usually have increased plasma calcitonin levels. Although parathyroid lesions are usually negative for calcitonin, parathyroid tumor showing immunopositivity for calcitonin and increased plasma calcitonin have been reported [5]. Hyper calcemia is common in hyperparathyroidism, but can be seen as a complication of many different tumors that secrete hormone factors. Although parathyroid hormone-related peptide secretion is known to occur and has been implicated as a cause of hypercalcemia of various malignancies, ectopic parathyroid hormone secretion and expression has been identified has been identified in various tumors such as small cell carcinoma of the ovary [6]. Many tumors, neuroendocrine and non-neuroendocrine, can be associated with ectopic parathyroid hormone secretion including renal cell carcinoma, ovarian tumors, lymphoma, endometrial carcinoma (adenosquamous), prostate carcinoma, and gastric cancer [7–14]. Thus, hormone secretion as well as hormone immunostains is very helpful in addition to relevant transcription factors and other markers. Immunostains are most useful when utilized as a panel in evaluating endocrine tumors and tissues.

Transcription Factors

Transcription factors can be very helpful in identifying and classifying tumors. Transcription factors often come into use with specificities and sensitivities that seem to decrease over time, but they still remain very helpful in identifying and classifying tumors. Thyroid transcription factor-1 (TTF-1) is very useful in separating thyroid tissues and tumors from parathyroid. TTF-1 is positive in thyroid tissues, folliculogenic thyroid tumors, and in thyroid C cells and medullary thyroid carcinomas [1]. The intensity of staining C cells and medullary thyroid carcinoma is less than folliculogenic thyroid tissues and tumors. TTF-1 is present in most lung adenocarcinomas and neuroendocrine lung carcinomas [15–19]. TTF-1 is not however specific for thyroid (or lung) tissues and tumors or even for neuroendocrine tumors of the thyroid (or lung). TTF-1 immunopositivity can also be seen in neuroendocrine carcinomas, particularly high-grade neuroendocrine carcinomas, from many sites as well as in nephroblastoma, ovarian epithelial tumors, colon adenocarcinoma, breast cancer, diffuse gliomas, and other tumors [16, 20–26]. Different TTF-1 clones have different specificities with TTF-1 (8G7G3/1) being more specific and TTF-1 (SPT24) being more sensitive [27, 28]. Other transcription factors are helpful in identifying neuroendocrine carcinomas from other sites, such as CDX2 in gastrointestinal tumors (including midgut neuroendocrine tumors, a few rectal and pulmonary neuroendocrine tumors) while other neuroendocrine tumors (parathyroid, pituitary, medullary thyroid carcinoma, Merkel cell, and paragangliomas/pheochromocytomas) are typically negative for CDX2 [29].

As briefly introduced earlier, glial cells missing 2 (GCM2) transcription factor is involved in embryonic parathyroid development and expressed in parathyroid tissues and lesions [30, 31]. Mutations in *GCM2* gene have been associated with sporadic parathyroid tumorigenesis, familial isolated hyperparathyroidism, and other parathyroid diseases [30, 32]. In a study of 58 parathyroid lesions (40 adenomas, 2 atypical adenomas, 2 carcinomas, 9 hyperplasias, 4 parathyroid cysts, and 1 recurrent hyperplasia of an autograft gland), GCM2 immunopositivity was seen in all of the parathyroid tissues and none of the thyroid or thymus tissues evaluated [31]. Although this transcription factor appears sensitive and specific for parathyroid lesions, its staining intensity and extent is variable in parathyroid neoplasia and may be negative in cysts [31].

Another recently described transcription factor, PAX8, is also used to identify tumors or to determine the primary site of metastatic tumors, usually as part of a panel of immunohistochemical stains (Fig. 2). *PAX8* (paired-box gene 8) is part of the PAX family of genes and encodes a transcription factor involved in "embryogenesis and disease." A member of the paired-box gene family, *PAX8* is expressed in the development and organogenesis of the thyroid gland, Mullerian tract, and kidney [33, 34]. In a study of 2228 primarily epithelial tumors, nuclear PAX8 staining was identified in 91% (60 of 66) thyroid tumors (but 0 of 3 medullary thyroid carcinomas) and 83% (5 of 6) of parathyroid tumors [33]. In a study of 1601

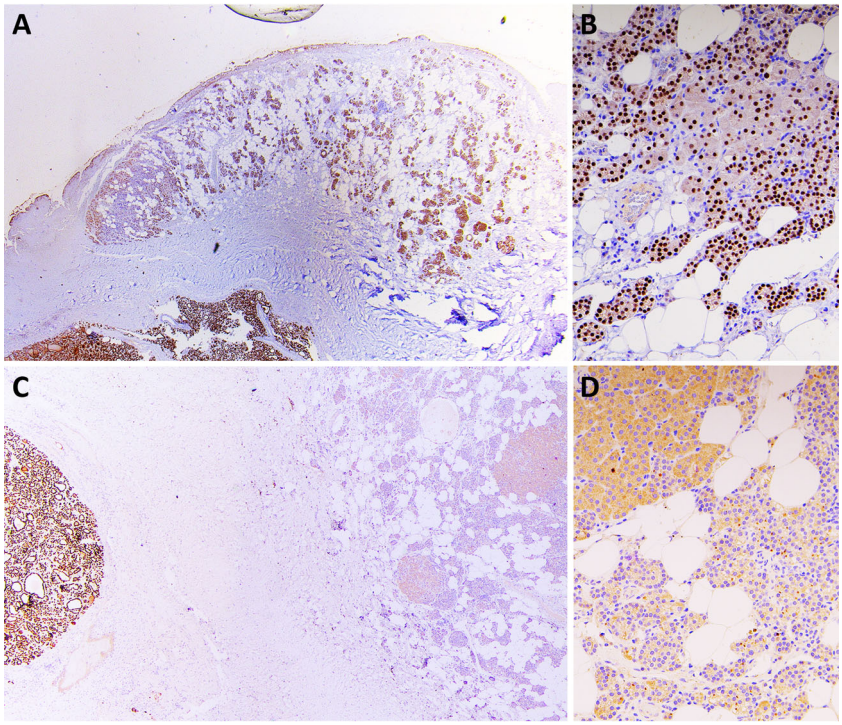

Fig. 2 PAX8 antibody in parathyroid pathology. Polyclonal antisera against PAX8 can be positive in the normal parathyroid and parathyroid proliferations (**a**–**b**); however, no reactivity is identified using monoclonal PAX8 antibody (**c**–**d**). Please note that the adjacent follicular epithelial proliferation is positive for both polyclonal (**a**) and monoclonal PAX8 (**c**) antibodies

non-neoplastic tissues, 933 primary and 496 metastatic neoplasms, PAX8 (polyclonal antibody) immunopositivity was identified in 65 of 65 thyroid folliculogenic tumors; 5 of 12 parathyroid hyperplasia/adenoma cases showed weak focal staining; 6 of 17 well-differentiated pancreatic neuroendocrine tumors; and 1 of 9 metastatic well-differentiated neuroendocrine carcinomas and 1 of 15 metastatic small cell carcinomas showed focal weak staining [34]. However, PAX8 expression is common in a variety of tumors, including the majority of renal cell carcinomas [33, 34]. Interestingly, renal cell carcinoma antigen may be positive in parathyroid tissue and other endocrine lesions including adrenal cortical [35]. PAX8 is also common in Mullerian tract tumors, but PAX8 expession is uncommon in breast, lung (except squamous cell carcinoma), and head and neck tumors [33, 34].

GATA3 is a transcription factor with a role in the pathogenesis of some parathyroid disorders (Fig. 1) [36–38]. Parathyroid abnormalities have been identified in GATA3-deficient mice deficient due to dysregulation of GCM2 [38]. Haploinsufficiency of GATA3 is associated with HDR (hypoparathyroidism, deafness, and renal dysplasia) in humans. GATA3 activates transcription of parathyroid hormone and interacts with GCM2 and MAFB activating parathyroid hormone gene expression by interacting with the transcription factor SP1SP1 [36]. In the diagnostic setting, GATA3 (clone L50-823) was recently studied in 2040 epithelial and 460 mesenchymal or neuroectodermal tumors [39]. GATA3 is known to have a role in differentiation of breast epithelium, urothelium, and T-lymphocytes [39]. GATA3 was expressed in the vast majority of breast and urothelial carcinomas but was uncommon in lung adenocarcinomas, ovary and endometrial carcinomas, and adenocarcinomas of the colon and prostate. Paragangliomas (18 of 22) and pheochromocytomas (22 of 24) commonly expressed GATA3. The other neuroendocrine tumors in this study did not show staining with GATA3 (0/11 lung carcinoids, 0/30 lung small cell carcinomas, 0/18 small intestine carcinoids, and 0/4 Merkel cell carcinomas) [39]. Papillary thyroid carcinoma (3 of 55), follicular thyroid carcinoma (1 of 20), and adrenocortical carcinoma (3 of 27) showed infrequent expression of GATA3 [39]. The role for GATA3 in the diagnostic evaluation of parathyroid tissues and tumors has yet to be fully elucidated.

Classification of Parathyroid Disease

A great variety of parathyroid diseases and diseases affecting the parathyroid, sporadic, and familial, occur. But the main entities diagnostic pathologists face in the classification of parathyroid disorders are parathyroid adenoma, atypical parathyroid adenoma, parathyroid hyperplasia (primary, secondary and tertiary), and parathyroid carcinoma. With knowledge of the clinical, laboratory, and radiographic information, most parathyroid diseases' pathologists encounter are readily diagnosed without the need for additional modalities.

The distinction between parathyroid neoplasms with atypical features, particularly atypical parathyroid adenoma and parathyroid carcinoma, can be difficult. The diagnosis of parathyroid carcinoma requires unequivocal invasion (vascular, perineural, or invasion into adjacent structures) and/or

metastases (Fig. 3). Parathyroid carcinomas are usually larger than adenomas, but there can be overlap in size. Carcinomas are usually associated with higher serum calcium levels than adenomas, but these can overlap. Parathyroid carcinomas usually have higher mitotic rates than parathyroid adenomas, but mitotic activity can be seen in adenomas. Atypical mitoses are usually seen only in parathyroid carcinoma. Atypical parathyroid adenomas have some features often seen in carcinomas, such as mitotic activity, cytologic atypia, fibrous bands, adherence to adjacent structures, trabecular growth, and tumor cells within the capsule, but lack unequivocal invasion or metastases. In these difficult cases, ancillary immunohistochemical markers would be of greatest utility.

Parafibromin

CDC73 (*HRPT2*) is a putative tumor suppressor gene on 1q21-q31 that is inactivated in some disorders affecting the parathyroid gland such as hyperparathyroidism jaw tumor syndrome (HPT-JT), some cases of familial isolated hyperparathyroidism, and other *CDC73*-related disorders [40–47]. The CDC73 protein associates with RNA polymerase II in the PAF1 transcription regulatory complex involved in inhibiting cyclin D1 expression and proliferation [48–50]. Patients with HPT-JT, an autosomal dominant disorder, may develop hyperparathyroidism, fibro-osseous jaw tumors, kidney cysts, hamartomas, and Wilms tumors. Hyperparathyroidism is the presenting feature in about 80% of patients with HPT-JT and usually develops by late adolescence. Patients with HPT-JT are at an increased risk of parathyroid adenoma or carcinoma with up to 15% developing parathyroid carcinoma. Somatic inactivating *CDC73* mutations can be seen in some sporadic parathyroid carcinomas. *CDC73* inactivation is reported in up to 75% of sporadic carcinomas, but may be higher as mutations may not be included in the coding region sequenced in clinical testing and some research studies. Inactivating *CDC73* mutations are implicated in a large proportion of parathyroid carcinomas, but are uncommon in adenomas, except in the setting of HPT-JT [51, 52]. Additionally, germline *CDC73* mutations are present in a subset of carcinomas clinically thought to be sporadic [53]. The *CDC73* (*HRPT2*) gene encodes parafibromin (CDC73 protein). Numerous studies have evaluated nuclear or nucleolar parafibromin as a single marker and in combination with other markers in parathyroid tissues and lesions. Table 2 summarizes parafibromin findings in representative studies.

Because *CDC73* mutation has shown strong concordance with parafibromin immunohistochemical expression, the utility of parafibromin immunohistochemistry has been stuied in parathyroid tumors [54, 57, 66, 67]. Loss of parafibromin is more common in parathyroid carcinoma than in benign parathyroid lesions (Fig. 4). In 2004, Tan et al. developed an anti-parafibromin monoclonal antibody and stained 52 definitive parathyroid carcinoma specimens (22 on full sections and 30 on tissue arrays) from 48 patients, 6 equivocal carcinomas (3 on tissue sections and 3 on arrays), 88 benign lesions (48 sporadic adenomas, 25 sporadic primary hyperplasias, and 13 MEN1-related tumors—all evaluated on sections), and 9 HPT-JT associated adenomas (7 evaluated on sections and 2 on array) [54]. The staining was categorized as diffuse

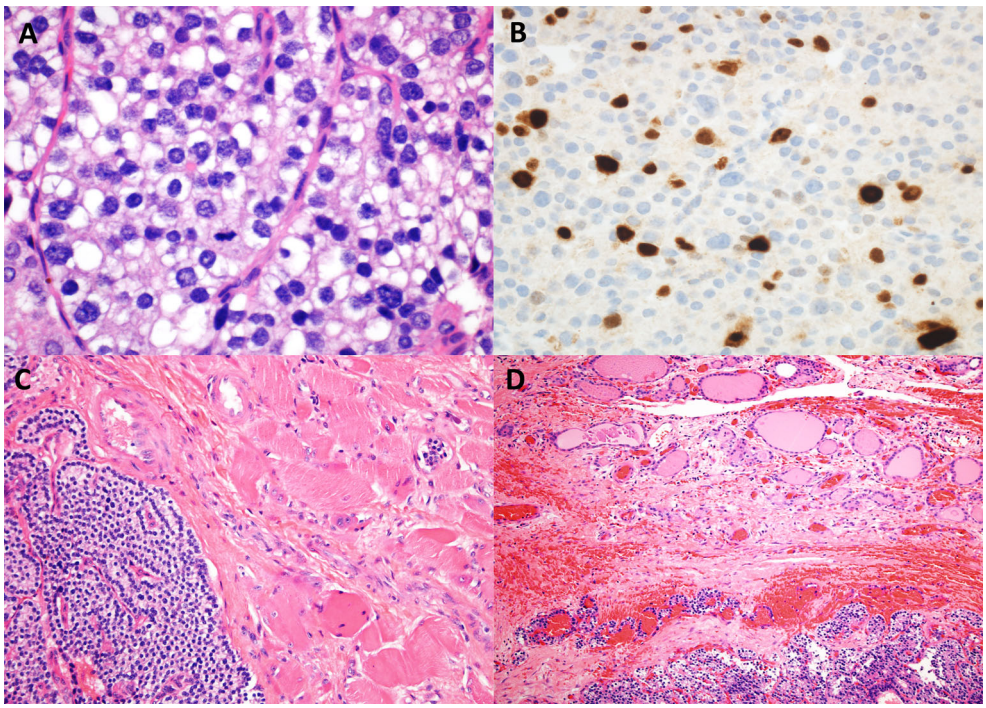

Fig. 3 Parathyroid carcinoma. Parathyroid carcinoma is often composed of monotonous cells with increased nuclear to cytoplasmic ratios and mitotic activity (**a**). Ki-67 (MIB1 antibody) shows increased proliferative activity in parathyroid carcinoma (**b**). Parathyroid carcinoma can invade into the skeletal muscle of the neck (**c**). Parathyroid carcinoma can invade into the thyroid (**d**)

Table 2 Parafibromin findings in representative studies

Study	Tissue	N	Total loss parafibromin	Partial loss parafibromin	No loss parafibromin
Tan et al. 2004 [54]	Definitive Carcinoma	52	26 (50%; absence of nuclear staining in all tumor tissue)	24 (46%; absence of nuclear staining in variably sized regions)	2 (5%; all nuclei positive)
	Equivocal carcinoma	6	1 (17%)	2 (33%)	3 (50%)
	Sporadic adenoma	48	0	0	48 (100%)
	HPT-JT adenoma	9	6 (67%)	2 (22%)	1 (11%)
	Sporadic hyperplasia	25	0	1 (4%)	24 (96%)
	MEN1-related tumor	13	0	0	13 (100%)
	Normal Parathyroid	6	0	0	6 (100%)
Gill et al. 2006 [55]	Sporadic carcinoma	11	8 (73%; > 99% nuclei negative)	2 (18%; all other staining patterns)	1 (9%; > 95% nuclei positive)
	HPT-JT tumor	4	3 (75%)	1 (25%)	0
	Sporadic adenoma	79	0	0	79 (100%)
	MEN2A adenoma	3	0	0	3 (100%)
	Sporadic hyperplasia	2	0	0	2 (100%)
	MEN1 hyperplasia	2	0	0	2 (100%)
	Secondary hyperplasia	6	0	0	6 (100%)
	Tertiary hyperplasia	4	0	0	4 (100%)
	Normal parathyroid	4	0	0	4 (100%)
Juhlin et al. 2007 [56]	Unequivocal Carcinoma	22	1 (4%, completely negative)	14 (68%; 25–83% positive nuclei)	7 (32%; almost 100% positive nuclei)
	Equivocal carcinoma	11	0	5 (45%), 52–80% positive nuclei	6 (55%)
	Sporadic adenoma	25	0	0	25 (100%)
Cetani et al. 2007 [57]	Carcinoma	11	11 (100%)*	0	0
	Sporadic adenoma	22	1 (5%)**	21 (95%; number positive nuclei ranged from 10 to 80%)	
Fernandez-Ranvier et al. 2009 [58]	Carcinoma	16	5 (31.1%; complete loss)	11 (68.8%; positive when no specific immunostaining detected)	
	Atypical adenoma	2	0	2 (100%)	
	Adenoma	18	0	18 (100%)	
	Hyperplasia	14	0	14 (100%)	
	Parathyromatosis	16	0	16 (100%)	
Kim et al. 2012 [60]	Carcinoma	8	3 (38%; > 95% nuclear positivity)	3 (38%; all other staining patterns)	2 (25%; > 99% nuclei negative)
	Adenoma	18	1 (6%)		17 (94%)
Guarnieri et al. 2012 [61]	Carcinoma	12	8 (67%; no nuclear staining at all)#	4 (33%)	
	Atypical adenoma	13	2 (15%) ##	11 (85%)	
	Adenoma	17	3 (18%)###	14 (82%)	
Wang et al. 2012 [62]	Carcinoma	15	9 (60%)	6 (40%)	
	Adenoma	19	1 (4%)	18 (96%)	
	Hyperplasia	8	0	8 (100%)	
	Normal parathyroid	6	0	6 (100%)	
Truran et al. 2014 [63]	Carcinoma	24	11 (46%)	13 (54%; positive if any nuclear staining present)	
	Adenoma	16	0	16 (100%)	
Karaarslan et al. 2015 [64]	Carcinoma	2	2 (100%)	0 (positive if any nuclear staining present)	
	Atypical adenoma	6	0	6 (100%)	

Table 2 (continued)

Study	Tissue	N	Total loss parafibromin	Partial loss parafibromin	No loss parafibromin
	Adenoma	84	0	84 (100%)	
Rhyanen et al. 2017 [65]	Carcinoma	32	4 (13%; > 99% nuclei positive)	21 (66%; between > 99% nuclei positive and > 95% negative)	7 (22%; > 95% nuclei positive)
	Atypical adenoma	28	1 (4%)	17 (63%)	9 (33%)
	Adenoma	73	0	17 (24%)	54 (76%)

*10 (of 11) had *HRPT2* mutation or LOH

**case had *HRPT2* mutation

All 3 with germline, and 4 of 5 with somatic mutation were parafibromin negative

One with germline mutation

One with somatic mutation

positive ("staining of all parathyroid nuclei; heterogeneity of staining without loss"), focal loss ("absence of nuclear staining in variably sized regions"), and diffuse loss ("absence of nuclear staining in all tumor tissue"). Staining intensity was evaluated in cases with diffuse staining. Of the 52 definitive carcinomas, 26 (50%) showed diffuse loss, 24 (46%) showed focal loss, and 2 (4%) showed diffuse staining (1 moderate and 1 strong intensity). Of the 6 equivocal carcinomas (features of carcinoma but without vascular invasion, invasion of surrounding tissue or metastasis), 1 showed diffuse loss, 2 showed focal loss, 1 showed diffuse weak staining, and 2 showed diffuse strong staining. Eight of the 9 HPT-JT adenomas had loss (6 diffuse and 2 focal loss) of parafibromin, and one showed diffuse weak staining. None of the 48 sporadic showed diffuse or focal loss of parafibromin. Seventeen (35%) sporadic adenomas showed diffuse moderate staining, and 31 (65%) showed diffuse strong staining. Only one (4%) sporadic primary hyperplasia showed focal loss of parafibromin, with the remaining cases showing diffuse staining [2 cases (8%) weak diffuse; 7 cases (28%) moderate diffuse; and 15 cases (60%) strong diffuse staining]. Two of 13 (15%) MEN1-related tumors showed diffuse moderate staining, and 11 (73%) showed diffuse strong staining. One (17%) of 6 normal parathyroids showed diffuse moderate staining, and 5 (83%) showed diffuse strong staining. The authors identified 2 adenomas with loss of parafibromin that they reclassified as "equivocal, but higly probable carcinoma" due to "severe architectural atypia, nuclear atypia, and abundant mitotic figures." One was associated with relapse and was reclassified as definitive carcinoma. The authors reported the loss of nuclear parafibromin has 96% sensitivity and 99% specificity in diagnosing definite carcinoma [54].

Many parafibromin immunohistochemical studies followed. Gill et al. (2006) evaluated parafibromin in 115 parathyroid tissues [55]. Diffuse nuclear staining in "all or nearly all (>95%) of tumor cells was considered diffusely strongly positive, regardless of the intensity of staining." [55] "Negative staining was indicated by complete absence of staining of all (>99%) of the tumor nuclei." [55] "Weak staining was indicated by all other staining patterns (usually staining of approximately 50% of parathyroid cells)." [55] Three of 4 HPT-JT-related tumors had complete absence of nuclear parafibromin and one had focal weak staining. Eight (of 11) sporadic carcinomas showed complete absence of nuclear parafibromin, 2 showed focal weak staining, and 1 showed diffuse strong staining. Of 100 non-HPT-JT benign parathyroid tissues (79 sporadic adenomas, 3 MEN2A adenomas, 2 sporadic hyperplasias, 2 MEN1 hyperplasias, 6 secondary hyperplasias, 4 tertiary hyperplasias, and 4 normal parathyroids), 98 showed strong diffuse nuclear positivity and 2 showed weak staining [55].

Somatic *HRPT2* mutations are uncommon in parathyroid adenomas, but are not uncommonly seen in cystic parathyroid

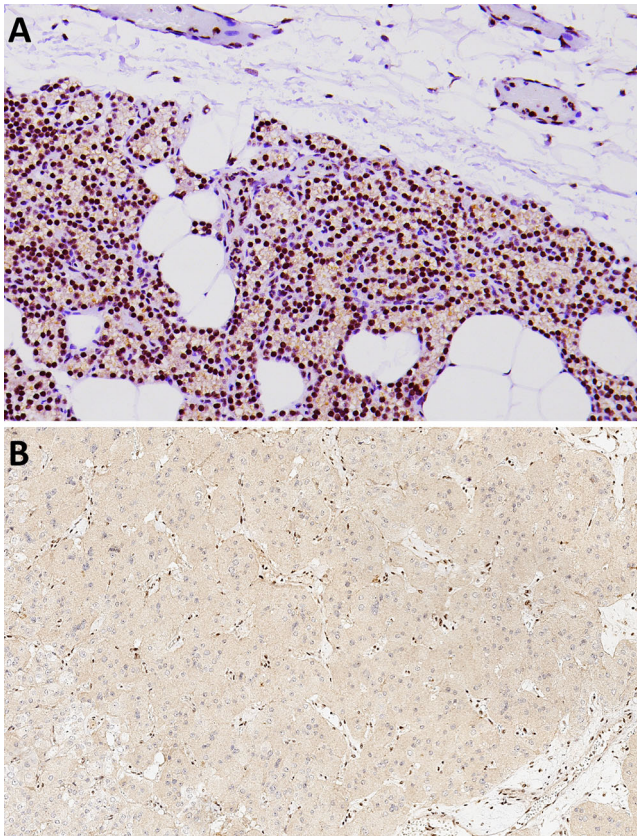

Fig. 4 Parafibromin immunohistochemistry. Parafibromin is a nuclear protein encoded by *HRPT2/CDC73*. Non-tumorous elements and normal parathyroid tissue along with parathyroid proliferations harboring wild-type *HRPT2* genotype show an intact nuclear expression (**a**). Parathyroid carcinomas often show loss of nuclear expression for parafibromin (**b**) whereas trapped non-tumorous elements retain nuclear reactivity. The latter serves as an internal positive control

adenomas [40, 53, 68]. Thus, Juhlin et al. (2006) studied 41 parathyroid adenomas with cystic change with known *HRPT2* mutation status, and found 3 parafibromin negative cases, all with *HRPT2* mutation [69]. Two additional cases had aberrantly size parafibromin on Western blot, one with negative nuclear but positive cytoplasmic staining and the other with partly positive nuclear staining—both had wild type *HRPT2*. Cyclin D1 levels were highly variable among parathyroids with and without *HRPT2* mutation. Thus, cyclin D1 and *HRPT2* were not mutually exclusive [69].

In 2007, Juhlin et al. found partial parafibromin loss (25–83% nuclei positive) in 14 of 22 (68%) and complete nuclear loss in 1 (4%) unequivocal parathyroid carcinomas, while the remaining 7 cases showed parafibromin expression in "almost 100%" of the nuclei [56]. Of 11 equivocal carcinomas, 5 (45%) had partial parafibromin loss (52 to 80% positive nuclei), and 6 (55%) showed no loss ("were completely positive for parafibromin nuclear expression"). All 25 sporadic adenomas showed parafibromin expression. Three of 6 carcinomas (5 unequivocal and 1 equivocal) with *HRPT2* mutation (4 germline, 2 somatic) showed reduced parafibromin expression. Four different antibodies were utilized and showed comparable results [56].

In a study by Cetani et al. (2007) in which "tumors were scored as positive if specific nuclear staining was detected, and the staining was quantified according to percentage of positive cells, independent of the intensity of staining." [57] Tumors were scored as "negative when no tumor cells showed a specific nuclear staining." [57] They found loss of parafibromin immunostaining to have a sensitivity (95% CI) of 100 (68–100) and a specificity (95% CI) of 88 (69–97) for differentiating parathyroid carcinomas from adenomas, including atypical adenomas [57]. All 11 parathyroid carcinomas studied showed loss of parafibromin, and 10 of 11 showed *HRPT2* gene abnormalities: 2 germline *HRPT2* mutations; 1 germline *HRPT2* mutation and LOH; 1 somatic *HRPT2* mutation; 5 somatic *HRPT2* mutations and LOH; and 1 with LOH only [57]. Twenty-one of 22 (95%) sporadic adenomas were positive for parafibromin (10–80% positive nuclei). The one adenoma with *HRPT2* mutation was parafibromin negative. Cyclin D1 expression was not related to parafibromin expression [57].

Kim et al. (2012) used the 3 parafibromin staining categories similar to that used by Gill et al. in 2006 [diffusely strong positive (> 95% cells diffuse nuclear staining with strong intensity), negative staining (> 99% cells absence of nuclear staining), and weak staining (all other staining patterns)] to evaluate 8 parathyroid carcinomas and 18 adenomas [60]. Three of 8 carcinomas were negative (1 without clinical follow-up, 1 with local recurrence, and 1 with local and distant metastasis), 3 showed weak staining (1 with no recurrence, 1 with local relapse, and 1 with local relapse who died), and 2 (both with no recurrence) showed diffuse strong parafibromin staining. Relapses and distant metastases were only identified in cases with weak or absent parafibromin staining. Seventeen of 18 adenomas showed diffuse strong parafibromin, and one was parafibromin negative. By combining the negative staining and weak staining into "loss of parafibromin expression", parafibromin had sensitivity for diagnosing carcinoma of 74% and specificity of 94.4% [60].

Guarnieri et al. (2012) evaluated parafibromin expression and *CDC73* mutation in 15 parathyroid carcinomas, 14 atypical adenomas, and 17 typical adenomas [61]. Parafibromin nuclear immunoreactivity was recorded as "IHC-negative or IHC-positive, respectively" [61]. Samples were considered "IHC-negative only if they did not reveal any neuclear staining at all." [61] In their series, "parafibromin immunostaining showed a sensitivity of 67% and a specificity of 82% in diagnosing carcinoma" [61]. Loss of parafibromin (no nuclear parafibromin) was identified in 8 of 12 (67%) carcinomas evaluated. Three of 15 (20%) carcinomas had concomitant germline *CDC73* mutations (one with a somatic mutation also), and 6 had somatic mutations only. All 3 with germline mutations and 4 of the 5 cases with only somatic mutations in

which parafibromin was evaluated were parafibromin negative. One parafibromin negative carcinoma did not have germline or somatic mutation. All 3 patients with local or distant recurrence had parafibromin negative tumors—one with neither germline nor somatic mutation, one with germline mutation, and one with germline and somatic mutation. The mean follow-up for carcinomas was 88 months (range 2–245). Two of 13 atypical adenomas were parafibromin negative, one with germline mutation and one had neither germline nor somatic mutation. One atypical adenoma had germline and somatic mutations, but was parafibromin positive. No other atypical adenomas showed germline or somatic mutations. No patient with an atypical adenoma had recurrence or metastasis with average followup 76 months (range 27–210). Three of the 17 typical adenomas had parafibromin loss, one of which had a somatic mutation. That case was the single (of 17) typical adenoma that had somatic mutation, and none had germline mutations. No patient with typical adenoma had recurrence or metastases with mean follow up of 104 months (46–197). Overall *CDC73* mutation had a diagnostic sensitivity of 60% and specificity of 94%. Diagnosing carcinoma, parafibromin had a sensitivity of 67% and specificity of 82%. In typical adenomas with negative family histories and no other tumors suggestive of HPT-JT, clinical evaluation and routine histologic evaluation were suggested to be "almost always sufficient for diagnosis and appropriate disposition." [61] However, genetic analysis, both tumor and germline, "is probably warrented in most atypical adenoma cases, but the role of immunohistochemistry is less certain." [61] Although parafibromin immunostaining was not favored to replace molecular analysis in improving diagnostic accuracy, combined molecular and immunohistochemical testing may be most effective [61].

In recent nationwide study from Finland, parafibromin was considered positive in tissue microarray sections if > 95% of nuclei were positive, negative if > 99% of nuclei were negative [65]. "Counts between these cut-offs were considered as weak positives" [65]. Among carcinomas, parafibromin was positive in 7 of 32 (22%), weak positive in 21 (66%), and negative in 4 (13%). In atypical adenomas, parafibromin was positive in 9 of 27 (33%), weak positive in 17 (63%), and negative in 1 (4%). In adenomas, parafibromin was positive in 54 of 71 (76%), weak positive in 17 (24%), and none were negative. One carcinoma and one atypical adenoma occurred in patients with germline *CDC73* mutation [65].

In a meta-analysis of parafibromin in parathyroid cancer, Hu et al. (2016) searched Pubmed, Embase, and Cochrane Library for terms "parafibromin," "*CDC73*," "*HRPT2*," and "parathyroid" and identified 202 patients from 10 studies with parathyroid carcinoma [70]. Significant heterogeneity existed among the studies. Parafibromin sensitivity ranged from 29 to 100% with pooled estimate of 68%, and specificity ranged from 61 to 100% with a pooled estimate of 95%. Scoring criteria and parafibromin antibody influenced sensitivity. If atypical adenomas were included, parafibromin specificity decreased. They concluded parafibromin had limited sensitivity, but satisfactory specificity. With the significant heterogeneity among studies, the authors suggested a standardized immunohistochemical protocol and scoring system be utilized in future studies [70].

Parafibromin in Secondary Hyperparathyroidism in Chronic Kidney Disease

Parathyroid carcinoma in the setting of secondary hyperparathyroidism due to chronic kidney disease is very uncommon. Tominaga et al. (2008) evaluated 7 distant metastases from 5 parathyroid carcinomas in hemodialysis patients for parafibromin and found strong parafibromin immunopositivity in 3 lung, 2 regional lymph node, and one chest wall metastases (only one lung metastasis did not show staining for parafibromin) [71]. All but one primary parathyroid showed strong diffuse nuclear staining, the other showed weakly positive staining. They concluded *HRPT2* may not play a significant role in parathyroid carcinogenesis in secondary hyperparathyroidism due to chronic kidney disease [71]. Thus this caveat in the evaluation of parafibromin in the setting of secondary hyperparathyroidism in chronic kidney disease should be kept in mind in the diagnostic setting.

Nucleolar Parafibromin

As nucleolar parafibromin localization had been suggested to have tumor suppressive effects in vitro [72, 73], Juhlin et al. (2011) evaluated nucleolar localization of parafibromin immunostain 82 parathyroid tumors [74]. Three of 23 parathyroid carcinomas and one of 16 atypical adenomas had absence of nucleolar parafibromin in all tumor cells in the presence of nuclear parafibromin expression in all tumor cells or subsets of tumor cells [74]. All nucleolar parafibromin negative cases had *HRPT2* mutation. All 43 adenomas and normal rims of parathyroid and *HRPT2*-transfected COS and HeLa cells showed strong nucleolar and nuclear parafibromin expression in the "vast majority of cells analyzed". The 3 carcinomas with absence of nucleolar parafibromin had somatic *HRPT2* mutations that were thought to lead to premature parafibromin truncation. Two of the 3 cases with complete nucleolar absence of parafibromin were associated with distant metastases. Absence of nucleolar parafibromin in cases with nuclear parafibromin expression and *HRPT2* mutations suggests nucleolar and nuclear evaluation of parafibromin expression may increase the parafibromin sensitivity in difficult cases [74].

Immunohistochemical Panel Approach to Diagnosis of Malignancy in Parathyroid

In addition to parafibromin, many immunohistochemical markers have been studied, alone or in combination with other markers in the evaluation of parathyroid lesions, such as MIB-1/Ki67 [3, 62–65, 75–84], galectin-3 [58, 62–64, 75, 76, 85, 86], APC [59, 75, 85], PGP9.5 [63, 67, 75, 87], HBME-1 [64], cyclin D1 [63, 80, 81, 88–91], p27 [3, 58, 76, 81, 92, 93], hTERT [94], and Rb [84, 92, 95–98], among many others in the evaluation of parathyroid lesions (Fig. 5).

Mitotic activity is usually higher in parathyroid carcinomas than in adenomas and hyperplasias (Fig. 3). But mitotic figures can be seen in adenomas and hyperplasias. Proliferative activity of parathyroid lesions has been evaluated by immunostaining for Ki-67 with MIB-1 antibody in numerous studies (Fig. 3) [3, 62–65, 75–84]. Proliferative activity varies among parathyroid diseases, but there is overlap in Ki-67 indices between parathyroid adenomas, hyperplasias, and carcinomas. For example, in a study of 22 histologically normal parathyroid glands, 33 hyperplasias, 43 adenomas, and 17 carcinomas, Ki-67 was significantly higher in carcinomas (LI = 8.4 ± 1.9) than in adenomas (LI = 2.7 ± 0.2) and hyperplasia (LI = 3.3 ± 0.4) [99]. p27, a cyclin-dependent kinase inhibitor, has also been evaluated in parathyroid disease. p27 had a significantly lower labeling index in carcinomas (13.9 ± 2.6) than in adenomas (56.8 ± 3.4), hyperplasias (69.6 ± 7.5), and normal parathyroid glands (89.6 ± 1.4). Thus, Ki-67 and

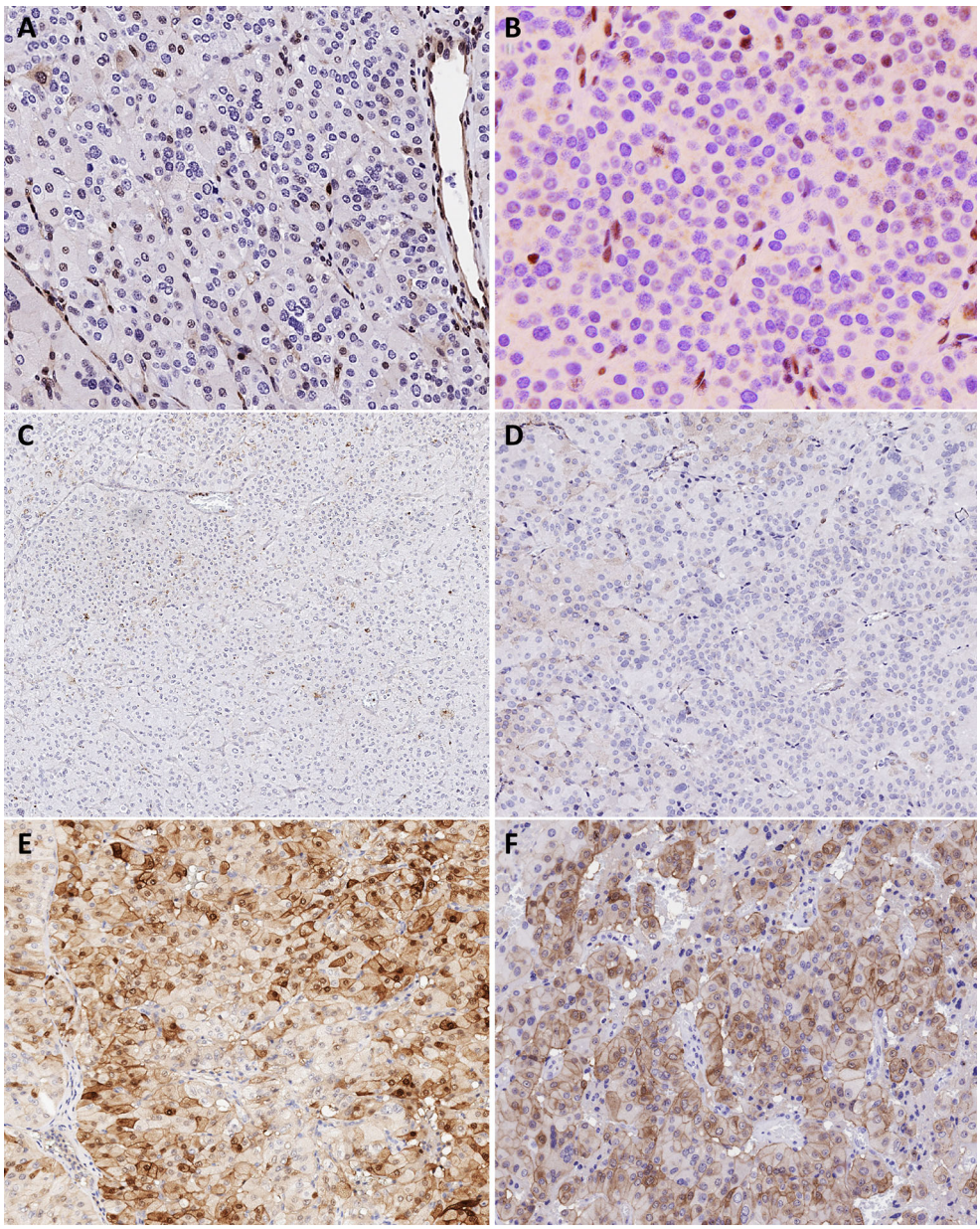

Fig. 5 Other biomarkers of parathyroid carcinoma. Several biomarkers have been proposed in the distinction of parathyroid carcinomas. While a panel approach is often performed by some experts. Loss of nuclear parafibromin expression is considered the hallmark of parathyroid carcinoma in the appropriate morphological setting. Other findings include loss of expression for p27 (**a**), Rb (**b**), APC (**c**), and bcl-2 (**d**), as well as positivity for PGP9.5 (**e**) and galectin-3 (**f**). Please note that multiglandular benign parathyroid disease can also be positive for galectin-3

p27 may be helpful diagnostically [99]. Ki-67 and p27, as well as keratin 14, have also been evaluated in oxyphil parathyroid carcinomas [3]. The Ki-67 labeling index was higher in oxyphil carcinomas (4.9 ± 1.1) than in oxyphil adenomas (1.9 ± 0.2) or chief cell adenomas (2.7 ± 0.4). The p27 labeling index was lower in oxyphil carcinomas (23.3 ± 10.4) than oxyphil adenomas (66.0 ± 2.9) or chief cell adenomas (60.0 ± 6.6). Keratin 14 expression was not identified in any oxyphil parathyroid carcinomas, but it was present in 35 of 38 oxyphil adenomas. The authors suggested a panel of Ki-67, p27, and keratin 14 may be helpful in diagnosis of oxyphil parathyroid tumors [3] (Fig. 6).

Bergero et al. (2005) found galectin-3, Ki-67, p27, and bcl2 showed differential expression between 26 parathyroid carcinomas and 30 adenomas studied [76]. Galectin-3 was positive in 24 of 26 (92.3%) of carcinomas and 1 of 30 (3.3%) adenomas. Ki-67 was 6.7% (range 1–38%) in carcinomas and 1.9% (0.5–13%) adenomas. p27 was decreased in 61.5% of carcinomas and 33.3% of adenomas. Bcl2 was decreased or absent in 61.5% of carcinomas and 20% of adenomas. Galectin-3 had the highest sensitivity (92.3%) and specificity (96.7%) of the markers studied. The sensitivity and specificity, respectively, for the other markers were Ki-67 (cutoff 6%) 42.3 and 93.3%, bcl2 61.5 and 66.7%, and p27 61.5 and 66.7%. The best sensistivity (96.2%) and specificity (90%) for 2 or more markers in a panel were galectin-3 and Ki-67 (cutoff 6%) [76].

Fernandez-Ranvier et al. (2009) evaluated parafibromin, galectin-3, Ki-67, Rb, p27, and mdm-2 in 16 parathyroid carcinomas, 16 parathyromatosis, 2 atypical adenomas, 18 adenomas, and 14 hyperplasias on tissue microarray [58]. "For parafibromin, galectin-3, and Rb proteins, immunostaining was considered positive when specific staining was present and negative when no specific immunostaining was detected." [58] Five of 16 (31.1%) carcinomas showed complete loss of parafibromin, while all other cases showed parafibromin staining. Distiguishing parathyroid carcinoma from all other groups studied (atypical adenomas, parathyromatosis, parathyroid adenoma, and parathyroid hyperplasia), parafibromin immunohistochemistry alone had a 31.3% sensitivity, 100% specificity, and 83.3% overall accuracy [58]. Galectin-3 was positive in 93.3% of carcinomas, 2 of 2 atypical adenomas, 18.7% (1 of 16) parathyromatosis, 5.6% (1 of 18) adenomas, and 14.3% (2 of 14) hyperplasias (focal weak in secondary hyperplasia). Distiguishing parathyroid carcinoma from all other groups studied, galectin-3 immunohistochemistry alone had a 93.3% sensitivity, 84% specificity, and 86.1% overall accuracy [58]. Elevated Ki-67 (> 5%) was present in 60% (9 of 15) carcinomas, 0 of 2 atypical adenomas, 6.7% (1 of 16) parathyromatosis, 5.6% (1 of 18) adenomas, and 0 of 14 hyperplasias. Distiguishing parathyroid carcinoma from all other groups studied, Ki-67 immunohistochemistry alone had a 60% sensitivity, 95.9% specificity, and 87.5% overall accuracy [58]. Loss of Rb was noted in 33.3% (5 of 15) carcinomas and 1 of 7 hyperplasias, while all other cases were positive for Rb. Distiguishing parathyroid carcinoma from all other groups studied, Rb immunohistochemistry alone had a 33.3% sensitivity, 97.9% specificity, and 84.1% overall accuracy [58]. p27 positivity (> 30% nuclei staining) was seen in 13.3% (2 of 15) carcinomas, 92.9% (13 of 4) hyperplasias, and all other cases studied. Distiguishing parathyroid carcinoma from all other groups studied, p27 immunohistochemistry alone had a 86.6% sensitivity, 51% specificity, and 59.3% overall accuracy [58]. Mdm2 positivity (> 50% cells staining) was seen 20% (3 of 15) carcinomas, 0 of 2 atypical adenomas, 20% (3 of 15) parathyromatosis, 5.6% (1 of 18) adenomas, and 35.7% (5 of 14) hyperplasias. Distiguishing parathyroid carcinoma from all other groups studied, mdm2 immunohistochemistry alone had a 18.3% sensitivity, 80% specificity, and 32.8% overall accuracy [58]. No single marker was definitive for malignancy, but a combination of parafibromin loss, Rb loss, and galectin-3 overexpression were generally able to identify carcinomas [58].

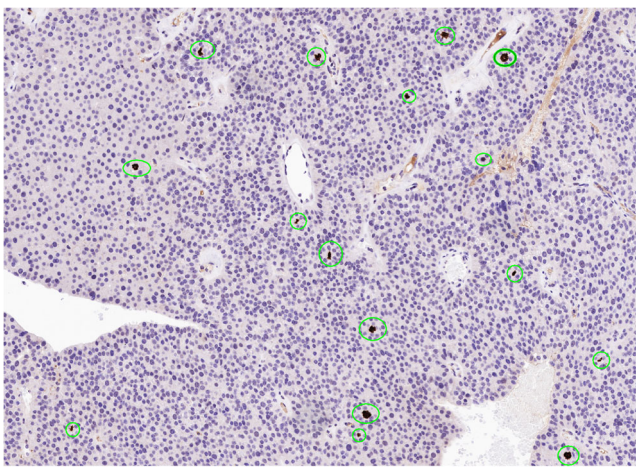

Fig. 6 PhosphoHistone-H3 immunohistochemistry. The use of phosphoHistone-H3 immunohistochemistry can facilitate an objective mitotic count in a parathyroid neoplasm. This biomarker can also be used to distinguish apoptotic cells from mitotic figures. The mitotic figures are highlighted in the photomicrograph. While an increased mitotic activity (> 5 per 50 HPF) in association with prominent macronucleoli and coagulative necrosis has been considered a triad of high-risk pathological features, increased mitotic activity in the absence of invasive growth is not sufficient for the diagnosis of parathyroid carcinoma

Osawa et al. (2009) found hTERT nuclear expression in 6 of 6 parathyroid carcinomas and an atypical adenoma (which was associated with multiple recurrences), while none of the 18 typical adenomas or 5 normal parathyroids showed hTERT expression [94]. Ki-67 > 4% was present in all hTERT positive cases. The authors suggested hTERT expression may be associated with telomerase activation in parathyroid carcinomas [94].

Erovic et al. (2012) evaluated 10 parathyroid carcinomas and 25 adenomas for 34 proteins involved in angiogenesis, inflammation, cell adhesion, cell cycle, and apoptosis by

immunohistochemistry on tissue microarray [92]. They suggested a panel including Bcl-2a, parafibromin, Rb, and p27 may be helpful in evaluating parathyroid tumors. They also identified potential treatment targets as systemic adjuvant treatments are needed for patients with metasatic parathyroid carcinoma [92].

Wang et al. (2012) evaluated 15 parathyroid carcinomas, 19 adenomas, 8 hyperplasias, and 6 normal parathyroids with Ki-67, galectin-3, fragile histidine triad (FHIT), and parafibromin immunostains [62]. Complete loss of parafibromin was seen in 9 of 15 (60%) carcinomas and 1 of 19 (4%) adenomas. All other specimens were parafibromin positive. Galectin-3 was positive in 11 of 15 (73%) carcinomas, 5 of 19 (26%) adenomas, 1 of 8 (12%) hyperplasias, and 0 of 6 normal parathyroids. Ki-67 proliferation index was elevated in 4 of 15 (27%) carcinomas, 1 of 19 (5%) adenomas, and none of the hyperplasias or normal parathyroids. The combination of loss of parafibromin and galectin-3 positivity had sensitivity of 87% for carcinoma. The specificity reached 100% if both galectin-3 positivity and high Ki-67 were included [62].

Truran et al. (2014) studied 24 parathyroid carcinomas and 16 adenomas with parafibromin, galectin-3, PGP9.5, Ki-67, and cyclin D1 [63]. "Parafibromin was considered positive when any nuclear staining was seen in the presence of a positively stained control." [63] Of the carcinomas, 46% (11 of 24) were parafibromin negative, 33% (8 of 24) were PGP9.5 positive, 57% (13 of 23) were galectin-3 positive, 22% (5 of 23) had high (> 4%) Ki-67, and 10% (2 of 21) were cyclin D1 negative. At least one abnormal immunohistochemical result was identified in 19 of the 24 carcinomas. None of the adenomas showed staining suggestive of carcinoma. They concluded a panel including parafibromin, galectin-3, PGP9.5, and Ki-67 was better than any single marker [63]. Karaarslan et al. (2015) evaluated 84 parathyroid adenomas, 6 atypical adenomas, and 2 carcinomas with parafibromin, galectin-3, Ki-67 and HBME-1 and found parafibromin expression, negative galectin-3, and Ki-67 proliferation < 1% were helpful identifying benign parathyroid tumors [64].

Kruijff et al. (2014) evaluated PGP9.5 and parafibromin and WHO histologic criteria in 81 atypical parathyroid tumors [87]. Mortality and recurrences rates of 15 and 38%, respectively, for the 13 WHO criteria-positive and parafibromin negative cases; 7 and 36% for 14 WHO criteria-positive and parafibromin positive; 0 and 10% for the 21 WHO criteria-negative and parafibromin negative; and 0 and 0% for 33 WHO criteria-negative and parfibromin positive tumors. PGP9.5 positivity correlated with the groups. They found WHO criteria are essential in differentiating benign from malignant parathyroid tumors and suggested that atypical adenomas with negative parafibromin staining have a "low but real recurrence risk and should be considered tumors of low malignant potential" [87].

APC (adenomatous polyposis coli) was also studied with parafibromin and other markers. APC and parafibromin were evaluated by Juhlin et al. (2010) in 1 parathyroid carcinoma, 5 atypical adenomas, and 54 typical adenomas [59]. Complete loss of APC and decreased parafibromin expression were identified in the carcinoma and in 2 of 5 atypical adenomas. None of the typical adenomas had APC loss, including 2 with *HRPT2/CDC73* mutation and loss of parafibromin [59]. Hosny Mohammed et al. (2016) evaluated 21 parathyroid carcinomas, 73 adenomas, and 3 atypical adenomas by for parafibromin, galectin-3, Ki-67, and APC [85]. Loss of parafibromin was noted in 33% (7 of 21) of carcinomas and 1 of 73 (1%) adenoma. APC loss was seen in 95% (20 of 21) of carcinomas and 52% (38 of 73) of adenomas. Ki-67 was elevated (> 5%) in 86% (18 of 21) carcinomas, 67% (2 of 3) of atypical adenomas, and none of the typical adenomas. They concluded that a panel showing parafibromin loss, high Ki-67, and APC loss is helpful in diagnosing parathyroid carcinoma [85]. Kumari et al. evaluated 227 parathyroid neoplasms with parafibromin, APC, galectin-3, PGP9.5, and Ki-67, and found the combination of parafibromin loss, galectin-3 positivity, and PGP9.5 positivity had 50% sensitivity and 97.9% specificity for carcinoma [75].

Thus, in an ideal setting, a panel of immunoperoxidase stains showing loss of parafibromin (nuclear or nucleolar) in addition to other markers such as galectin-3 positivity, Ki-67 > 5%, PGP9.5 positivity, Rb loss, bcl2 loss, decrease in p27, hTERT expression, decrease in mdm2, and APC loss, would be a helpful diagnostic adjunct in difficult parathyroid cases.

Prognostic and Predictive Biomarkers in Parathyroid Disease

Witteveen et al. (2011) evaluated prognostic significance of CASR and parafibromin expression and *CDC73* mutation in 23 parathyroid carcinomas [100]. Decreased CASR expression was identified in 7 (30%) and was associated with a 16-fold increased risk of local or distant metastases. *CDC73* mutation was present 4 (17%) of carcinomas and was associated with a 7-fold increased risk of local or distant metastases. Loss of parafibromin was identified in 13 (59%) carcinomas and was associated with a 4-fold increased risk of local or distant metastases. Thus, not only are these diagnostic markers, but they also have prognostic significance [100].

Cetani et al. (2013) evaluated *CDC73* mutation and parafibromin immunostaining in 35 apparently sporadic parathyroid carcinomas with a median follow-up of 7 years [66]. Thirteen of 32 (41%) evaluable cases had nonsense or frameshift *CDC73* mutations and were sequenced, and 6 of these were germline. *CDC73* mutation was associated with

increased recurrence or metastasis (92.3%), but not survival. The type of mutation, somatic or germline, was not associated with outcome. Of the 34 carcinomas evaluated for parafibromin, 17 showed parafibromin loss (< 5% nuclei staining) and 17 (50%) were parafibromin positive (median 30%, range 10–80% positive nuclei). Loss of parafibromin was associated with increased recurrence or metastasis, and there was an inverse association between percentage of positive cells and mortality. The 10-year survival was lower with parafibromin negative tumors (23%) compared to parafibromin positive tumors (87%). In the 31 cases with both parafibromin immunostain and *CDC73* mutation evaluation available, 11 had both *CDC73* mutation and loss of parafibromin, 2 had *CDC73* mutation and positive parafibromin (10 and 30% parafibromin positive cells), and 4 showed loss of parafibromin (< 5% positive cells) and wild type *CDC73*. Tumors with *CDC73* mutation and parafibromin loss had a high likelihood of recurrence and metastasis (92.3%). Also cases with both *CDC73* mutation and parafibromin loss had a lower 10 year overall survival (18%) than those with neither (84%). Loss of parafibromin predicted clinical outcome and mortality better than *CDC73* mutation. In addition to diagnostic and prognostic utility for parafibromin expression and *CDC73* mutation status, the authors suggested *CDC73* mutation analysis has "added value" in possibly identifying germline mutations leading to screening of relatives [66]. Thus, parafibromin may have utility in raising the possibility of familial parathyroid disease.

In addition to biomarkers, clinical and pathologic features may also raise the possibility of germline associated disease. Individuals less than 45 years of age with primary hyperparathyroidism, history of failed parathyroidectomy, and family history of hypercalcemia and/or other endocrinolopathy may benefit from further evaluation to rule out an underlying genetic predisposition [101]. Individuals with germline mutations can manifest with synchronous, symmetrical multiglandular parathyroid involvement, a cystic parathyroid lesion, or a parathyroid carcinoma. Individuals with multiglandular disease should have MEN1 and MEN4 excluded, whereas cystic change in a parathyroid, particularly in a young individual, may raise the possibility of a *CDC73/HRPT2*-driven disorder such as HPT-JT syndrome or familial isolated hyperparathyroidism [101, 102]. Individuals with parathyroid carcinoma may also benefit from genetic evaluation to evaluate for the possibility of a *CDC73/HRPT2*-driven germline genetic disorder.

Recently, Gill et al. expended our knowledge of clinical and histologic features of parafibromin-deficient parathyroid neoplasms [102]. Parafibromin-deficient parathyroid neoplasms were more common in younger patients that had larger tumors with distinct morphologic features [102]. In addition to microcystic change in approximately half of the cases in their series, the presence of a thick capsule, prominent arborizing vasculature, extensive sheet-like growth, eosinophilic tumor cytoplasm, nuclear enlargement with coarse chromatin, and perinuclear clearing were also identified [102].

Discussion

The identification and classification of most parathyroid tissues and lesions are generally straightforward, but in difficult cases immunoperoxidase studies may be helpful. Standard widely available immunohistochemical markers, often used as a panel, such as keratins, chromogranin-A, synaptophysin, TTF-1, parathyroid hormone, and thyroglobulin are helpful in identifying parathyroid and differentiating it from other tissues and tumors (thyroid tissue, folliculogenic and medullary thyroid tumors, paragangliomas, and other tumors in the neck or metastatic to the neck).

Immunohistochemical markers have been evaluated for utility in diagnostic classification of parathyroid tumors such as parathyroid adenoma versus carcinoma. The most studied markers have been Ki-67 and parafibromin. Generally, a Ki-67 index of > 5 is worrisome for parathyroid carcinoma, but proliferative indices show overlap. Parathyroid carcinoma is often in the differential diagnosis of a parathyroid adenoma/atypical adenoma, but parathyroid carcinoma is quite rare. Thus, even with numerous studies in the literature, the number of parathyroid carcinomas studied remains limited. Also, how "loss" of parafibromin is defined in these studies is highly variable—from total nuclear loss of parafibromin in all tumor cells, nucleolar loss of parafibromin, partial/variable loss, loss in varying percentages of tumor cells, variable intensities of staining, to no loss of parafibromin in any tumor cell nuclei—the evaluation of parafibromin in the literature is not straightforward. From the literature, it appears that complete nuclear or nucleolar loss of parafibromin is most helpful in supporting a diagnosis of parathyroid carcinoma. One must also keep in mind caveats in the evaluation of parafibromin. HPT-JT associated parathyroid adenomas also may also show loss of parafibromin. Parathyroid carcinomas in the setting of chronic renal failure, even in the metastatic setting, may retain parafibromin. Although one must keep these caveats in mind, loss of parafibromin, particularly complete nuclear or nucleolar loss, generally does appear to be helpful in identifying parathyroid carcinoma.

Ki-67 proliferation index of > 5% and complete nuclear or nucleolar loss of parafibromin in all tumor cells are helpful in evaluation of parathyroid tumors, but other immunohistochemical findings such as galectin-3 positivity, PGP9.5 positivity, Rb loss, bcl2 loss, decrease in p27, hTERT expression, decrease in mdm2, and APC loss also have promise as a diagnostic adjuncts in difficult parathyroid cases. However, even fewer cases have been evaluated for these markers. Additional studies will continue to evaluate the diagnostic

utility of immunohistochemical and molecular studies in the evaluation of problematic parathyroid lesions. Similar to many aspects of endocrine pathology, a panel of multiple markers will likely provide most diagnostic utility. The prognostic utility of these markers is also beginning to be studied in parathyroid carcinoma.

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