REVIEW

Atypical parathyroid adenomas: challenging lesions in the differential diagnosis of endocrine tumors

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Abstract

Atypical parathyroid adenomas represent a group of intermediate form of parathyroid neoplasms of uncertain malignant potential which show some atypical histological features that represent a challenge for the differential diagnosis with parathyroid carcinomas. They may occur as sporadic or as a part of hereditary syndromes. The molecular signature of these neoplasms is still unknown and the germline *CDC73* mutations appears to be the most common anomaly in this setting suggesting that these cases might represent variants of the hyperparathyroidism-jaw tumor syndrome. The identification of markers predicting the outcome is of great importance to guide an adequate postoperative monitoring and, the same time, relieve of the anxiety of relatively strict monitoring patients not at risk. This review will summarize the current knowledge of the clinical, biochemical, molecular and histological profile of atypical parathyroid adenomas.

Key Words

- ▶ parathyroid adenoma
- ▶ parathyroid carcinoma
- ▶ primary hyperparathyroidism
- ▶ *CDC73*
- ▶ parafibromin
- ▶ PGP9.5
- ▶ galectin-3

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Introduction

Parathyroid tumors are a heterogeneous group of tumors that affect 0.1–0.3% of the general population. They are mostly hyperfunctioning, that is produce an excessive and uncontrolled secretion of PTH determining primary hyperparathyroidism (PHPT), the third most common endocrine disease after diabetes and thyroid cancer (Bilezikian *et al.* 2018). Approximately 85% of the patients have a single parathyroid adenoma, 10% hyperplasia and 3% double adenoma. Atypical parathyroid adenoma and carcinoma, in contrast, are rarer being the latter the rarest parathyroid tumor (<1%) (Cetani *et al.* 2016, Saponaro *et al.* 2018). PHPT occurs as sporadic (90%) or as familial (10%) disease. Among the latter, multiple endocrine neoplasia (MEN) type 1 (MEN1), type 2A (MEN2A),

type 4 (MEN4) and the hyperparathyroidism-jaw tumor syndrome (HPT-JT) are caused by known germline genetic mutations and are associated with a broad spectrum of endocrine and non-endocrine tumors (Cardoso *et al.* 2017). Most patients with HPT-JT are at increasing risk of parathyroid carcinoma (up to 37%) and develop single or multiple cystic parathyroid adenomas and occasionally atypical adenomas (Marx & Goltzman 2019). Because of the underlying genetic basis, these diseases may have different clinical features compared to the sporadic counterpart.

Atypical parathyroid adenomas represent a group of intermediate form of parathyroid neoplasms of uncertain malignant potential which show some atypical

histological features (i.e. solid growth pattern, fibrous bands and cellular atypia), which represent a challenge for the differential diagnosis with parathyroid carcinomas. In this regard, it should be reminded that, at variance with parathyroid carcinoma, atypical parathyroid adenoma lacks evident signs of local invasion and/or metastasis. In some cases, the initial diagnosis of atypical adenoma needs to be revised to carcinoma because of the occurrence of local or distant metastasis during the follow-up (Sandelin *et al.* 1994). The reverse, even less frequently, may also occur when the initial diagnosis of parathyroid carcinoma is not confirmed by an expert endocrine pathologist (Ippolito *et al.* 2007, Cetani *et al.* 2008, Kumari *et al.* 2016, Ryhänen *et al.* 2017). Finally, there are cases in which the differential diagnosis between atypical parathyroid adenoma and carcinoma may be a major challenge also for an experienced pathologist. The macroscopic appearance at surgery is of limited help since in both cases the parathyroid lesions may appear firm and adherent to the adjacent structures, features that in the suspicion for malignancy may guide the surgeon to perform a more radical surgical approach.

The question of whether atypical parathyroid adenomas could represent an early stage of a parathyroid carcinoma, which is excised before the development of the molecular signature of malignancy responsible for invasive properties remains to be established. Immunohistochemical markers have been sought to help identifying atypical parathyroid adenoma carrying the risk of malignant behavior, but unfortunately, current data in small series of parathyroid typical and atypical adenoma and carcinoma have shown an overlap between the different tumors.

Our understanding of the molecular pathogenesis of parathyroid tumors has significantly increased over the last two decades, especially for parathyroid carcinoma. Conversely, no major progress has been made to define the molecular mechanisms underlying atypical parathyroid adenomas.

The clinical profile and outcome (recurrence rate and overall survival) of patients with atypical parathyroid adenoma seems to be less severe than that of patients with parathyroid carcinoma. In view of the diagnostic difficulties between these two conditions at histology the identification of markers capable of predicting the outcome of patients with atypical adenoma (low risk vs high risk) would be crucial in order to plan an adequate clinical management.

The aim of this review is to summarize the current knowledge of the clinical, biochemical, molecular and histological profile of atypical parathyroid adenomas.

Medline search strategy

We conducted a systematic search for the full articles through the public Medline database (<https://www.ncbi.nlm.nih.gov/pubmed>; last access: December 21, 2018) using as keywords either 'atypical parathyroid adenoma' or 'parathyroid atypical adenoma' or 'equivocal parathyroid adenoma' or 'parathyroid equivocal adenoma' and yielded 144, 181, 70 and 56 articles, respectively, after a filtration for the use of English language in the text. A further specific manual search was made checking our topic from the reference lists of relevant studies, thus allowing the finding of seven additional articles. Titles and abstracts of all identified articles were screened and full-text articles of studies that met the predefined eligibility criteria were obtained and carefully reviewed. We finally extracted data from a total of 92 different full articles after removal of duplicates and exclusion of articles where the presence of atypical features in parathyroid tumors were not based on histological examination of surgically removed tissue material. The reports described a total of 672 patients with a diagnosis of atypical parathyroid adenoma, mainly sporadic and including 24 members of FIHP, HPT-JT and MEN1 kindreds. Articles included 55 case series with comparative analyses and 36 case report studies dealing with 41 cases, 15 of which had a familial history of PHPT.

We reviewed all available information on demographic data, clinical presentation, pre-operative biochemistry, operative findings and procedures, histopathology and postoperative outcome from the case series and reports collected.

Epidemiology

Six hundred seventy-two patients have been reported according to the criteria of the research. All but 24 cases, which included members of FIHP, HPT-JT and MEN1 kindreds, were sporadic or otherwise not specified (Table 1). The pathological diagnosis of atypical parathyroid adenoma was made mostly according to the World Health Organization (WHO) criteria (see below) (DeLellis *et al.* 2004, 2017). The largest series has been reported by Schneider *et al.* (2015).

Table 1 Demographic, biochemical, clinical, surgery-related features of patients with atypical parathyroid adenoma derived from the literature revision.

	Total cases (<i>n</i> = 672) ^a		Sporadic cases (<i>n</i> = 331) ^b		Familial cases (<i>n</i> = 24) ^c	
	<i>n</i> (%)	Value	<i>n</i> (%)	Value	<i>n</i> (%)	Value
Sex (total <i>n</i> = 466; sporadic <i>n</i> = 281; familial <i>n</i> = 23)						
Male	185 (40)		116 (41)		11 (48)	
Female	281 (60)		165 (59)		12 (52)	
Age at diagnosis (years) (total <i>n</i> = 103, sporadic <i>n</i> = 44, familial <i>n</i> = 24)						
Mean ± s.d.		43.3 ± 18.9		48.1 ± 18.5		25.6 ± 8.1
Median (range)		44 (10–86)		50 (16–80)		28 (10–37)
Clinical manifestations (total <i>n</i> = 242, sporadic <i>n</i> = 107, familial <i>n</i> = 13)						
Renal involvement	71 (29)		25 (21)		4 (31)	
Bone involvement	44 (18)		30 (28)		2 (15)	
Both	6 (3)		3 (3)		0 (0)	
Palpable lesion (total <i>n</i> = 247, sporadic <i>n</i> = 151, familial <i>n</i> = 3)	37 (15)		15 (10)		2 (67)	
Serum calcium level (mg/dL) (total <i>n</i> = 79 ^d , sporadic <i>n</i> = 34, familial <i>n</i> = 19)						
Mean ± s.d.		13.4 ± 2.4		14.0 ± 2.6		12.8 ± 1.6
Median (range)		12.9 (10.2–23.2)		13.3 (10.2–23.2)		12.1 (11–16)
Serum PTH level (ng/L) (total <i>n</i> = 74 ^d , sporadic <i>n</i> = 26, familial <i>n</i> = 20)						
Mean ± s.d.		782 ± 712		951 ± 871		637 ± 697
Median (range)		430 (73–3242)		507 (124–3242)		209 (73–2164)
Adherence to adjacent structures (total <i>n</i> = 230, sporadic <i>n</i> = 151, familial <i>n</i> = 4)	98 (43)		78 (52)		2 (50)	
Initial surgery (total <i>n</i> = 275, sporadic <i>n</i> = 193, familial <i>n</i> = 15)						
<i>En bloc</i> resection	65 (24)		41 (21)		3 (20)	
Bilateral cervical exploration	52 (19)		46 (24)		5 (33)	
Simple PTx	155 (56)		105 (54.5)		7 (47)	
Sternotomy/thoracotomy	3 (1)		1 (0.5)		0 (0)	
Tumor size (cm) (total <i>n</i> = 55, sporadic <i>n</i> = 21, familial <i>n</i> = 13)						
Mean ± s.d.		2.7 ± 1.4		3.2 ± 1.6		2.3 ± 1.1
Median (range)		2.5 (0.7–7.2)		3 (1–7.2)		2.3 (0.7–4)
Tumor weight (g) (total <i>n</i> = 24, sporadic <i>n</i> = 13, familial <i>n</i> = 1)						
Mean ± s.d.		10.8 ± 20.4		12.6 ± 25.1		1.1
Median (range)		4.15 (0.3–101)		3.8 (0.3–101)		1.1
Cystic features ^e (total <i>n</i> = 125, sporadic <i>n</i> = 31, familial <i>n</i> = 11)	37 (30)		7 (22)		5 (45)	
Outcome (total <i>n</i> = 478, sporadic <i>n</i> = 236, familial <i>n</i> = 15)						
Cured	457 (96)		229 (97)		9 (60)	
Persistence	5 (1)		2 (1)		0 (0)	
Recurrence	16 (3)		5 (2)		6 (40)	
Months to recurrence after first surgery (total <i>n</i> = 10, sporadic <i>n</i> = 3, familial <i>n</i> = 6)						
Mean ± s.d.		94 ± 69		76 ± 82		126 ± 58
Median (range)		120 (12–204)		48 (12–168)		132 (24–204)
Follow-up (months) (total <i>n</i> = 57, sporadic <i>n</i> = 20, familial <i>n</i> = 13)						
Mean ± s.d.		62.6 ± 61.4		38.6 ± 39.2		104 ± 91
Median (range)		47 (0.25–252)		21 (0.25–120)		96 (2–252)

The figures in parentheses indicate the number of patients with available information. Serum calcium, the most represented normal values were 8.5–10.5 and 8.8–10.4 mg/dL. Plasma PTH, the most represented normal values were 11–65, 15–60 and 15–65 ng/L.

^aIncludes one case with non-functioning atypical parathyroid adenoma. ^bIncludes one case with *CDC73* germline mutation and one case of clinical Multiple endocrine neoplasia type 1 with no family history. ^cFamilial cases include 17 Familial Isolated Hyperparathyroidism, 6 Hyperparathyroidism-jaw tumor and 1 multiple endocrine neoplasia type 1. ^dData from non-functioning atypical parathyroid adenoma were not included. ^eDetected at neck ultrasound and/or pathology.

In most consecutive series of patients undergoing surgery for PHPT, the incidence of atypical parathyroid adenoma ranged from 0.5 to 4.4% (de Meneses Montenegro *et al.* 2006, Ippolito *et al.* 2007, Juhlin *et al.* 2010, O'Neal *et al.* 2011, Ricci *et al.* 2012, Chandramohan *et al.* 2014,

Haglund *et al.* 2015, McCoy *et al.* 2015, Quinn *et al.* 2015, Schneider *et al.* 2015, Ozolins *et al.* 2016, Mamedova *et al.* 2017). An incidence as high as 14% has been reported in the Asian countries (Cakir *et al.* 2016, Kumari *et al.* 2016, Hu *et al.* 2018).

At variance with benign disease, in whom the women predominate over men with a ratio of 3–4:1, but similar to parathyroid carcinoma in which the ratio between gender is comparable, atypical parathyroid adenomas occur with a ratio of 1.5:1. The median age at diagnosis is 44 years, which is, as in PC, a decade earlier than the typical age of benign disease. As expected, the familial cases were younger compared to their sporadic counterpart (median age 28 (10–37) vs 50 (16–80) years) (Table 1).

Pathogenesis

Genetic alterations

The pathogenic mechanisms underlying the origin of atypical adenomas are currently unknown and a few studies on a small sample size have been carried out so far.

Our effort should be directed to understand whether atypical adenomas might represent a pre-malignant lesion or might not have a true malignant potential, thus sharing genetic features with parathyroid carcinoma or benign parathyroid adenoma, respectively.

Clonality in tumor origin has important implications for cancer risk assessment and treatment. A step-wise accumulation of mutations within the lineage of a single cell supports a malignant progression model for which aggressive tumors bear at equal or even greater frequency the same genetic alterations early observed in benign neoplasia and gain more malignant potential throughout the lifetime (Vogelstein *et al.* 2013). Multiclonal tumor origin is the predominant mode of tumorigenesis of some cancer types, like colorectal cancer, whereas other tumors, such as many of the myeloid cell lineages, seem to rather have a monoclonal tumor origin. If parathyroid tumorigenesis would follow the multiclonal origin model we would expect that known molecular events characterizing parathyroid adenomas, that is 11q loss and/or *MEN1* mutations, would also be present in carcinomas at a frequency $\geq 35\%$ (Pardi *et al.* 2013, Brewer *et al.* 2019). The finding that such alterations occur at a very low frequency in parathyroid carcinoma suggests that most parathyroid cancers arise *de novo*, rather than evolve from a preexisting benign adenoma (Costa-Guda *et al.* 2013, Brewer *et al.* 2019).

Somatic loss-of-function mutations of the *CDC73* tumor suppressor gene, encoding parafibromin, are the most common genetic abnormality found in sporadic parathyroid carcinomas (up to 70%) (Cetani *et al.* 2018). Conversely, *CDC73* mutations are very rarely detected

in benign adenomas (Howell *et al.* 2003, Cetani *et al.* 2004a, Krebs *et al.* 2005, Bradley *et al.* 2006, Guarnieri *et al.* 2012). Of note, germline mutations of the *CDC73* gene are present in about 90% of patients with HPT-JT and in one-third of patients with apparently sporadic parathyroid carcinoma even in the absence of family history, suggesting a high risk for the patients and relatives to develop HPT-JT-related tumors. This inherited syndrome is characterized by the asynchronous development of parathyroid tumors, a variety of kidney lesions, benign ossifying fibroma of mandible/maxilla and uterine tumors (Cardoso *et al.* 2017). As mentioned before, the prevalence of parathyroid carcinoma in these patients is much higher than in sporadic cases (up to 37 vs 1%, respectively). In this setting, rare patients have a parathyroid carcinoma that has been progressed from a benign or atypical parathyroid adenoma (Brewer *et al.* 2019). However, as underlined in the pathology section, since the histological diagnosis of atypical adenomas and carcinomas could be very challenging, as to whether in HPT-JT, there is a true progression from benign to malignant phenotype remains unclear.

On the basis of the evidence that *CDC73* has an important role in parathyroid carcinoma, alterations of this gene have also been searched for in atypical parathyroid adenomas.

Inactivating *CDC73* mutations, including two large deletions spanning exons 1–10 of the gene were identified in 24 of 63 (38%) cases and in all but one (1.5%) case were at germline level (Table 2). Seventy-three percent of germline mutations were found in familial cases (11 patients with FIHP and 4 with HPT-JT) and 7 in apparently sporadic PHPT (Table 2). Somatic *CDC73* mutation screening was only performed in seven studies which included 27 neoplasms (Bradley *et al.* 2006, Juhlin *et al.* 2006, Mizusawa *et al.* 2006, Cetani *et al.* 2007, Guarnieri *et al.* 2012, Sulaiman *et al.* 2012a,b). Only four somatic *CDC73* mutations were detected. However, three mutations represented the somatic hit associated with germline mutations (in two members of FIHP families and in a 58-year-old man with apparently sporadic PHPT) (Bradley *et al.* 2005, Kelly *et al.* 2006, Guarnieri *et al.* 2012). The latter patient and one FIHP relative remained normocalcemic up to the latest evaluation 27 and 24 months after surgery, respectively, whereas no information on the outcome was reported for the remaining patient. Interestingly, Sulaiman *et al.* described a PHPT patient carrying a germline *CDC73* mutation who developed a benign and an atypical adenoma (Sulaiman *et al.* 2012a). A somatic hit, different from the germline *CDC73* mutation,

Table 2 Summary of *CDC73* genetic studies in atypical parathyroid adenomas.

Authors	Number of samples	Sporadic/familial (associated disease)	Analyses (number of samples)	DNA source	Mutation ^a (number of carriers, sporadic/familial)	<i>CDC73</i> gene				Outcome of mutated/wild-type patients (number of patients)
						Predicted effect	Germline/somatic	Large deletion (number of carriers)	Germline/somatic	
Muscarella <i>et al.</i> (2018)	7 (3) ^b	ns	Sanger sequencing (3), MLPA (7), qRT-PCR (7), arrayCGH (7)	Blood (6 tissues)	No			No		ns
Van der Tuin <i>et al.</i> (2017)	1	F (HPT-JT) ^c	Sanger sequencing	Blood	c.3_15dup	p.Ser6Glyfs*5	G			Recurrence
Ryhänen <i>et al.</i> (2017)	16	ns	NGS, MLPA	Blood	c.271C>T (1)	p.Arg91*	G	No		Recurrence/cured
Mamedova <i>et al.</i> (2017)	2	2 Sp ^d	NGS, MLPA	Blood	No			Exon 1-10 (1)	G	Cured
Bricaire <i>et al.</i> (2013)	2	1 F (FIHP) 1Sp	Denaturing HPLC; Sanger sequencing	Blood	c.308-9T>A (F) c.271C>T (Sp)	Intron 3 acceptor splice site (VUS) p.Arg91*	G G			ns
Pazienza <i>et al.</i> (2013) ^e	1	1 F (HPT-JT)	Sanger sequencing	Blood	c.242_253del	p.Glu81_Pro84del	G			ns
Carpten <i>et al.</i> (2002)	2	F (FIHP) ^f	Sanger sequencing	Blood	No			Exon 1-10 (2)	G	ns
Korpi-Hyövähti <i>et al.</i> (2014)	1	ns	Sanger sequencing, CNV, aCGH, SNP	Blood and tissue	c.131+1delG	Intron 1 donor splice site	G ^g			ns
Sulaiman <i>et al.</i> (2012a)	3	Sp	microarray Sanger sequencing, aCGH, qRT-PCR	Blood and tissue	No					Cured
Guarnieri <i>et al.</i> (2012)	14	1 F (FIHP) 13 Sp	Sanger sequencing	Blood and tissue	c.679_680delAG (1 Sp) ^h c.231C>G (1 Sp) ^h c.685_688delAGAG (F)	p.Arg227Lysfs*31 p.Arg77Pro p.Arg229Tyrfs*27	G S G			Cured/Cured
Sarquis <i>et al.</i> (2008)	1	F (HPT-JT)	Sanger sequencing	Blood	c.96G>A	p.Trp32*	G			Recurrence
Cetani <i>et al.</i> (2007)	4	Sp	Sanger sequencing LOH analysis	Blood and tissue	No				No	Cured (3) Persistence (1)

(Continued)

Table 2 Continued

Authors	Number of samples	Sporadic/familial (associated disease)	Analyses (number of samples)	DNA source	CDC73 gene				Outcome of mutated/wild-type patients (number of patients)
					Mutation ^a (number of carriers, sporadic/familial)	Predicted effect	Germline/somatic	Large deletion (number of carriers)	
Juhlin <i>et al.</i> (2007, 2011)	1	Sp	Sanger sequencing	Blood	c.127_128insC	p.Trp43Serfs*23	G		ns
Bradley <i>et al.</i> (2006)	1	F (FHP)	Sanger sequencing	Blood and tissue	c.356delA ^h c.284T>C ^h	p.Gln119Argfs*14 p.Leu95Pro	G S		ns
Juhlin <i>et al.</i> (2006)	1	Sp	Sanger sequencing LOH analysis	Blood and tissue	c.126_131+18del	Intron 1 donor splice site	S	LOH	Cured
Kelly <i>et al.</i> (2006)	2	F (FHP)	Sanger sequencing	Blood	c.140_144del (2) ^h c.61_64del (1) ^h	p.Lys47Argfs*17 p.Lys21Glufs*5	G S		Cured
Mizusawa <i>et al.</i> (2006)	3	F (FHP)	Sanger sequencing	Blood and tissue	c.520_523del (2) ⁱ	p.Ser174Lysfs*27	G		Cured
Bradley <i>et al.</i> (2005)	1	F (FHP)	Sanger sequencing	Blood	c.131+1G>A	Intron 1 donor splice site	G		Cured
Cetani <i>et al.</i> (2004a,b)	2	F (FHP)	Sanger sequencing	Blood	c.131+1G>A (2) ^j	Intron 1 donor splice site	G		Persistence (1) Recurrence (1)
Cavaco <i>et al.</i> (2004)	2	F (HPT-JT)	Sanger sequencing	Blood	c.766_767del (2) ^j	p.Val256Lysfs*10	G		ns

^aMutations are numbered in relation to the cell division cycle 73 (CDC73) cDNA reference sequences (GenBank accession number NM_024529.4). Nucleotide +1 corresponds to the A of the translation initiation codon (ATG). All mutations were annotated based on the last recommendations of the Human Genome Variation Society (HGVS) (<https://www.hgvs.org/>). ^hMLPA was performed in seven samples and Sanger sequencing in three samples. The remaining four samples of the study were previously sequenced in Guarneri *et al.* (2012). ⁱThe mutation was firstly identified by Carpten *et al.* (2002) in the same case. ^jOne of the two cases has a suspicious familial history for Calve's disease but not for HPT. The authors classified the case as HPT-JT after the finding of the CDC73 mutation. ^kThe authors also reported the molecular data of the same patient previously described by Guarneri *et al.* (2012). ^lThe mutation was carried by two members of the same kindred, first described by Teh *et al.* (1998). ^mThe patient also had a benign adenoma that harbors a different CDC73 mutation at somatic level. ⁿRepresent the two hits in the same patient. ^oThe mutation was carried by two members of the same kindred. ^pFounder mutation associated with the HPT-JT syndrome in six Roma families from Portugal.

CGH, comparative genomic hybridization; CNV, copy number variations; F, familial; FHP, familial isolated hyperparathyroidism; G, germline; HPLC, high-pressure liquid chromatography; HPT-JT, hyperparathyroidism-jaw tumor; LOH, loss of heterozygosity; MLPA, multiple-ligation probe assay; NGS, next-generation sequencing; ns, not specified; qRT-PCR quantitative reverse transcriptase polymerase chain reaction; S, somatic; SNP, single nucleotide polymorphism; Sp, sporadic; VUS, variant of unknown significance.

was found in the benign but not in the atypical adenoma, which was diploid at the *CDC73* locus. Nonetheless, the benign adenoma harbored less cytogenetic anomalies, compared to the atypical adenoma and parathyroid carcinomas evaluated in the same study. Further studies in the atypical adenoma, using the genome-wide high-resolution array-CGH technique, revealed a wide DNA copy number alterations at several chromosomal loci (extensive gains on 1p, 16, 17, 19, 20 and 22, small gains on chromosome 11 and 12, including *MEN1* and *CDK4* loci, respectively). Interestingly, a similar profile was found in three carcinomas included in the study. Finally, the atypical adenoma also displayed loss at 9p23–24.1 loci that was also identified in a sporadic parathyroid adenoma. Despite the interesting molecular signature of this atypical adenoma, being based on a single tumor, the authors suggested doing further studies using a larger sample size to confirm the data.

In summary, exclusive *CDC73* somatic mutations are very rare in atypical parathyroid adenomas, suggesting a minor pathogenic role, if any, in these neoplasms. Nevertheless, an interesting study generating parathyroid-specific *Cdc73*-knockout mice, resulted in mice deleted for one or both *Cdc73* alleles that developed parathyroid tumors having in 75% of the cases nuclear pleomorphism, fibrous septa and galectin-3 overexpression, consistent with the histological diagnosis of atypical parathyroid adenomas. Parathyroid tumors of *Cdc73*^{+/-} and conditional parathyroid-specific mice (*Cdc73*^{+/-}/*PTH-Cre* and *Cdc73*^{L/L}/*PTH-Cre*) had significantly increased proliferation rate, higher mean serum calcium and PTH levels than wild-type littermates. Thirty-three percent of female *Cdc73*^{+/-} mice developed uterine neoplasms, resembling HPT-JT syndrome in human (Walls *et al.* 2017).

The oncosuppressor *MEN1* gene and *CCND1* oncogene have been clearly established as drivers of benign parathyroid tumorigenesis (Brewer *et al.* 2019). A small subset of patients with parathyroid carcinoma carries *MEN1* mutations and their frequency is much lower than that reported in sporadic parathyroid adenoma (6 vs 35%, respectively) (Brewer *et al.* 2019). Loss-of-function *MEN1* mutations have been searched for in ten atypical parathyroid adenomas and only one germline mutation (c.253A>T, p.127S) in exon 2 was detected in a *MEN1* patient (Cetani *et al.* 2002, Juhlin *et al.* 2006, Mizusawa *et al.* 2006, Sulaiman *et al.* 2012a, Pal *et al.* 2018).

Other genes have been evaluated as possible contributors for the pathogenesis of atypical parathyroid adenomas. Loss-of-function mutations of the calcium-sensing receptor (*CASR*), which are responsible for

familial hypocalciuric hypercalcemia type 1 and neonatal severe hyperparathyroidism and FIHP, were not detected at germline level in three relatives of FIHP and HPT-JT families bearing atypical adenomas (Mizusawa *et al.* 2006). Such mutations have also not been detected in sporadic parathyroid adenomas (Hosokawa *et al.* 1995, Cetani *et al.* 1999). Only one case of apparently sporadic parathyroid adenoma was found to harbor a *CASR* mutation in the germline (Guarnieri *et al.* 2010).

Genetic testing of β -catenin (six cases), enhancer of zeste homolog 2 (*EZH2*) and zinc finger X-linked (*ZFX*) (12 cases) genes was negative. Remarkably, such mutations also appear to be very rare in parathyroid adenomas (Cromer *et al.* 2012, Sanpaolo *et al.* 2016, Romano *et al.* 2017).

Allelic loss (LOH) at different chromosomal loci including 12 tumor suppressor genes (*CDKN1A*, *CDKN2A*, *TP53*, *CDC73*, *VHL*, *APC*, *PTEN*, *NM23*, *KAI1*, *MEN1*, *RB1*, and *NF2*) was identified in 25 of 38 (66%) sporadic atypical parathyroid adenomas (nine cases at *CDKN1A*, four at *RB1* and six at *CDC73* and *PTEN*) (McCoy *et al.* 2015). Similarly, this molecular profile was partially shared by parathyroid carcinomas. Of interest, LOH of *NM23*, *VHL* and *APC* was only observed in parathyroid carcinomas but not in atypical adenomas. Notably, the same authors in a previous study found a different LOH profile between benign adenoma and parathyroid carcinoma, particularly at *CDC73* and *RB1* locus, which were more frequently associated with malignant disease (Yip *et al.* 2008).

Epigenetic alterations

The role of epigenetic alterations in atypical parathyroid adenomas has been investigated in even fewer studies.

Global hypomethylated genome together with hypermethylation of specific genes has been described in many human malignant tumors. A global hypermethylation, compared with normal parathyroid glands, has been reported in parathyroid carcinomas though all of the hypermethylated genes in parathyroid carcinomas were also hypermethylated, at lesser extent, in benign parathyroid tumors (Starker *et al.* 2011, Barazeghi *et al.* 2016, Verdelli & Corbetta 2017). Sulaiman and coworkers investigated the methylation state of genes commonly involved in the pathogenesis of human cancer and particularly of parathyroid tumors (i.e. *CDKN2A*, *PAX1*, *RASSF1A*, *CTNNB1*, *SFRP1*, *APC*, *CDC73*, *MEN1*, *CASR* and *VDR*). They found a concordant hypermethylation of promoter 1A of *APC*, *CTNNB1* and *RASSF1A* in three atypical adenomas as well as

in three parathyroid carcinomas and 66 parathyroid adenomas. Conversely, they did not find hypermethylation of *MEN1* or *CDC73* promoter among the three categories of tumors, regardless of their mutational state. Finally, hypermethylation of *SFRP1* gene, likely involved in the activation of the Wnt/ β -catenin signaling pathway, was found in parathyroid carcinomas but not in atypical adenomas and benign adenomas, suggesting a putative role of this protein as a marker for parathyroid malignancy (Sulaiman *et al.* 2013).

Several studies have outlined an important role of miRNAs for the prediction of prognosis and outcome in many human cancers (Kwok *et al.* 2017). miRNAs are small endogenous non-coding RNA molecules (18–25 nucleotides) which may repress protein expression. The only study addressing the role of miRNAs in atypical parathyroid adenoma was published in 2018, investigating the epigenetic signature of miRNAs in 19 atypical parathyroid adenomas compared with parathyroid carcinomas and adenomas (Verdelli *et al.* 2018). An overexpression of miR-372 was found in 14 of 19 (74%) cases. The level of overexpression was similar to that observed in parathyroid cancers and significantly higher than in benign parathyroid adenomas. A significant reduction of mRNA levels of its target genes, namely *CDKN1A* (P21), large tumor suppressor kinase 2 (LATS-2) and *CCDN1* (cyclin D1), as well as an inhibitory effect at protein level was also identified (Verdelli *et al.* 2018).

Although epigenetic investigation is limited and additional studies are necessary, these data suggest a potential role of epigenetic alterations in the pathogenesis of atypical parathyroid adenomas.

Pathological features

The overlapping of histopathologic features between carcinomas and atypical adenomas can sometimes make the diagnosis of atypical adenoma very challenging (DeLellis 2011). According to the histopathological criteria proposed by the WHO, the diagnosis of malignancy in parathyroid gland should be limited to tumors with clear and unequivocal signs of invasive growth, such as neoplastic infiltration of adjacent tissues (as the soft tissues or the thyroid gland), vascular invasion, perineural space invasion and/or documented metastases (WHO 4th edition) (DeLellis *et al.* 2017). The WHO and Armed Forces Institute of Pathology (AFIP) defined an unequivocal vascular invasion when the neoplastic cells are located inside capsular or pericapsular vessels,

attached to the vessel wall, generally associated with fibrin thrombi, in presence or not of endothelial covering (Rosai *et al.* 2014). Capsular invasion is evident when a tongue or mushroom-like protrusion of the neoplasm goes over the fibrous capsule and variably extends into adjacent soft tissue. Other histological features of parathyroid carcinoma, variably represented, include the presence of foci of coagulative necrosis, variable degree of pleomorphism, including the presence of macronucleoli in some cases, and the presence of broadbands of fibrous connective tissue. However, none of the latter features is specific of malignancy, since variably observed both in benign and malignant tumors of the parathyroid gland (Rosai *et al.* 2014). The term ‘atypical adenoma’ is referred to a group of lesions of the parathyroid gland showing some histological features exhibited by carcinomas, in the absence of unequivocal evidence of invasive growth (Rosai *et al.* 2014, DeLellis *et al.* 2017). This subset of parathyroid lesions has also been labeled as tumors of uncertain malignant potential. Both macroscopically and microscopically, atypical adenoma can simulate a carcinoma, exhibiting the following features: (i) adherence to contiguous structures, in absence of clear invasion; (ii) banding fibrosis with or without hemosiderin deposition; (iii) entrapment of neoplastic cells within the fibrous capsule; (iv) solid/trabecular growth patterns and (v) mitotic activity (Rosai *et al.* 2014) (Fig. 1). The banding fibrosis appears as collagenous bands originating from the fibrous capsule and subdividing the tumor in variable-sized nodules. In some cases, the fibrous bands may create the entrapment of neoplastic cells in the context of the capsule, simulating capsular invasion (known as pseudocapsular) (DeLellis 2008). Moreover, fibrous bands in association to cystic degeneration may create adherence of the tumor to the surrounding structures, giving the macroscopic impression of malignancy at the time of surgery (Fernandez-Ranvier *et al.* 2007). Most carcinomas have a prevalent solid growth pattern associated to a variable quote of trabecular organization. More rarely, carcinomas can exhibit a follicular pattern, a spindle cell pattern or rarely a carcinosarcomatous pattern (Rosai *et al.* 2014). The growth pattern, as well as the cellular composition (chief cells, oncocyctic cells or clear cells) is not specific of malignancy. Since many carcinomas exhibit bland cytological features, the presence of cellular atypia and pleomorphism are not useful for the diagnosis of carcinoma (Schantz & Castleman 1973). As in other endocrine tumors, the presence of enlarged hyperchromatic or even bizarre nuclei is not specific of malignancy, since variably observed in

Figure 1

Representative histopathological images of atypical parathyroid adenoma. (A) This atypical parathyroid adenoma has irregular nodules composed of monotonous chief cells separated by prominent fibrous bands with hemosiderin deposition and marginal cystic degeneration (hematoxylin and eosin (H&E) staining, original magnification $\times 200$). (B) An entrapment of the tumor cells within the fibrous capsule of the neoplasm is shown (H&E staining, original magnification $\times 100$). (C) This tumor is composed mostly of chief cells with a minority of clear cells. The neoplastic cells show mild nuclear pleomorphism and trabecular growth pattern (H&E staining, original magnification $\times 200$). (D) This tumor shows a prominent cystic degeneration (H&E staining, original magnification $\times 100$).

parathyroid adenomas (Rosai *et al.* 2014, DeLellis *et al.* 2017). Nonetheless, some of the atypical features may occasionally be also found in benign adenomas. Of note, Sulaiman and coworkers found the presence, although at limited extent, of almost one of such characteristics (i.e. trabecular growth pattern, cellular pleomorphism or fibrous bands) in an up to 33% of benign adenomas (Sulaiman *et al.* 2012b). A mitotic activity in excess of 5 mitoses per 50 high power microscopic fields, in combination with the presence of foci of coagulative necrosis and macronucleoli, has been proposed as a triad to label parathyroid lesions with a high risk of malignant behavior, in terms of disease recurrence (Bondenson *et al.* 1993). The presence of atypical mitosis and focal areas of necrosis, though considered as criteria of malignancy, has also been described in a minority of atypical parathyroid adenomas (Katz *et al.* 2012, Ricci *et al.* 2012, Nair *et al.* 2013, Ramaswamy *et al.* 2017, Ryhnen *et al.* 2017, Pal *et al.* 2018, Silva-Figueroa *et al.* 2018).

Most of the studies included in this review have based the diagnosis of atypical adenoma on WHO criteria. However, there is no general consensus about the number of single histological atypical features to consider in order to classify a parathyroid neoplasm as atypical, rather than malignant or benign. As a matter of fact, the histological diagnosis of atypical parathyroid adenoma is strictly related to the lack of evidence of unequivocal findings of malignancy. Having said that, the correct diagnosis mainly depends on the meticulous examination of serial histological sections and their

interpretation is at risk of subjective bias. Indeed, even between experienced endocrine pathologists exists some degree of inter-observer variability, with obvious impact on over- or under-diagnosis of specific types of parathyroid neoplasms, making difficult the comparison of data between different institutions.

Some authors, according to the criteria of Seethala and coworkers (Seethala *et al.* 2009), defined the diagnosis of atypical adenomas when at least two of the following features were present, namely intraoperative adherence, bands of fibrosis, pronounced trabecular growth, strong mitotic activity, necrosis, diffuse sheet-like monotonous small cells with high nuclear/cytoplasmic ratio, cellular atypia and nucleolomegaly, in the absence of unequivocal signs of malignancy (Mishra & Newman 2014, McCoy *et al.* 2015, Cakir *et al.* 2016, Ozolins *et al.* 2016). Conversely, Kumari and coworkers, according to the histological criteria of Chan, established the diagnosis of atypical adenoma when one to three of the above features were present (Chan 2007, Kumari *et al.* 2016).

When histological data from different studies included in this review were pooled together, thick fibrous bands and trabecular growth pattern were the most representative morphological characteristics of atypical parathyroid adenomas (76 and 67%, respectively), followed by the presence of mitotic figures/high mitotic activity (52%), pseudocapsular invasion (42%), cellular pleomorphism (30%) and nuclear atypia (24%) (Fig. 2). Increased mitotic activity best characterized carcinomas compared to atypical adenomas and benign adenomas

Table 3 List of papers comparing results of immunohistochemistry with different markers between atypical parathyroid adenomas, benign adenomas and carcinomas.

Authors	Tissue samples	S/F	Ki-67 over-expression ¹	CASR loss ²	Cyclin D1 over-expression ³	Parafibromin loss	APC loss ⁴	Galectin-3 overexpression	PGP9.5 over-expression ⁵	P27 loss ⁶	MDM2 over-expression ⁷	RB loss ⁸
Sungu <i>et al.</i> (2018)	AA (n = 14) PC (n = 10) PA (n = 21)	ns ns ns	36% 50% Nd	7% 50% ^b 0% ^b	71% ^a 70% ^b 23.8% ^{ab}	nd nd nd	nd nd nd	71% ^{a9} 40% ⁹ 14% ^{a9}	nd nd nd	nd nd nd	nd nd nd	nd nd nd
Ryhänen <i>et al.</i> (2017)	AA (n = 28) PC (n = 32) PA (n = 72)	ns ns ns	Nd Nd Nd	nd nd nd	nd nd nd	4% ^{a10} 13% ^{b10} 0% ^{b10}	nd nd nd	nd nd nd	nd nd nd	nd nd nd	nd nd nd	nd nd nd
Quinn <i>et al.</i> (2015)	AA (n = 34) PC (n = 18) AA (n = 10)	ns ns S	3.9 6.8 0%	nd nd nd	nd nd nd	21% ¹¹ 38% ¹¹ 2% (focal) ⁸	nd nd nd	nd nd nd	nd nd nd	nd nd nd	nd nd nd	nd nd nd
Ozolins <i>et al.</i> (2016)	PC (n = 8) PA (n = 8) AA (n = 3)	S S S	87% 0% 67% ^a	nd nd nd	nd nd nd	100% ⁸ 0% ⁸ 0% ¹²	nd nd nd	nd nd 33%	nd nd nd	nd nd nd	nd nd nd	nd nd nd
Hosny Mohammed <i>et al.</i> (2017)	PC (n = 21) PA (n = 73) AA (n = 19)	ns ns ns	86% ^b 0% ^{ab} Nd	nd nd nd	nd nd nd	33% ^{b12} 1.4% ^{b12} 32% ¹³	67% 95% ^b 52% ^b	15% 4% 47% ¹⁴	nd nd 32%	nd nd nd	nd nd nd	nd nd nd
Kumari <i>et al.</i> (2016)	PC (n = 14) PA (n = 194) AA (n = 6)	ns ns S	Nd Nd 17%	nd nd nd	nd nd nd	50% ¹³ 10% ¹³ 0% ^{c8}	9% 22% nd	43% ¹⁴ 10% ¹⁴ 50% ¹⁵	64% 15% nd	nd nd nd	nd nd nd	nd nd nd
Karaarslan <i>et al.</i> (2015)	PC (n = 2) PA (n = 84) AA (n = 22)	ns ns S	0% 1% Nd	nd nd nd	nd nd nd	100% ^{bc8} 0% ^{b8} 36% (diffuse) ^{a16}	nd nd nd	100% ^{b15} 20% ^{b15} 32% ⁹	nd nd 22%	nd nd nd	nd nd nd	nd nd nd
Agarwal <i>et al.</i> (2016)	PC (n = 9) PA (n = 144) AA (n = 54)	S S ns	Nd Nd Nd	nd nd nd	nd nd nd	28% (focal) 55% (diffuse) ¹⁶ 13% (diffuse) ^{a16}	26% 26% nd	45% ^{b9} 15% ^{b9} nd	45% 22% 35% ^c	nd nd nd	nd nd nd	nd nd nd
Kruijff <i>et al.</i> (2014)	PC (n = 27) AA (n = 13) PC (n = 15)	ns S and F S	Nd Nd Nd	nd nd nd	nd nd nd	48% ⁸ 15% ^{c8} 67% ^{ac8}	nd nd nd	nd nd nd	81% ^c nd nd	nd nd nd	nd nd nd	nd nd nd
Guarnieri <i>et al.</i> (2012)	PA (n = 17) AA (n = 2) PA (n = 17)	15 S 2 F S S	Nd 0% 0%	nd nd nd	nd nd nd	18% ^{a8} 100% (total) ⁸ 18% (total) ⁸	nd 50% ^a 6% ^a	nd nd nd	nd nd nd	nd nd nd	nd nd nd	nd nd nd
Sulaiman <i>et al.</i> (2012a)	AA (n = 2) PA (n = 13) AA (n = 5)	ns ns S	50% 38% 20%	nd nd nd	nd nd nd	29% (partial) nd 40% (partial) ^{a8}	nd nd 40%	nd nd nd	nd nd nd	nd nd nd	nd nd nd	nd nd nd
Demiralay <i>et al.</i> (2011)	PC (n = 1) PA (n = 54) AA (n = 2) [§]	ns 52 S 2 F S	ND ND 0%	nd nd nd	nd nd nd	100% (partial) 3.7% ^a 0% ⁸	100% ^b 0% ^{b*} nd	nd nd 100% ^a	nd nd nd	nd nd 50%	nd nd 0%	nd nd 0%
Juhlin <i>et al.</i> (2010)	PC (n = 16) PA (n = 18)	S S	60% ^b 6% ^b	nd nd	nd nd	31% ^{b8} 0% ^{b8}	nd nd	92% ^b 6% ^{ab}	nd nd	87% ^b 28% ^b	20% ^b 6% ^b	33% 0%
Fernandez-Ranvier <i>et al.</i> (2009)												

(Continued)

Table 3 Continued

Authors	Tissue samples	S/F	Ki-67 over-expression ¹	CASR loss ²	Cyclin D1 over-expression ³	Parafibromin loss	APC loss ⁴	Galectin-3 overexpression	PGP9.5 over-expression ⁵	P27 loss ⁶	MDM2 over-expression ⁷	RB loss ⁸
Juhlin <i>et al.</i> (2006)	AA (n = 1)	S	0%	nd	100%	100% ^{a8}	nd	nd	nd	nd	nd	nd
Cetani <i>et al.</i> (2007)	PA (n = 44)	S	Nd	nd	86%	2.5% ^a (n = 40) ⁸	nd	nd	nd	nd	nd	nd
	AA (n = 4)	S	Nd	nd	50%	50% ^{a8}	nd	nd	nd	nd	nd	nd
	PC (n = 11)	S	Nd	nd	73% ^b	100% ^{b8}	nd	nd	nd	nd	nd	nd
	PA (n = 22)	S	Nd	nd	36% ^b	49% ^{ab8}	nd	nd	nd	nd	nd	nd
Tan <i>et al.</i> (2004)	AA (n = 6)	S	Nd	nd	nd	17% (diffuse) ^{bc17}	nd	nd	nd	nd	nd	nd
						33% (focal)	nd	nd	nd	nd	nd	nd
	PC (n = 52)	S	Nd	nd	nd	50% (diffuse) ^{bc17}	nd	nd	nd	nd	nd	nd
						46% (focal)	nd	nd	nd	nd	nd	nd
Stojadinovic <i>et al.</i> (2003)	PA (n = 48)	S	Nd	nd	nd	0% ^{ab17}	nd	nd	nd	nd	nd	nd
	PA (n = 9)	F (HPT-JT)	Nd	nd	nd	67% (diffuse)	nd	nd	nd	nd	nd	nd
	AA (n = 8)	S	0%	nd	13%	23% (focal)	nd	nd	nd	57%	63% ^a	nd
	PC (n = 20)	ns	27% ^b	nd	18%	nd	nd	nd	nd	82% ^b	18% ^b	nd
	PA (n = 45)	ns	2% ^b	nd	9%	nd	nd	nd	nd	20% ^b	93% ^{ab}	nd

¹High Ki-67 proliferative index was defined if >5% tumor nuclei stained. ²Global loss was defined as <10% of membrane staining. ³Overexpression was defined as >5% of specific nuclear staining.

⁴Complete loss was defined as <10% of cytoplasmic immunostaining. ⁵Overexpression was defined as >50% of cytoplasmic and/or nuclear immunostaining. ⁶Loss of expression was defined as <30% of tumor cell stained positive. ⁷Overexpression was defined as >50% of tumor cell nuclei stained positive. ⁸Complete loss was defined as nuclei of all neoplastic cells had absent staining. ⁹Positive staining was defined as >30% of tumor cytoplasmic or membranous cell immunostaining. ¹⁰Complete loss was defined as >99% of the nuclei of the neoplastic cells were negative, positive staining if >95% were positive, weak positives if >2 and <95% of nuclei were positive. ¹¹Criteria for scoring not reported. ¹²The intensity of staining was rated as either (0) negative, (1) low, (2) moderate or (3) high. Area of distribution was determined by percentage and it was scored from 0 to 100. The ultimate score was the outcome of multiplying the percentage of tumor cells stained (0–100) by staining intensity (0–3). A cut-off score of 100 was used to differentiate between negative (<100) and positive (>100) results. ¹³Complete loss was defined as <10% nuclear staining of tumor cells. Any staining >10% was taken as positive staining. ¹⁴Overexpression was defined as >30% of positive immunostaining. ¹⁵Cases with >60% of immunostaining were considered as positive (3+), with staining of 31–60% of the tumor cell were 2+ and with staining of <30% of the tumor cell were 1+. No reaction was seen as negative staining. ¹⁶Diffuse positive staining was defined as >90% staining of parathyroid tumor nuclei. Partial loss was defined as the absence of nuclear staining in 10–90% of tumor nuclei. Complete loss was defined as nuclear staining in <10% of tumor nuclei. ¹⁷Diffuse positive staining was defined as staining of all parathyroid tissue nuclei. Focal loss was defined as the absence of nuclear staining in variably sized regions. Diffuse loss was defined as the absence of nuclear staining in all tumor tissue. ¹⁸These samples derived from primary tumor and recurrence of the same patient. *Immunohistochemistry was performed in 36/54 specimens. ^aPA vs AA; ^bPA vs PC; ^cAA vs PC with $P < 0.05$.

AA, atypical adenoma; APC, adenomatous polyposis coli; CASR, calcium-sensing receptor; F, familial; MDM2, mouse double minute 2 homolog; nd, not done; PA, parathyroid adenoma; PC, parathyroid carcinoma; PGP9.5, protein gene product 9.5; RB, retinoblastoma; S, sporadic.

Figure 2

Main histological features reported in atypical parathyroid adenomas.

(up to 81 vs 38 and 7%, respectively), whereas fibrous bands were similarly present in carcinomas and atypical adenomas compared to benign adenomas (up to 100 vs 83 and 9%, respectively) (Table 4).

Immunohistochemical markers

In the last decades, a great effort has been made to evaluate the role of immunohistochemical markers as an aid to routine histology in the differential diagnosis of parathyroid tumors especially to evaluate the risk of malignancy in tumors with atypical features.

Few markers have displayed the desirable sensitivity and specificity. Ideally such markers would have near 100% of specificity and high sensitivity. Therefore, the risk of rely on such markers in cases with equivocal diagnoses might result in misdiagnosis and subsequent over or under-treatment of the patients.

Early studies focused on markers that control cell-cycle and apoptosis, namely Ki-67, P53, P27, RB (Cryns *et al.* 1994, Lloyd *et al.* 1995, Vargas *et al.* 1997, Cetani *et al.* 2004b). In more recent years, novel and promising markers have been used including parafibromin, galectin-3, APC and PGP9.5, either as single or panel of markers (Table 3).

Before examining the results of the different studies, it should be kept in mind that there are some limitations in the interpretation of the immunohistochemical stains, namely (i) the lack of a general consensus on the cut-off value of staining to classify the specimens as positive or negative; (ii) the use of various scoring systems; (iii) the use of different immunohistochemistry protocols,

including different antibodies and dilution range and (iv) the relative low number of samples. These limitations highly account for the different results among studies published in the literature.

Ki-67

The cell-cycle-associated Ki-67 antigen is a well-known proliferative index used in several human cancers (Menon *et al.* 2019). Ki-67 labeling index is mostly reported as the percent of positively stained cells and a cut-off value >5% of tumor nuclei stained is commonly accepted as a high proliferative index. Abbona and coworkers firstly used the Ki-67 to successfully differentiate parathyroid carcinomas, especially those with clinically aggressive behavior, from benign tumors (Abbona *et al.* 1995). Eighty-five percent of specimen diagnosed as atypical adenomas has a Ki-67 proliferative index <5% (100/117). Studies on atypical adenomas have shown a variable rate of Ki-67 overexpression ranging from 0 to 67% (Stojadinovic *et al.* 2003, Ozolins *et al.* 2016, Hosny Mohammed *et al.* 2017) (Table 3). Ozolins and coworkers found that a high Ki-67 expression was of diagnostic value for parathyroid carcinoma, being detected in 87% of cases compared with 0% in both benign and atypical adenomas (Ozolins *et al.* 2016). Similarly, two other studies found an almost exclusive staining in carcinomas (60 and 27%), being absent in atypical adenomas and only present in 6 and 2% of benign adenomas (Stojadinovic *et al.* 2003, Fernandez-Ranvier *et al.* 2009). A higher expression of Ki-67 in carcinomas (mean value 6.7%, up to 70%) as compared to atypical and benign adenomas (mean values 0.3%, up to 2% and mean value 0.4%, up to 28%) was also detected by Kumari and coworkers (Kumari *et al.* 2016). Other authors did not find any utility of Ki-67 staining to distinguish carcinoma from atypical adenoma (Quinn *et al.* 2015, Hosny Mohammed *et al.* 2017). In this regard, one study unexpectedly found low Ki-67 expression both in carcinomas and atypical adenomas (100 and 83%, respectively) (Karaarslan *et al.* 2015) (Table 3). When Ki-67 overexpression was combined with galectin-3 overexpression and/or parafibromin loss (see below), the sensitivity and specificity in the differential diagnosis greatly increased (Hosny Mohammed *et al.* 2017). In summary, the proliferation marker Ki-67 is mainly overexpressed in malignant tumors and appears of limited diagnostic value to distinguish atypical adenomas from carcinomas.

Table 4 Comparison of histopathological features between atypical parathyroid adenomas, carcinomas and adenomas among different studies.

Authors	Number of cases	Trabecular growth pattern	Thick fibrous band	Cystic/hemorrhagic areas	Mitotic activity (rate of >1 per 10 hpf)	Nuclear atypia	Cellular pleomorphism	Pseudocapsular invasion
Sungu <i>et al.</i> (2018)	AA = 14 PC = 10		64% 100%		29% 50%	29% 40%		0% 70%
Ryhänen <i>et al.</i> (2017)	AA = 28 PC = 32		93% ^a 88% ^a	44% 31%	9% (3.6%) ^a 25% ^a	5% ^b 6% ^b		
Kumari <i>et al.</i> (2016)	PA = 72 AA = 19 PC = 14		3% ^a 53% ^a 93% ^a	53%	1% ^a 26% ^a 43% ^a	0% ^b 47% ^a 100% ^a		32% ^a 86% ^a
Quinn <i>et al.</i> (2015)	PA = 194 AA = 34 PC = 18		0% ^a 79% 94%		7% ^a	100% ^a 85% 89%	44% 56%	0% ^a 56% ^a 100% ^a
Juhlin <i>et al.</i> (2010)	AA = 5 PC = 1		60% 100%			40% 0%		80% 0%
Ippolito <i>et al.</i> (2007)	AA = 16 PC = 11	20% 100%	81% 82%		25% 73%	37.5% 82%		0% 19%
Fernandez-Ranvier <i>et al.</i> (2007)	AA = 7 PC = 26	91% 29%	71% 69%		29% 65%	14% 54%		9% 50% ^{cd}
Stojadinovic <i>et al.</i> (2003)	AA = 8 PC = 16 PA = 45	75% ^{ac} 94% ^{ac} 0% ^{ac}	100% ^{ac} 100% ^{ac} 9% ^{ac}	100% ^{ac} 0% ^{ac} 56% ^{ac}	38% ^{ac} 81% ^{ac} 7% ^{ac}	12% ^{ac} 75% ^{ac} 2% ^{ac}		6% ^{cd} 2% ^{cd}

^a $P < 0.001$; ^b $P = 0.003$; ^cComparison refers to adenoma (typical and atypical) versus carcinoma. ^d $P < 0.002$.

Parafibromin

Parafibromin is a promising molecular marker for diagnosing parathyroid carcinoma, also confirmed by the evidence in favor of a strong association of *CDC73* mutations with malignancy (Cetani *et al.* 2018). Different criteria have been used to evaluate parafibromin immunostaining among studies. Our group as well as others have defined as negative the complete or diffuse absence of nuclear parafibromin staining in all neoplastic cells in the presence of an internal positive control represented by the endothelial cells within the parathyroid tumors and/or rim of normal parathyroid tissue surrounding the tumor (Juhlin *et al.* 2006, 2007, 2010, Cetani *et al.* 2007, 2013, Fernandez-Ranvier *et al.* 2009, Guarnieri *et al.* 2012, Sulaiman *et al.* 2012a, Kruijff *et al.* 2014, Karaarslan *et al.* 2015). At variance, other authors have regarded partial or focal loss as sufficient to indicate negative staining, providing a higher diagnostic sensitivity as demonstrated by Hu and coworkers in their meta-analysis (Hu *et al.* 2016).

Nuclear parafibromin loss (diffuse and/or focal) has been shown in up to 100% of parathyroid carcinomas in the majority of the studies, and rarely in benign adenomas (up to 13%) (Juhlin *et al.* 2006, 2010, Cetani *et al.* 2007, Guarnieri *et al.* 2012, Sulaiman *et al.* 2012a, Kruijff *et al.* 2014, Quinn *et al.* 2015, Agarwal *et al.* 2016, Kumari *et al.* 2016, Ozolins *et al.* 2016, Ryhänen *et al.* 2017) and seems to predict the negative clinical outcome (Cetani *et al.* 2007, Witteveen *et al.* 2011) (Table 3). Hu and coworkers conducted a meta-analysis which included ten studies with a total of 202 patients with parathyroid carcinomas. The sensitivity of parafibromin staining ranged from 29 to 100% and specificity from 61 to 100%. The use of different parafibromin antibody ($P < 0.00$) and scoring criteria ($P < 0.05$) accounted for the difference in sensitivity (Hu *et al.* 2016).

A significant heterogeneity of the loss of parafibromin staining ranging from 0 to 50% has been observed in atypical parathyroid adenomas across studies (Table 3). Of note, two studies reported a positive parafibromin expression either in atypical or benign adenomas, and loss of staining was observed in 100 and 31% of parathyroid carcinomas, respectively, suggesting that atypical adenomas might be considered as benign lesions (Fernandez-Ranvier *et al.* 2009, Karaarslan *et al.* 2015). In line with these results, Kruijff and coworkers found that atypical adenomas mostly had a positive staining of parafibromin (61%). These authors also showed an apparent cure of the disease in these cases and recurrence

(10%) in those with loss of parafibromin staining (Kruijff *et al.* 2014). On the contrary, Quinn and coworkers and Ryhanen and coworkers did not detect difference in the loss of parafibromin between carcinomas and atypical adenomas (38 vs 21%, $P=0.34$ and 13 vs 4%, $P=0.34$, respectively) (Quinn *et al.* 2015, Ryhnen *et al.* 2017). In the meta-analysis of Hu and coworkers, 94 patients with atypical parathyroid adenomas were included in six surveys. Meta-regression analysis showed that the inclusion of atypical adenomas in the control group, represented by parathyroid adenomas and hyperplasias, influenced the specificity of parafibromin staining. Indeed, the specificity of parafibromin staining decreased to 63% if only atypical adenomas were used as control, while it increased to 95% if other benign lesions were also included (Hu *et al.* 2016).

Recently, Gill and coworkers reported the results of parafibromin immunohistochemistry in 815 parathyroid tumors of patients undergoing surgery for PHPT during the 12-year period of the study (Gill *et al.* 2019). The following indications for immunohistochemistry were used: (i) suspected or confirmed parathyroid carcinoma; (ii) atypical parathyroid adenomas; (iii) multiglandular PHPT; (iv) recurrent parathyroid tumors; (v) PHPT at young age; (vi) familial PHPT with the exclusion of genetic-established syndromic form; (vii) PHPT with severe hypercalcemia; (viii) large size of parathyroid tumors and (ix) operative suspicion of malignancy. They found a complete loss of parafibromin expression in 5.2% of these tumors, compared to 0.19% in truly unselected parathyroid tumors included in the study. The authors pointed out that these parafibromin-negative tumors have a distinctive morphology including a sheet-like growth pattern often interrupted by an arborizing vasculature, eosinophilic rather than oxyphilic cytoplasm, nuclear enlargement, speckled chromatin with prominent nucleoli, but relatively preserved nuclei-to-cytoplasm ratio and a characteristic perinuclear cytoplasmic clearing. The authors proposed to consider the parafibromin-negative tumors not fulfilling WHO criteria for malignancy (i.e. adenomas and atypical adenomas) a distinct subtype of parathyroid neoplasms that requires a long-term follow-up because of the risk, although low, of metachronous disease and aggressive behavior (Gill *et al.* 2019).

Of special note, in addition to its well-known nuclear localization, parafibromin also localizes to the nucleolar compartment. Juhlin and coworkers investigated the expression of nucleolar parafibromin in 16 atypical adenomas and 23 carcinomas. They found that one (6%) of atypical adenomas and three (13%) of the carcinomas,

which expressed nuclear parafibromin in all or subsets of the tumor cells, did not display the protein at nucleolar level (Juhlin *et al.* 2011). Remarkably, all three carcinomas but not the atypical adenoma harbored *CDC73* mutations which predicted the loss of the three nucleolar localization signals of parafibromin. The authors suggested that the search for nucleolar staining might increase the sensitivity for the detection of carcinoma and atypical adenoma in cases positive to nuclear parafibromin, compared to the search for nuclear parafibromin alone. Very recently, the same authors published an additional study on nucleolar parafibromin immunostaining (Juhlin *et al.* 2019). Three of 46 (6.5%) atypical adenomas displayed loss of nucleolar parafibromin expression, confirming the previous results obtained in a smaller cohort of atypical adenomas (Juhlin *et al.* 2011). The lack of *CDC73* mutational status of samples with negative nucleolar staining raise the question of whether this staining pattern might be of relevance in the clinical practice. Indeed, the authors have acknowledged that the significance of the presence or loss of nucleolar expression is currently of uncertain significance and no other researchers have investigated the nucleolar localization of parafibromin.

Adenomatous polyposis coli

Adenomatous polyposis coli (APC) is a ubiquitously expressed tumor suppressor protein that acts as an antagonist of the Wnt signaling pathway, regulating β -catenin concentrations and interacting with E-cadherin. APC is uniformly expressed in normal parathyroid tissue and adenomas, and loss of APC expression has been observed in up to 100% of carcinomas (Juhlin *et al.* 2006, 2010). Loss of APC in atypical adenomas ranges between 23% and 67% of cases among the studies (Table 3). Its role as a tool for help differentiating parathyroid neoplasms is of limited value because of the overlap in the loss of APC expression between benign and malignant lesions (Agarwal *et al.* 2016, Kumari *et al.* 2016).

Galectin-3

Galectin-3 is a member of the lectin family and is involved in regulatory functions critical in cancer such as apoptosis, angiogenesis, metastasis, immune surveillance, gene expression and inflammation. Galectin-3 overexpression seems to be related to the ability of the tumor to evade apoptosis. Galectin-3 was firstly studied in parathyroid tumors by Bergero and coworkers who found a diffuse overexpression in >90% (24/26) of parathyroid carcinomas

(metastatic or not) and in only 3% (1/30) of adenomas, highly suggesting that galectin-3 could be a marker in the differential diagnosis between benign and malignant parathyroid tumors (Bergero *et al.* 2005).

Galectin-3 expression has been evaluated in 56 atypical parathyroid adenomas either alone or combined with other markers (Table 3). Galectin-3 overexpression (range 32–100%) was found at a rate similar to that observed in parathyroid carcinoma, indicating that this marker, when used alone, does not help to differentiate these two categories of parathyroid neoplasms (Table 3). In contrast, galectin-3 overexpression is rarely found in benign parathyroid adenoma (Kumari *et al.* 2016). Of note, Karaaslan and coworkers found a significant difference in the percentage of diffuse staining (>30% extent of staining) of galectin-3 between carcinomas, atypical adenomas and adenomas, but when considering the presence of focal positivity ($\leq 30\%$ extent of staining) alone, the distinction between the three groups was no more seen (Karaaslan *et al.* 2015).

Protein gene product 9.5 (PGP9.5)

PGP9.5 is the product of ubiquitin carboxyl-terminal esterase L1 (*UCHL1*) gene. Its expression is highly specific to neurons and cells of the neuroendocrine system and their tumors. PGP9.5 is also expressed during embryonic development of the parathyroid glands in the rat. Marked overexpression has been reported in some cancers (non-small-cell lung, colon-rectal, esophageal, gastric, prostate and hepatocellular cancers) and has been associated with advanced staging. PGP9.5 overexpression has been found as a statistically significant classifier for carcinomas and HPT-JT-associated tumors (Haven *et al.* 2004, Howell *et al.* 2009). PGP9.5 overexpression in atypical parathyroid adenomas ranges between 22 and 35%, whereas in carcinomas between 35 and 71% (Howell *et al.* 2009, Kruijff *et al.* 2014, Truran *et al.* 2014, Agarwal *et al.* 2016, Kumari *et al.* 2016). Conversely, in benign adenomas PGP9.5 overexpression ranges from 0 and 22% (Howell *et al.* 2009, Truran *et al.* 2014, Agarwal *et al.* 2016, Kumari *et al.* 2016) (Table 3). PGP9.5 overexpression was the strongest predictor for recurrent hypercalcemia after the first operation either in atypical adenomas or carcinomas ($P=0.003$) (Kruijff *et al.* 2014).

Panels of immunohistochemical markers

In addition to studies evaluating the expression of single immunohistochemical markers, the pathological

evaluation of parathyroid lesions may benefit from the use of a combination of multiple markers (Table 3). As a matter of fact, a panel of markers may better characterize the complexity of the molecular alterations in the context of the same neoplastic lesion.

Stojadinovic and coworkers firstly used a panel of markers focused on proteins involved in cell-cycle regulation and affecting cell proliferation (P53, mdm2, P21, P27, cyclin D1, Ki-67 and BCL2) in a cohort of 8 atypical adenomas, 20 carcinomas and 45 benign adenomas (Stojadinovic *et al.* 2003). The authors found that P53 was indiscriminately negative across the different categories of tumors (Stojadinovic *et al.* 2003). Conversely, previous studies detected P53 overexpression in up to 52% of benign parathyroid adenomas and 22% of carcinomas (Cryns *et al.* 1994, Kishikawa *et al.* 1999, Stojadinovic *et al.* 2003). Therefore, excluding P53 from the panel, they found that atypical parathyroid adenomas had mostly overlapping molecular profiles, either with the benign counterpart or carcinoma. The phenotype P27(+) BCL-2(+) Ki-67(–) MDM2(+) was typical of non-malignant tumors being present in adenomas (76% in typical and 29% atypical adenomas) but absent in carcinomas, suggesting that this profile might be useful to distinguish benign from malignant neoplasms. Nonetheless, no distinctive molecular profile was exclusively associated with parathyroid atypical adenomas (Stojadinovic *et al.* 2003).

Expression of parafibromin, RB, galectin-3, Ki-67, P27 and MDM2 was investigated in parathyroid carcinomas, atypical adenomas and benign neoplasms with a tissue microarray technique (Fernandez-Ranvier *et al.* 2009). The loss of parafibromin and RB expression, associated with galectin-3 and Ki-67 overexpression, could differentiate parathyroid carcinoma from other parathyroid tumors, including atypical adenomas, which displayed a profile of benign tumors. Conversely, the expression of both P27 and MDM2 proteins was heterogeneous among the different categories. Of note, this study included only two cases of atypical adenomas and therefore no conclusions on the utility of these immunohistochemical panels can be drawn (Fernandez-Ranvier *et al.* 2009).

Juhlin and coworkers showed that loss of APC expression associated with a reduced expression of parafibromin was common in atypical or malignant tumors (Juhlin *et al.* 2010). Loss of parafibromin but not of APC was also found in a few benign adenomas, suggesting that APC seems to be preferred to parafibromin in the differential diagnosis of malignant tumors for its higher specificity.

A panel consisting of parafibromin, APC, galectin-3 and PGP9.5 was investigated in two studies (Agarwal *et al.* 2016, Kumari *et al.* 2016). An intermediate level of immunostaining for each single marker was detected in atypical adenomas compared to carcinomas and adenomas (Table 3). No association was found in the various combined immunoprofile between atypical and benign adenomas in a subgroup of specimen selected for having a gland size >10g (Agarwal *et al.* 2016). However, Agarwal and coworkers showed that parafibromin loss segregated with atypical parathyroid adenomas, suggesting that such tumors might be prone to malignant progression (Agarwal *et al.* 2016). Conversely, Kumari and coworkers reaffirmed the importance of histology as a diagnostic tool for predicting a malignant behavior of parathyroid tumors. They suggested that the combination of positive staining for parafibromin and negative staining for PGP9.5 and/or galectin-3 could be associated with a positive outcome in atypical parathyroid adenoma and carcinoma, and proposed to consider them as a benign neoplasm or a cancer with low-risk of recurrence, respectively (Kumari *et al.* 2016).

Karaaslan and coworkers evaluated the immunostaining of parafibromin, galectin-3, Ki-67 and Hector Battifora mesothelial cell-1 (HBME-1), a common molecular marker of tumors mostly used as a diagnostic and prognostic tool for thyroid carcinoma. The latter marker was not informative, whereas the finding of retained parafibromin expression associated with galectin-3 negative or weak positive immunostaining and a Ki-67 proliferation index <1% was the signature of benign adenoma, but not of carcinoma and atypical adenoma (Karaaslan *et al.* 2015).

Additional immunohistochemical markers

Calcium-sensing receptor (CASR) has been evaluated in 14 atypical adenomas in comparison with 21 carcinomas and 10 adenomas (Sungu *et al.* 2018). A global loss of CASR was detected in 7% of atypical adenomas and 50% of carcinoma and in any adenomas, suggesting that CASR might be of help in distinguishing carcinomas from adenomas (either atypical or benign adenoma) (Sungu *et al.* 2018). In line with these results, loss of CASR protein was previously reported in about 30% of sporadic parathyroid carcinomas and was associated with a worse prognosis of the patients (Haven *et al.* 2004, Witteveen *et al.* 2011).

Overexpression of cyclin D1 has been reported in about 65–90% of parathyroid carcinomas and in 18–40% of benign adenomas (Brewer *et al.* 2019). Conversely, its overexpression in atypical adenomas ranges between 13 and 71% (Stojadinovic *et al.* 2003, Cetani *et al.* 2007, Sungu *et al.* 2018). The variability of results among the different categories of parathyroid tumors suggests that this marker has a limited diagnostic role (Table 3).

A strong membranous staining of E-cadherin has been detected in benign parathyroid tumors, and its loss has a key role in the tumorigenesis of parathyroid carcinomas and other malignancies (Fendrich *et al.* 2009). Membranous distribution pattern of E-cadherin was found in 14/16 (87.5%) and a mixed membranous and cytoplasmatic distribution in 2/16 atypical adenomas (Schneider *et al.* 2015). During the follow-up (median 30 months), all but one patients were cured.

Estrogen receptor (ER) signaling is associated with tumorigenesis of different tissues, where loss of ERB1 is associated with a worse prognosis. No statistical difference in the intensity of nuclear estrogen receptor β type 1 (ERB1) and 2 (ERB2) staining has been detected in atypical adenomas ($n=7$) compared with carcinomas ($n=6$), benign adenomas ($n=161$), normal parathyroid gland and tumor rims (Haglund *et al.* 2015). An inverse correlation between ERB1 expression level and tumor weight was found.

No difference in the expression level of programmed death-ligand 1 (PD-L1), a transmembrane protein expressed in several normal tissues and immune cells and involved in the interaction of tumor cells with host immune response, was observed between atypical adenomas and carcinomas (Silva-Figueroa *et al.* 2018). In this study, the authors also investigated the tumor-infiltrating lymphocytes (CD3⁺ and CD8⁺) and macrophages (CD68⁺). Atypical adenomas displayed a significant greater expression of CD3 compared with carcinomas, whereas there was no difference in the densities of CD8 and CD68 between groups, although parathyroid carcinomas showed a greater expression of CD68 compared with atypical adenomas, suggesting that the macrophage inflammatory process might be a potential contributor to disease aggressiveness. The authors concluded that CD3⁺ might represent a novel promising biomarker useful to differentiate atypical adenomas from carcinomas.

An accumulation of alpha-smooth muscle actin (α -SMA), a marker of activated fibroblasts, was observed in fibrous bands and capsule in atypical adenomas ($n=5$) and carcinomas ($n=4$) but also in the

parenchyma of adenomas and normal parathyroid glands (Verdelli *et al.* 2015).

Telomerase activation is implicated in human cancer progression and shown to represent a specific feature of parathyroid carcinoma but not of parathyroid adenoma (Onoda *et al.* 2004). Osawa and coworkers investigated the nuclear staining of human telomerase reverse transcriptase (hTERT), the catalytic subunit bearing the enzymatic activity of telomerase, in different parathyroid neoplasms (Osawa *et al.* 2009). They found that one typical parathyroid adenoma showed a positive staining either in the primary tumor or in two recurrent parathyroid tissues of the same patient whereas no staining was observed in benign adenomas and four normal parathyroid glands. Conversely, all six carcinomas had positive nuclear staining.

A diffuse nuclear expression of glial cells missing 2 (GCM2), a transcription factor exclusively expressed in the parathyroid gland, was shown in normal parathyroid glands and 58 benign and malignant tumors, including two atypical parathyroid adenomas, without difference among parathyroid lesions (Nonaka 2011).

Alterations of some proteins involved in the Wnt/ β -catenin signaling may lead to nuclear accumulation of β -catenin and activation of gene transcription. Nuclear accumulation of active unphosphorylated β -catenin has been reported by some authors in parathyroid carcinoma and parathyroid adenoma (Björklund *et al.* 2008, Svedlund *et al.* 2010). We studied the staining of nuclear β -catenin in three atypical adenomas, 18 carcinomas and 63 adenomas (Cetani *et al.* 2010). In agreement with other authors, all tumors were immune negative, suggesting that β -catenin immunostaining is not useful in the differential diagnosis of these neoplasms (Cetani *et al.* 2010).

Clinical profile

The presenting symptoms and signs include symptomatic nephrolithiasis, fatigue, bone pain, brown tumor and depression. Notably, none of these signs are specific for patients with atypical parathyroid adenoma. Renal manifestations (nephrocalcinosis, nephrolithiasis and kidney failure) occur in 29% of patients, bone involvement (osteitis fibrosa cystica, osteoporosis and pathological fractures) in 18% and both manifestations in 3% of cases. On physical examination, 15% of patients with atypical adenomas have a palpable neck lesion (Table 1 and Supplementary Table 1, see section on [supplementary data](#) given at the end of this article), but a higher rate (32%)

has been reported in the cohort of Quinn *et al.* (2015). Yener and coworkers described the unique patient with atypical adenoma presenting with spontaneous tendon rupture, a rare presentation of PHPT (Yener *et al.* 2014).

The biochemical profile of patients with atypical parathyroid adenoma is more similar to that of patients with carcinoma than classic adenoma and is characterized by moderate hypercalcemia (median 12.9 mg/dL) associated with elevated PTH levels (mean values 12 times above the upper value of normal range) (Table 1).

Most parathyroid tumors are solid, but a few of them can be cystic or partly cystic. In a cohort of 907 patients with benign and atypical parathyroid adenomas, Hu and coworkers found an incidence of 4% of cystic parathyroid adenomas, based on ultrasound or pathology (Hu *et al.* 2018). The authors defined parathyroid lesion as cystic when the cystic areas occupied more than 50% of the estimated volume of the parathyroid gland. Interestingly, atypical adenomas ($n=56$) were more common among cystic rather than solid lesions (9/37, 24.3% vs 47/870, 5.4%, $P=0.001$) (Hu *et al.* 2018). The overall data of the literature reported a frequency of 30% of cystic atypical parathyroid adenomas based on imaging and/or pathology, 86% of them were found at histology (Table 1 and Supplementary Table 1).

At surgery, a firm and/or adherent lesion to the neighboring tissues, which can raise the suspicion of malignancy, may be found in up to 74% of the patients (Schneider *et al.* 2015) (Supplementary Table 1). Taking into account all studies evaluating the presence of adherence, it has been described in 43% of cases (Table 1). Christakis and coworkers, in a series of 23 atypical adenomas and 31 carcinomas, reported a higher rate of pre-operative (clinical, biochemical and imaging) or intraoperative features of malignancy in the latter compared to the former ($P=0.023$ and $P=0.006$, respectively). The presence of adherence to adjacent structures was the best intraoperative predictor of carcinoma ($P=0.033$) (Christakis *et al.* 2016).

About one quarter of patients with the histological diagnosis of atypical parathyroid adenoma undergo *en bloc* resection mostly based on operative findings (macroscopic aspect of the tumor and/or presence of adherence to adjacent structures). The remaining patients undergo bilateral exploration and/or minimally invasive parathyroidectomy (PTx). Ectopic parathyroid adenomas account for 4–22% in different case series (Phitayakorn & McHenry 2006, Roy *et al.* 2013). Of note, five were at ectopic sites (1.8%), mainly in the anterior mediastinum (Table 1) (Pal *et al.* 2018).

The median size and weight of atypical parathyroid adenomas are 2.5 cm (range 0.7–7.2 cm) and 4.15 g (range 0.3–101 g), respectively (Table 1). Agarwal and coworkers found that atypical adenomas were more common in tumors weighing ≥ 7 g (20.4%) than in those < 7 g (6.9%) and that the incidence of atypia reached 33% in tumors ≥ 10 g (Agarwal *et al.* 2016). Similarly, O'Neal and coworkers found that the rate of atypical parathyroid adenomas was significantly higher than that of carcinomas among tumors weighting ≥ 2 g (17.5 vs 1.3%, $P < 0.05$) (O'Neal *et al.* 2011). Nevertheless, one study found a higher rate of large tumor, based on diameters measured by pre-operative neck ultrasound, in the groups of atypical parathyroid adenoma and carcinoma than in classical adenoma group ($P < 0.001$) (Cakir *et al.* 2016).

Despite the more severe clinical and biochemical profile of patients with atypical adenomas, compared with that with benign disease, most patients (96%) are cured with PTx. However, a note of caution should be taken into account since the median follow-up is rather short (median 47 (0.25–252) months) (Table 1).

The overall rate of recurrence of atypical parathyroid adenomas is 3% and is higher in familial than in sporadic cases (40 vs 2%, $P < 0.0001$) (Table 1). The interval between the initial PTx and the first recurrence of tumor ranged from 12 months to 17 years (mean 101 months) (Supplementary Table 1).

Survival data derived from longitudinal retrospective studies at single institutions found an overall survival up to 93% after a follow-up of 5 and 10 years (Christakis *et al.* 2016, Ryhnen *et al.* 2017). However, all deaths were unrelated to the disease.

Impact of molecular and immunohistochemical profile on outcome

Few studies have reported data on the impact of molecular profile on clinical outcome of patients with atypical adenomas so far (Table 2). These studies were mainly focused on *CDC73* mutations. Of note, 42% of patients carrying germline *CDC73* had recurrence and/or persistence of disease after a median and mean follow-up of 120 (range 23–252) and 110 ± 71 months, respectively (Table 2). The only patient carrying a somatic *CDC73* mutation was cured after a 120-month follow-up. Ninety-five percent of *CDC73* mutation-negative patients were cured by surgery.

The impact of immunohistochemical markers on the outcome of patients having an atypical parathyroid adenoma has also been evaluated in few studies with discrepant results. As to whether parafibromin staining may predict the recurrence risk of atypical adenomas and therefore classify the tumors as having low or high malignant potential has been one of the main aims of the studies. One of the first study addressing this issue was published in 2013 and included 54 atypical adenomas (Kruijff *et al.* 2014). The authors were able of demonstrating that patients with parafibromin-positive tumors had no risk of recurrence whereas the only two cases (10%) with recurrence had loss of parafibromin expression. The authors suggested that patients with atypical adenomas with retained parafibromin expression should be managed as tumor having a benign behavior, whereas those with loss of parafibromin expression have a real, even if low, risk of recurrence, and should be classified as tumors of low malignant potential (Kruijff *et al.* 2014). At variance with these results, Agarwal and coworkers found loss of parafibromin staining in 8/22 (36%) patients with large atypical parathyroid adenomas (≥ 7 g) and none had recurrence of the disease after a median follow-up of 40 months (Agarwal *et al.* 2016). All patients whose tumors were parafibromin positive were also cured by PTx.

When we considered the overall studies on parafibromin immunostaining that included the outcome of the patients only seven were selected. Data on parafibromin staining are available in 130 patients with atypical parathyroid adenoma with a follow-up ranging between 1 and 210 months. Loss of parafibromin expression was observed in 40 (31%) cases and the recurrence rate did not differ between patients with retained or lost parafibromin expression (4 vs 12.5%, respectively; $P = 0.135$) (Cetani *et al.* 2007, Fernandez-Ranvier *et al.* 2009, Guarnieri *et al.* 2012, Kruijff *et al.* 2014, Agarwal *et al.* 2016, Kumari *et al.* 2016, Ryhnen *et al.* 2017).

Additional clinical considerations and surveillance

The diagnosis of atypical parathyroid adenomas should always be performed by an expert parathyroid pathologist because of the challenge in distinguishing parathyroid atypical adenoma from carcinoma. In our opinion, the histological slides should always be reviewed when

the initial diagnosis was made by an inexperienced parathyroid pathologist. Once the histopathological diagnosis has been confirmed, parafibromin immunohistochemistry should be carried out, as suggested by other authors (Cardoso *et al.* 2017, Van Der Tuin *et al.* 2017), together with the germline testing of *CDC73* gene. Conversely, Gill and coworkers suggested that for atypical adenomas without concerning clinical features of family history the genetic screening should only be performed in patients with parafibromin loss (Gill *et al.* 2019).

When a *CDC73* germline mutation is identified, the genetic testing should be offered to first-degree relatives, even if asymptomatic, to identify mutation carriers who are at high risk of developing HPT-JT-associated tumors. At the same time, the identification of relatives not carrying the *CDC73* mutation would allow reassuring them from the anxiety of developing the disease and prevent unnecessary screening.

No specific guidelines for the surveillance of patients with atypical parathyroid adenomas after parathyroid surgery exist so far. Some authors, based on their own experience, suggest a close follow-up for the risk, although low, of recurrence, regardless of the results of immunostaining (Kumari *et al.* 2016, Hu *et al.* 2018). Conversely, others advise a close follow-up only in patients with atypical tumors of large size and/or complete loss of parafibromin expression (Kruijff *et al.* 2014, Agarwal *et al.* 2016, Ryhänen *et al.* 2017, Gill *et al.* 2019).

At our institution, regardless of the results of parafibromin immunostaining, we recommend annual follow-up evaluations including biochemical testing and neck ultrasound for the first 5 years after initial surgery and every 2–3 years thereafter. An appropriate screening for *CDC73*-related tumors is also recommended in patients carrying germline *CDC73* mutations.

Conclusions

The diagnosis of atypical adenoma is still challenging. On the basis of studies reported in the literature, patients with atypical parathyroid adenoma at diagnosis have a clinical and biochemical profile more severe than the benign counterpart and, to some extent, similar even though less severe, to that of patients with parathyroid carcinoma. The molecular signature of these neoplasms is still unknown and the germline *CDC73* mutations appears to be the most common anomaly in this setting suggesting that these cases might represent HPT-JT variants. This latter finding should prompt

a genetic analysis in first-degree relatives. No specific immunohistochemical signature has currently been identified. Despite the lack of prospective studies the outcome of patients with atypical parathyroid adenomas is benign in the majority of cases.

Future directions

A collaborative approach aimed to collect data on a large number of patients with atypical parathyroid adenoma is required to increase our knowledge on the molecular profile of the disease. A modern approach using whole exome sequencing of somatic and germline DNA samples could lead to the identification of a specific genetic signature of patients who are at risk of recurrence and guide an adequate postoperative monitoring and, at the same time, relieve of the anxiety of a relatively strict monitoring of patients not at risk.

Supplementary data

This is linked to the online version of the paper at <https://doi.org/10.1530/ERC-19-0135>.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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Author contribution statement

Filomena Cetani and Elena Pardi collected all the data from literature, conceived the work and wrote the manuscript. Liborio Torregrossa wrote and supervised the part related to pathological and immunohistochemical features. Claudio Marcocci proofread the manuscript.

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