RESEARCH ARTICLE

Association of Biochemical and Histological Features with Parafibromin, Galectin-3, and PGP9.5 in Parathyroid Neoplasms

Niraj Kumari[1], Nandita Chaudhary[2], Prabhakar Mishra[3], Amit Agarwal[4], Narendra Krishnani[5]

Abstract

Background: Carcinoma in parathyroid is diagnosed when there is recurrence or metastasis or fulfillment of histological criteria. Immunohistochemical (IHC) markers are used to assist in difficult cases. Associations of IHC markers with unfavorable clinical or histological features that predict aggressive behavior of parathyroid neoplasms have not been reported so far. We planned to study the direct association of IHC markers with biochemical and histological features in parathyroid neoplasms.

Materials and methods: IHC for parafibromin (PF), adenosis polyposis coli (APC), galectin-3 (Gal-3), and PGP9.5 was performed and correlated with biochemical and histological features.

Result: PF loss, Gal-3, and PGP9.5 overexpression alone or in combination showed significant association with one or more features like hypercalcemic crisis; low serum vitamin D; raised serum alkaline phosphatase (ALP); diffuse sheet pattern; predominant oncocytic histology; diffuse macronucleoli; thick fibrous bands; and capsular, vascular, and adjacent tissue invasion. The majority of histological features that significantly correlated with the expression or loss of IHC makers is included in the current criteria for diagnosing malignancy in parathyroid neoplasms.

Conclusion: The presence of hypercalcemic crisis and predominant oncocytic histology showed significant association with IHC markers related to parathyroid malignancy. Inclusion of these features in current criteria may make it more comprehensive for predicting malignancy in parathyroid neoplasms, though the search for reliable factors predicting malignancy still continues.

Keywords: Galectin-3, Hypercalcemic crisis, Oncocytic, Parafibromin, Parathyroid carcinoma, PGP9.5.

World Journal of Endocrine Surgery (2019): 10.5005/jp-journals-10002-1246

Introduction

Parathyroid carcinoma is a rare neoplasm accounting for 0.1–5% of all parathyroid neoplasms in patients with primary hyperparathyroidism and shows higher frequency in Asians than in Westerners.[1–6] The clinical signs and symptoms of benign and malignant parathyroid lesions show a significant overlap in the form of marked hypercalcemia and high serum parathyroid hormone levels, and definitive diagnosis of malignancy is based on histological criteria. Absolute diagnosis of parathyroid carcinoma requires documentation of distant or lymph node metastasis; however, malignant potential in parathyroid neoplasms can be predicted based on certain histological features in postoperative specimens as given by Bondeson and Chan et al. Some of these features in the histological criteria are considered absolute for diagnosing carcinoma where even one feature is sufficient to label a parathyroid neoplasm as carcinoma whereas others are minor features where a minimum number of four features are required to predict malignancy.[1,7–9]

There is evidence that though certain histological features have been used to predict malignant behavior in parathyroid neoplasms, these features have not shown consistency with the aggressive biological behavior of malignant neoplasms, as a proportion of histologically benign parathyroid tumors show recurrence or metastasis and histologically malignant tumors behave in a benign fashion without long-term recurrence or metastasis.[10–12] Subsequently, a number of immunohistochemical (IHC) markers either alone or as a panel have been studied to substantiate the histological criteria for predicting the risk of aggressive tumor behavior. These include parafibromin (PF), Gal-3, PGP9.5, APC, Ki-67, cyclin D1, p53, Rb, p27, and fragile histidine triad.[13–17]

[1,2,5]Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

[3]Department of Biostatistics and Health Informatics, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

[4]Department of Surgical Endocrinology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Corresponding Author: Narendra Krishnani, Department of Pathology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India, Phone: +91 522 2494246, e-mail: narendrakrishnani@yahoo.co.in

How to cite this article: Kumari N, Chaudhary N, Mishra P, Agarwal A, Krishnani N. Association of Biochemical and Histological Features with Parafibromin, Galectin-3, and PGP9.5 in Parathyroid Neoplasms. World J Endoc Surg 2019;11(1):6–14.

Source of support: Financial support received from intramural research funding

Conflict of interest: None declared

PF is a member of the Paf1 complex and inhibits cell growth by causing cell-cycle arrest at the G1 phase partly due to the downregulation of cyclin D1 and histone modification. PF is expressed in various normal tissues including the parathyroid gland, adrenal gland, kidneys, pancreas, heart, and skeletal muscle. Complete or partial loss of PF expression has been observed in hyperparathyroidism-jaw tumor (HPT-JT) syndrome and parathyroid, breast, lung, gastric, and colorectal cancers. PF expression has been related to tumor size, pathological

stage, and lymphovascular invasion in breast, colorectal, and gastric cancers.[18–21] Gal-3 is the only member of the galectin family having an antiapoptotic effect which has a role in several biological functions including tumor cell adhesion, proliferation, differentiation, angiogenesis, cancer progression, and metastasis. It is expressed in thyroid, colorectal, breast, gastric, and liver cancers; brain tumors; large cell lymphoma; and melanoma.[19–24] PGP9.5 is a neuron-specific protein, having opposing biological functions, acting both as ubiquitin carboxyl-terminal hydrolase and ligase. It was normally thought to be expressed in neuroendocrine and neural cells; however, it has been expressed in a variety of other normal tissues including mammary epithelial cells, melanocytes, distal renal tubular epithelium, fibroblasts, and germ cells. PGP9.5 has been associated with the progression of the disease stage in lung, colorectal, pancreatic, and mesenchymal tumors.[25–29] Tokumaru et al. showed that inactivation of PGP9.5 via promoter hypermethylation or gene deletion was significantly related to head and neck squamous cell carcinoma and pancreatic carcinoma, suggesting its tumor suppressor action.[25] Its overexpression is known to be strongly associated with parathyroid carcinoma and HPT-JT-related tumors.[12]

The sensitivity and specificity of PF, Gal-3, APC, and PGP9.5 for parathyroid carcinoma have been reported to be high, though it is variable in different studies.[12,13,19,22,30–37] The fact is that the sensitivity and specificity of these IHC markers in parathyroid neoplasms have been assessed with respect to the histological criteria[1–7,9] for diagnosing benign and malignant tumors which itself does not accurately predict the biological behavior of parathyroid carcinoma.[10,11] Whether these IHC markers have any direct association with worrisome histological feature required for diagnosing malignancy is not clearly known, neither has it been well evaluated. Therefore, this study was planned to find out if there is any direct association of PF loss or overexpression of Gal-3 and PGP9.5 with the individual biochemical and histological features in parathyroid neoplasms and whether any feature can independently predict the expression of an individual or a combination of IHC markers.

Materials and Methods

All consecutive parathyroidectomy specimens received in the Department of Pathology at a tertiary care referral center from January 1993 to December 2013 within a period of 21 years were retrieved and clinical, biochemical, and gross findings were recorded from hospital records and case files. All cases were reviewed histologically according to the criteria given by Chan et al. and DeLellis et al. as shown in Table 1.[1,7–9] The cases were categorized into five diagnostic categories—carcinoma, atypical adenoma, adenoma including lipoadenoma, double adenoma, and hyperplasia. The diagnosis of carcinoma or atypical adenoma was done according to the criteria mentioned in Table 1.

High serum calcium was defined as the serum calcium level >11 g/dL (normal range: 9–11 mg/dL) and hypercalcemic crisis as the serum calcium level >14 g/dL. Hypophosphatemia was defined as the serum phosphorus level of <3.0 mg/dL (normal range: 3–5 mg/dL), raised parathormone (PTH) was defined as the serum PTH level of >55 pg/mL (normal range: 9–55 pg/dL), raised alkaline phosphatase (ALP) was defined as the serum ALP levels of >150 IU/L (normal range: 35–150 IU/L), raised creatinine was defined as the serum creatinine level of >1.5 mg/dL (normal range: 0.5–1.5 mg/dL), and low vitamin D levels was defined as the serum vitamin D level of <9.0 ng/mL (normal range: 9–54 ng/dL).

Predominant oncocytic tumors were designated when >75% of tumor cells were oncocytes with large size and granular eosinophilic cytoplasm. This cutoff percentage for predominantly oncocytic tumor was based on previously reported cutoff values for oncocytic histology in thyroid, parathyroid, as well as other neuroendocrine tumors, ranging from 60 to 100%.[38] The subset of oncocytic tumors having >90% oncocytic cells was also analyzed separately. Similarly, a tumor with diffuse macronucleoli was categorized when >75% of tumor cells had prominent nucleolus.

IHC was performed for PF, APC, Gal-3, PGP9.5, and Ki-67. Mouse monoclonal antibodies were used for PF (clone—2H1) and Gal-3 (clone—B2C10) from Santa Cruz at a dilution of 1:20 and 1:50, respectively. Rabbit monoclonal antibody was used for APC (clone—EP701Y, Abcam), whereas rabbit polyclonal antibody was used for PGP9.5 (DAKO) at a dilution of 1:50 each. Ki-67 was used (DAKO, clone-MIB1) at 1:50 dilution. Antigen retrieval for PF, APC, Gal-3, and Ki-67 was done in citrate buffer (pH 6.0) and for PGP9.5 in Tris EDTA (pH 9.0). Primary antibodies were incubated for 1 hour at room temperature followed by secondary antibody (Ultra Vision Quanto Detection System—Thermo Scientific) for 30 minutes. The cutoff for positive expression of different antibodies was based on different studies in the literature.[12,18,19]

Interpretation of Immunohistochemistry Results

PF

- The proportion of positive nuclei out of total tumor nuclei was expressed in percentage. The complete loss was defined as <10% nuclear staining of tumor cells. Any staining more than 10% was taken as positive staining.

Table 1: Histologic criteria for diagnosis of malignancy in parathyroid neoplasms[1,7–9]

Absolute criteria of malignancy[a]	*Features associated with malignancy*[b]
Invasion into surrounding soft tissues	Capsular invasion without extension into surrounding soft tissues
Invasion of surrounding vital structures—thyroid, esophagus, pharynx, larynx, trachea, recurrent laryngeal nerve, and carotid artery	Mitosis >5/10 HPF
Vascular invasion	Broad intratumoral fibrous bands
Perineural invasion	Coagulative tumor necrosis
Histologically documented regional or distant metastasis	Diffuse sheet-like monotonous small cells with high N:C ratio
	Diffuse cellular atypia
	Macronucleoli present in many tumor cells

[a] Presence of any one of the following features qualifies for parathyroid carcinoma
[b] Presence of four or more of these features qualifies for parathyroid carcinoma while one to three of these features qualifies for diagnosis of a typical adenoma

- The rim of normal parathyroid tissue and stromal fibroblasts within the tumor were taken as positive internal controls.

APC

- Complete loss of APC was defined as <10% cytoplasmic immunostaining of any intensity.

Gal-3 and PGP9.5

- Both cytoplasmic staining and nuclear staining were taken as positive.
- Cases with >50% (PGP9.5) and >30% (Gal-3) moderate to strong immunostaining were considered immunoreactive.

Ki-67

- Ki-67 was counted as the number of consecutive positive nuclei in 1,000 cells manually and categorized into two groups with >5% and <5%.

For all antibodies, the intensity was graded as mild, moderate, and severe based on the subjective assessment. IHC results were then correlated with biochemical features, tumor weight, and histological features.

Statistical Analysis

The data were analyzed using statistical package for social sciences (SPSS) software version 20.0. Categorical variables were analyzed by Chi-square (univariate) and binary logistic regression (multivariate) tests. The Fischer exact test was applied to assess the level of significance, wherever the values were 5 or less. A *p* value of <0.05 was considered as statistically significant.

Results

A total of 246 parathyroid resections were received within a period of 21 years. Neoplastic lesions accounted for 227 cases comprising of 194 adenomas including three cases of lipoadenomas, 19 atypical adenomas, and 14 carcinomas. Nineteen cases having involvement of more than one gland (3 cases of double adenoma and 16 cases of hyperplasia) were excluded from the final analysis. Thus, 227 parathyroid neoplasms were included for further workup. Carcinoma showed a higher mean age of 47.4 years (range 30–66 years, median 51.0 years). Atypical adenoma and adenoma had nearly similar age of presentation with a mean of 40.7 years (17–76 years, median 40.0 years) and 41.6 years (12–76 years, median 41.0 years), respectively. The male-to-female ratio was 1.8:1 for carcinoma, 0.9:1 for atypical adenoma, and 0.4:1 for adenoma.

The presenting complaints (bone pain, trivial fractures, recurrent renal stones, pancreatitis, and proximal muscle weakness) and biochemical alterations (raised serum calcium, hypercalcemic crisis, low serum phosphorus, high serum PTH, and low serum vitamin D) were largely similar in all three diagnostic categories. Bony lesions (deformities or cystic tumors) were less in carcinoma than in adenoma and atypical adenoma (*p* value = 0.005), whereas high serum ALP was more common in carcinoma and atypical adenoma (*p* value = 0.04).

PF, Gal-3, and PGP9.5 were available in all cases, whereas APC was available in 192 cases only due to inadequate tissue. Complete loss of PF immunostaining was noted in 7/14 (50%) carcinoma, 6/19 (31.6%) atypical adenoma, and 19/194 (9.8%) adenoma cases. Complete loss of APC was seen in 1/11 (9.1%) carcinoma, 4/17 (23.5%) atypical adenoma, and 36/164 (22%) adenoma cases. Gal-3 was overexpressed in 6/14 (42.8%) carcinoma, 9/19 (47.4%) atypical adenoma, and 19/194 (9.8%) adenoma cases. Similarly, PGP9.5 was overexpressed in 9/14 (64.3%) carcinoma, 6/19 (31.6%) atypical adenomas, and 29/194 (14.9%) adenoma. All IHC markers except APC were significantly associated with carcinoma and atypical adenoma (*p* value = 0.001).

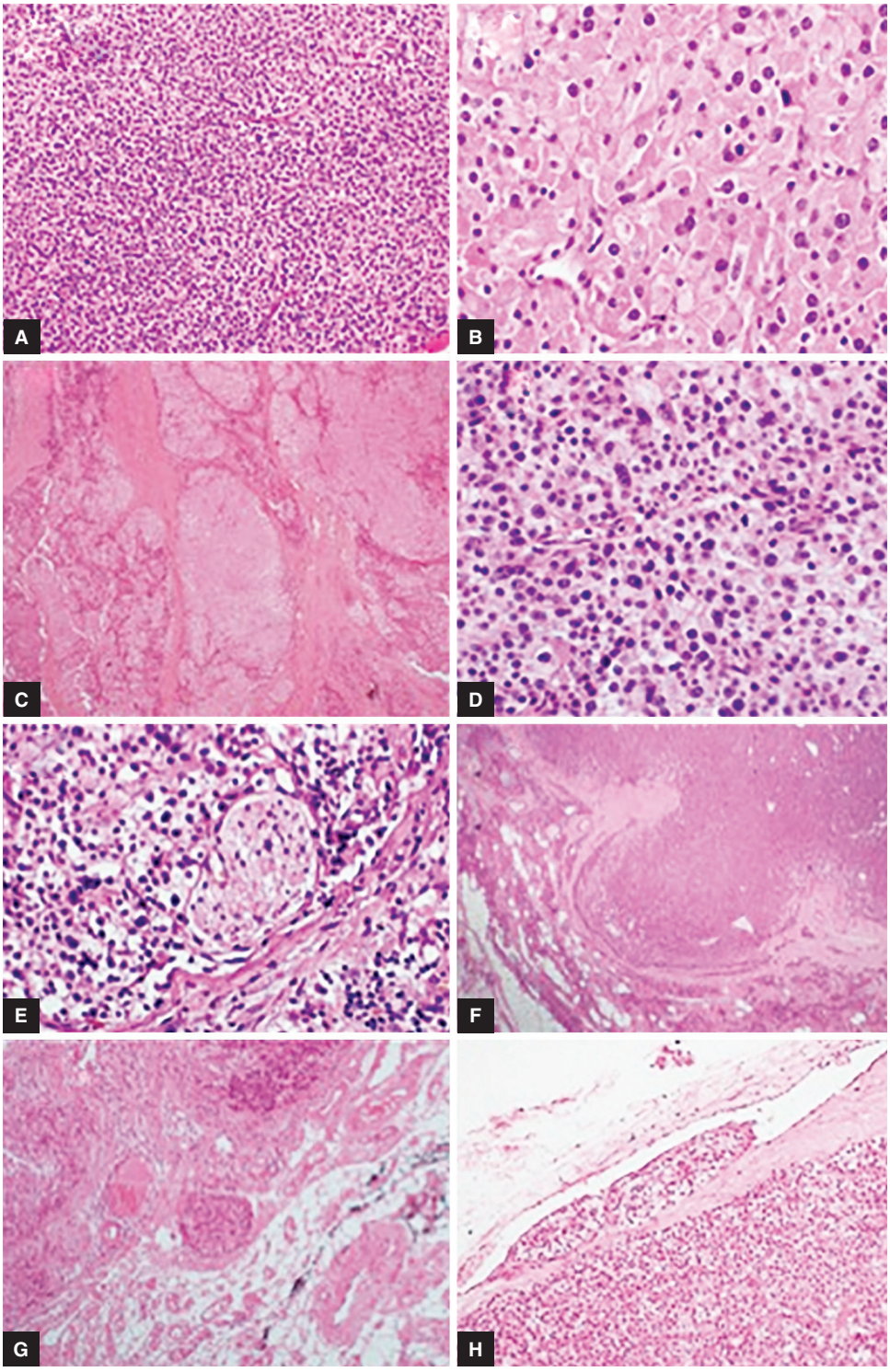
Univariate analysis showed significant association of PF loss with hypercalcemic crisis (*p* value 0.005), low serum phosphorus (*p* value 0.04), and low serum vitamin D (*p* value 0.04). Loss of APC expression was associated only with proximal muscle weakness (*p* value = 0.007). Gal-3 overexpression was associated with bony lesions (*p* value = 0.004) and low serum vitamin D (*p* value = 0.02). PGP9.5 overexpression was seen with high serum calcium (*p* value = 0.01), hypercalcemic crisis (*p* value = 0.001), and raised serum ALP (*p* value = 0.03). The correlation of individual IHC markers with clinical and biochemical features is shown in Table 2. In multivariate analysis, the Gal-3 overexpression was independently associated with bony lesions and deformities (*p* value = 0.001, odds ratio = 7.0, 95% CI = 2.1–22.5), whereas PGP9.5 was associated with hypercalcemic crisis (*p* value = 0.009, OR = 3.9, 95% CI = 1.4–10.9) and raised ALP (*p* value = 0.004, odds ratio = 4.0, 95% CI = 1.0–16.4).

The individual histological features of parathyroid neoplasms were also analyzed with loss of PF and APC and overexpression of Gal-3 and PGP9.5. Loss of PF was significantly associated with predominantly nested and sheet-like cellular arrangement, predominantly chief or oncocytic cell morphology at both cutoff values (75% and 90%), high cellularity, thick fibrous bands with the formation of expansile nodules, >5 mitosis/10 HPF, capsular invasion, vascular invasion, and adjacent tissue invasion on univariate analysis; however, on multivariate analysis only association was seen only with capsular invasion (*p* value = 0.001, odds ratio 21.6, 95% CI—4.8 to 97.7). None of the histological features showed any association with APC loss. Gal-3 overexpression was significantly associated with predominantly sheet-like arrangement, predominantly chief or oncocytic histology at both cutoff values (having either 75%

Table 2: Correlation of biochemical features with PF, APC, Gal-3, and PGP9.5

Biochemical features	*PF loss (%)*	*p value*	*APC loss (%)*	*p value*	*Gal-3 expression (%)*	*p value*	*PGP9.5 expression (%)*	*p value*
High serum calcium	82.8	0.2	81.6	0.1	81.2	0.2	88.1	0.01
Hypercalcemic crisis	31.0	0.005	15.8	0.6	21.9	0.1	31.0	0.001
Low serum phosphorus	82.8	0.04	68.4	0.7	75	0.2	76.2	0.1
High serum PTH	95.2	1.0	94.1	0.5	96.6	1.0	97.0	1.0
Low serum vitamin D	15.8	0.04	37.9	1.0	16	0.02	28.6	0.2
Raised ALP	17.4	0.7	78.0	0.8	85.3	0.1	86.4	0.03
Raised serum creatinine	17.8	0.8	24.3	0.3	12.9	0.4	17.9	0.8

The numbers are percentages as the total numbers available in each group were variable



Figs 1A to H: (A) Diffuse sheet-like pattern with high cellularity (H&E stain, 400× magnification); (B) Predominant oncocytic cells with abundant granular cytoplasm in a case of parathyroid carcinoma (H&E stain, 400× magnification); (C) Thick fibrous band forming expansile nodule in a case of atypical parathyroid adenoma (H&E stain, 100× magnification); (D) Diffuse macronucleoli with mitosis in a case of atypical parathyroid adenoma (H&E stain, 400× magnification); (E) Microphotograph shows a nerve bundle entrapped within the tumor cells (H&E stain, 400× magnification); (F) Microphotograph shows penetration of tumor capsule with tumor cells lying into the adjacent parathyroid parenchyma (H&E stain, 20× magnification); (G) Microphotograph shows tumor nodule outside the parathyroid parenchyma invading the adjacent adipose tissue (H&E stain, 100× magnification); (H) A tumor embolus seen attached to a vessel wall external to the main tumor (H&E stain, 200× magnification)

or 90% oncocytic cells), high cellularity, thick fibrous bands with expansile nodules, macronucleoli in >75% tumor cells, capsular invasion, and adjacent tissue invasion (Figs 1A to D). Multivariate analysis showed that Gal-3 overexpression was independently

associated with capsular invasion (p value < 0.001, odds ratio = 12.2, 95% CI = 3.1–48.2), predominantly oncocytic cell type (p value = 0.004, odds ratio = 7.7, 95% CI = 1.9–31.8), and predominantly sheet-like arrangement (p value = 0.007, odds ratio = 16.7, 95% CI = 2.1–128.5). PGP9.5 overexpression was significantly associated with predominant sheet-like or follicular pattern, predominantly chief or oncocytic cell type, thick fibrous bands with expansile nodules, mitosis >5/10 HPF, capsular invasion, vascular invasion, perineural invasion, and adjacent tissue invasion (Figs 1E to H). Similarly, on multivariate analysis, PGP9.5 overexpression was independently associated with predominantly oncocytic histology with greater or equal to 75% oncocytes (p value < 0.001, odds ratio = 9.0, 95% CI = 3.8–21.2), sheet-like arrangement (p value = 0.002, odds ratio = 8.8, 95% CI = 2.2–34.1), and adjacent tissue invasion (p value = 0.004, odds ratio = 10.4, 95% CI = 2.0–51.9). The correlation of histological features with individual IHC markers is shown in Table 3.

Coagulative tumor necrosis, a feature associated with malignancy, was present only in two cases of atypical adenoma and one case of carcinoma. One case of carcinoma with necrosis showed PF loss and PGP9.5 overexpression. Two cases of atypical adenoma showed Gal-3 overexpression. These findings were indicative of necrosis being a worrisome feature expressing IHC markers of the malignant phenotype.

The combination of PF loss + Gal-3 overexpression, PF loss + PGP9.5 overexpression, and PF loss + PGP9.5 and Gal-3 overexpression also showed significant association with certain biochemical and histological features as mentioned in Tables 4 and 5 (Fig. 2). Loss of PF and Gal-3 overexpression was independently associated with hypercalcemic crisis (p value = 0.005, odds ratio = 44.3, 95% CI = 3.1–627.4), predominantly oncocytic histology (p value < 0.001, odds ratio = 42.8, 95% CI = 8.2–221.6), capsular invasion (p value = 0.002, odds ratio = 44.0, 95% CI = 3.9–489.2), and diffuse macronucleoli (p value = 0.04, odds ratio = 91.3, 95% CI = 1.1–7105.8). Loss of PF and PGP9.5 overexpression was independently associated with predominantly oncocytic histology (p value < 0.001, odds ratio = 65.9, 95%, CI = 12.8–338.4) and adjacent tissue invasion (p value = 0.01, odds ratio = 63.1, 95% CI = 2.2–1810.1). Combination of PF loss with Gal-3 and PGP9.5 expression was independently associated with hypercalcemic crisis (p value = 0.007, odds ratio = 35.9, 95% CI = 2.6–495.9), predominantly oncocytic histology (p value < 0.001, odds ratio = 49.0, 95%, CI = 9.2–259.9), and capsular invasion (p value = 0.002, odds ratio = 50.9, 95% CI = 4.0–640.7).

All tumors with predominant oncocytic cell type using >75% as well as >90% cutoff value (n = 30) were correlated with PF and APC loss and Gal-3 and PGP9.5 expression which included 20 adenomas, 3 atypical adenomas, and 7 carcinomas (5 carcinomas had >90% oncocytic cells). The atypical adenoma and carcinoma using >75% cutoff value showed significant association with PF loss (p value = 0.002) or Gal-3 (p value = 0.002) and PGP9.5 expression (p value = 0.02) either individually or in combination (p value = 0.001). A similar association was also noted for carcinomas having >90% oncocytic cells. Among the seven oncocytic parathyroid carcinomas using >75% cutoff criteria, five cases (71%) showed strong expression of both Gal-3 and PGP9.5 and four of these also showed loss of PF. Of the remaining two cases, one showed positive expression of PGP9.5 alone while the other showed loss of both. The oncocytic carcinoma that showed loss of both Gal-3 and PGP9.5 showed retained nuclear expression of PF; however, this tumor was histologically malignant and was composed of >90% oncocytic cells. The remaining four out of five oncocytic carcinomas using >90% cutoff value showed complete PF loss in all cases, positive expression of both Gal-3 and PGP9.5 in three cases and expression of PGP9.5 alone in the remaining one case. Thus, oncocytic histology was associated with the expression of Gal-3 and PGP9.5 alone or in combination in a similar proportion of carcinomas using >75% as well as >90% cutoff criteria.

The Ki-67 index of >5% was significantly associated with thick fibrous bands, severe nuclear atypia, necrosis, capsular invasion,

Table 3: Correlation of histological features with PF, APC, Gal-3 and PGP9.5

Histological features		*PF loss, n = 32 (%)*	*p value*	*APC loss, n = 41 (%)*	*p value*	*Gal-3 expression, n = 34 (%)*	*p value*	*PGP9.5 expression, n = 44 (%)*	*p value*
Predominant pattern	Nests	17 (53.1)	0.01	29 (70.7)	0.9	24 (70.6)	0.8	34 (77.3)	0.3
	Trabeculae	03 (9.3)	1.0	03 (7.3)	0.7	02 (5.9)	0.7	02 (4.5)	0.3
	Sheets	08 (25)	<0.001	06 (14.6)	0.06	09 (26.4)	<0.001	10 (22.7)	<0.001
	Follicles	06 (18.8)	0.9	04 (9.8)	0.1	02 (5.9)	0.05	01 (2.3)	0.002
Predominant cell type	Chief	16 (50)	<0.001	29 (70.7)	0.2	17 (50)	<0.001	21 (47.7)	<0.001
	Oncocytic	15 (46.9)	<0.001	07 (17.1)	0.5	15 (44.1)	<0.001	20 (45.4)	<0.001
	Clear	01 (3.1)	1.0	01 (2.4)	1.0	0	0.6	0	0.5
High cellularity		07 (21.9)	0.008	04 (9.8)	1.0	07 (20.6)	0.01	07 (15.9)	0.09
Severe nuclear atypia		02 (6.2)	0.09	01 (2.4)	1.0	02 (5.9)	0.1	02 (4.5)	0.1
Thick fibrous bands		11 (34.4)	<0.001	03 (7.3)	0.5	09 (26.4)	0.001	11 (25)	<0.001
Macronucleoli >75%		04 (12.5)	0.05	03 (7.3)	0.6	06 (17.6)	<0.001	04 (9)	0.2
Mitosis >5/10 HPF		02 (6.2)	0.01	01 (2.4)	0.2	01 (2.9)	0.2	02 (4.5)	0.03
Necrosis		02 (6.2)	0.09	0	0.6	02 (5.9)	0.1	02 (4.5)	0.1
Capsular invasion		10 (31.2)	<0.001	03 (7.3)	1.0	09 (26.4)	<0.001	11 (25)	<0.001
Vascular invasion		05 (15.6)	0.002	01 (2.4)	0.6	04 (11.8)	0.06	06 (13.6)	0.002
Perineural invasion		02 (6.2)	0.05	0	1.0	01 (2.9)	0.3	03 (6.8)	0.007
Adjacent tissue invasion		06 (18.8)	<0.001	01 (2.4)	0.6	05 (14.7)	0.004	07 (15.9)	0.001
Lymph node metastasis		01 (3.1)	0.1	NA		0	1.0	01 (2.3)	0.1
Recurrence		01 (3.1)	0.1	0	1.0	01 (2.9)	0.1	01 (2.3)	0.1

Table 4: Correlation of biochemical features with combination of IHC markers

Biochemical and gross features	*PF loss, Gal-3 expression (%)*	*p value*	*PF loss, PGP9.5 expression (%)*	*p value*	*PF loss, Gal-3 + PGP9.5 expression (%)*	*p value*
High serum calcium	90.0	0.2	92.3	0.1	90.0	0.2
Hypercalcemic crisis	60.0	<0.001	53.8	<0.001	60.0	<0.001
Low serum phosphorus	90.0	0.1	84.6	0.1	90.0	0.09
High serum PTH	100.0	1.0	100.0	1.0	100.0	1.0
Low serum vitamin D	12.5	0.1	12.5	0.1	12.5	0.1
Raised ALP	83.3	0.5	86.7	0.3	90.9	0.2
Raised serum creatinine	18.2	1.0	15.4	1.0	10.0	0.6

The numbers are percentages as the total numbers available in each group were variable

Table 5: Correlation of histological features with combination of IHC markers

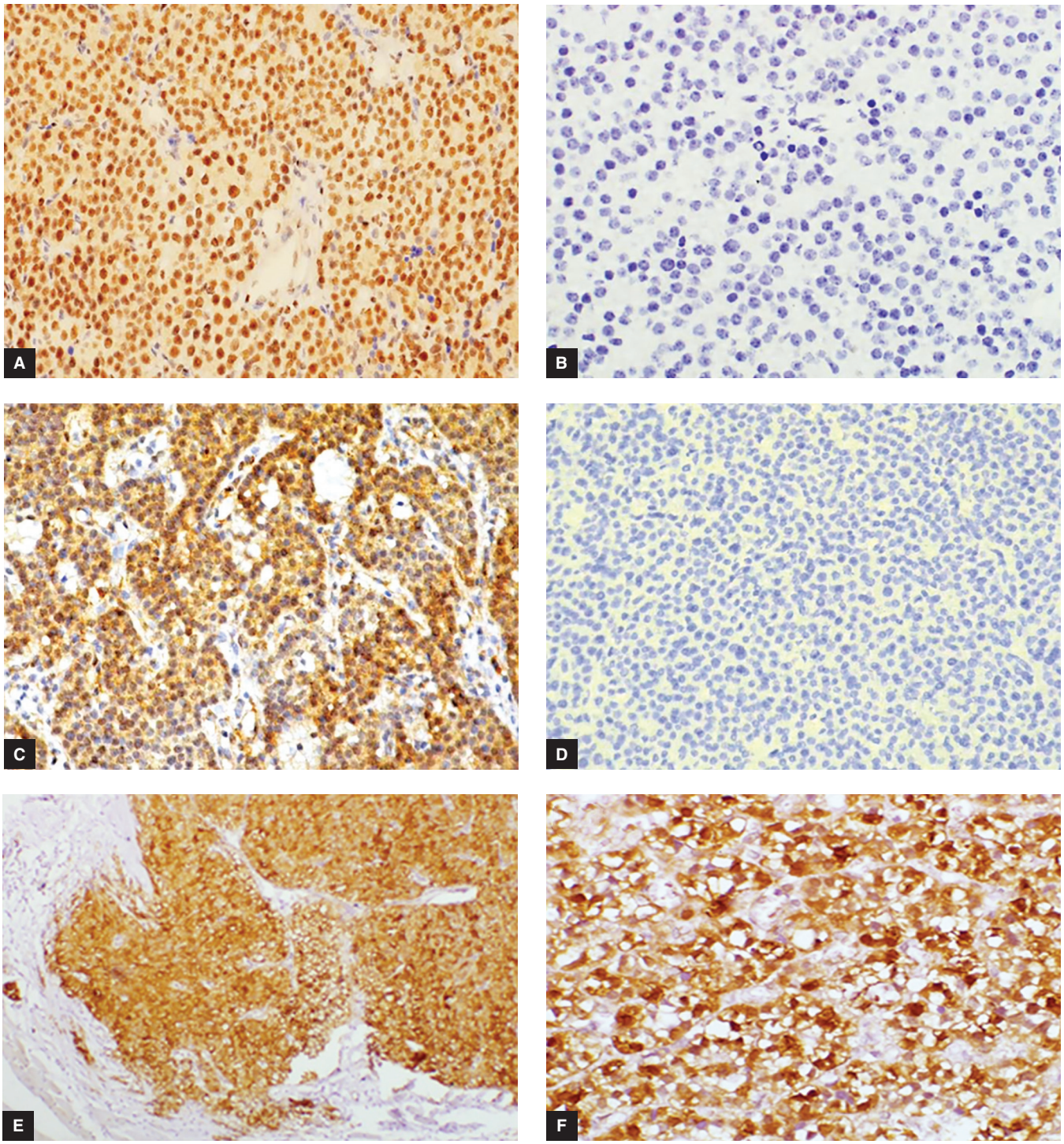
Histological features		*PF loss, Gal-3 expression, n = 12 (%)*	*p value*	*PF loss, PGP9.5 expression, n = 15 (%)*	*p value*	*PF loss, Gal-3 + PGP9.5 expression, n = 11 (%)*	*p value*
Predominant pattern	Nests	07 (58.3)	0.2	09 (60)	0.2	07 (63.6)	0.7
	Trabeculae	01 (8.3)	1.0	01 (6.7)	1.0	01 (9.1)	1.0
	Sheets	05 (41.7)	<0.001	06 (40)	<0.001	04 (36.4)	0.001
	Follicles	0	0.1	0	0.08	0	0.1
Predominant cell type	Chief	02 (16.7)	<0.001	02 (13.3)	<0.001	02 (18.2)	<0.001
	Oncocytic	10 (83.3)	<0.001	12 (80)	<0.001	03 (27.3)	<0.001
	Clear	0	1.0	0	1.0	0	01
High cellularity		03 (25)	0.04	04 (26.7)	0.02	02 (18.2)	0.16
Severe nuclear atypia		01 (8.3)	0.1	01 (6.7)	0.1	0	NA
Thick bands		07 (58.3)	<0.001	08 (53.3)	<0.001	06 (54.5)	<0.001
Macronucleoli >75%		03 (25)	0.006	03 (20)	0.02	02 (18.2)	0.05
Mitosis >5/10 HPF		01 (8.3)	0.06	02 (13.3)	0.006	01 (9)	0.06
Necrosis		01 (8.3)	0.1	02 (13.3)	0.03	01 (9)	0.1
Capsular invasion		07 (58.3)	<0.001	08 (53.3)	<0.001	06 (54.5)	<0.001
Vascular invasion		03 (25)	0.01	04 (26.7)	0.002	02 (18.2)	0.05
Perineural invasion		01 (8.3)	0.1	02 (13.3)	0.006	01 (9)	0.06
Adjacent tissue invasion		05 (41.7)	<0.001	05 (33.3)	<0.001	04 (36.4)	<0.001
Lymph node metastasis		NA		01 (6.7)	0.08	0	NA
Recurrence		01 (8.3)	0.06	01 (6.7)	0.08	01 (9)	0.06

vascular invasion, perineural invasion, adjacent tissue invasion, and lymph node metastasis (*p* value = <0.05).

Discussion

The histological criterion is currently considered as the gold standard for predicting malignant potential in parathyroid tumors; however, malignant parathyroid tumors diagnosed by the histological criteria may behave in a benign fashion.[1,7–11] The gray category of atypical adenoma, again based on the histological criteria, adds to the uncertainty. IHC markers have been used in conjunction with histological features to predict malignant behavior of parathyroid neoplasms with more accuracy. The panel of IHC markers studied in parathyroid tumors to differentiate between benign and malignant lesions has been found to be related to malignancy or disease progression in several other cancers.[20,21,23–29] The questions remain that if histology is not accurate and specific to predict the malignant behavior, how can the sensitivity and specificity of these IHC markers be derived taking histology as the gold standard? Therefore, we evaluated the direct association of IHC markers with individual biochemical and histological features. A previous study from the present authors studied the diagnostic categories of parathyroid neoplasms based solely on histological criteria and correlated the IHC markers with the histological categories; however, no recurrence or metastasis was seen in 33 cases of atypical adenoma and carcinoma except for one case of carcinoma in a follow-up period ranging from 1 to 162 months (mean 25 months).[38,39] Thus, our earlier finding questions the strength of histological criteria being the gold standard.

The sensitivity and specificity of PF loss in parathyroid carcinoma have been reported in the range of 4.5–100% and 89.5–100%, respectively, in different studies.[12,13,19,22,32–37] In the present study, sensitivity, specificity, and predictive accuracies of PF loss for carcinoma were 50%, 90.2%, and 87.5%, respectively, which shows that PF is more helpful in excluding malignancy rather than diagnosing malignancy. Reported sensitivity and specificity of Gal-3 expression in parathyroid carcinoma range between 54.2–93.3% and 73.7–100%, respectively.[12,13,19,22,32–37] Sensitivity, specificity, and predictive accuracies of Gal-3 in PC in the present study were 45.4%, 90.2%, and 83.7%, respectively, again emphasizing its role in excluding malignancy in parathyroid neoplasms. The reported sensitivity, specificity, and predictive accuracies of PGP9.5 expression in parathyroid carcinoma are 33.3–63.6%, 85–100% and 58.9–92% respectively, in different studies.[12,37] Sensitivity,



Figs 2A to F: (A) Intact expression of PF in a case of adenoma (IHC stain, 400× magnification); (B) Loss of expression PF in a case of atypical adenoma (IHC stain, 400× magnification); (C) Intact expression of APC in parathyroid adenoma (IHC stain, 400× magnification); (D) Loss of APC expression in atypical adenoma (IHC stain, 400× magnification); (E) Galectin expression in parathyroid carcinoma (IHC stain, 400× magnification); (F) Expression PGP9.5 in parathyroid carcinoma (IHC stain, 400× magnification)

specificity, and predictive accuracies of PGP9.5 in the present study were 45.4%, 85%, and 79.3%, respectively.

We could not find any literature evaluating the direct association of these IHC markers with clinicopathological features in parathyroid neoplasms. In the present study, IHC expression or loss was correlated with clinicopathological features that showed the presence of certain biochemical (hypercalcemic crisis and raised serum ALP) and histological (sheet-like pattern, predominance of oncocytic cell type (using both >75% and >90% cutoff criteria), capsular invasion, and adjacent tissue invasion) features were independently associated with either one or combination of antibodies. The oncocytic histology in four cases of histological

adenoma and three cases of atypical adenoma showed complete PF loss and overexpression of Gal-3 and PGP9.5 in 60–100% of tumor cells which again raises concern over their malignant potential. Moreover, it is believed that histologically benign or atypical parathyroid neoplasms with loss of PF should be considered as parathyroid neoplasms of low malignant potential, whereas intact PF staining in malignant parathyroid neoplasms indicates low malignant potential.[39–41] Some of the previously mentioned features are included in the histological criteria for diagnosing risk of malignancy as given by Chan et al., DeLellis et al., and Bondeson et al., such as capsular, vascular, and adjacent tissue invasion; thick fibrous bands; diffuse macronucleoli and diffuse sheet like pattern; however, clinical or biochemical features are not a part of histological criteria.[1,7–9] Observations of the present study indicate that predictive accuracy of the above diagnostic criteria can be enhanced by the inclusion of certain biochemical and histological features like hypercalcemic crisis, and predominant oncocytic cell type, as these features also positively correlate with IHC markers for parathyroid carcinoma. The importance of predominantly oncocytic histology can be affirmed by the fact that 7 of the 14 carcinomas in the present study were composed predominantly of oncocytic histology. This feature was significantly associated with carcinoma and also with Gal-3 and PGP9.5 expression irrespective of histological diagnosis. We tried to find out any difference in the association of these IHC markers between oncocytic adenoma and carcinoma and found that the oncocytic carcinomas showed significant association (p value = 0.002) with all the three markers, both singly and in combination, compared to adenoma. However, the oncocytic histology also showed significant association with PGP9.5 overexpression in adenomas. Published studies have shown that oncocytic tumors tend to be non-functional and, hence, are mostly diagnosed at a later stage with larger tumor size and higher tumor weight. In the present study, four cases of oncocytic parathyroid carcinoma using >75% cutoff were functional with raised PTH levels and hypercalcemic crisis was noted in two of these. In the remaining three cases, PTH levels were not available; however, two of these showed hypercalcemia. The number of oncocytic carcinoma in the present study is too and diffuse sheet like pattern less to derive any statistical significance and more studies with a greater number of cases are required to validate our findings.

Therefore, the basis of histological criteria for predicting the risk of malignancy is still debatable and the only proof of malignancy of histologically and/or immunohistochemically diagnosed parathyroid carcinoma is either metastasis or recurrence. This is also reinforced from findings of the present study where only 1 (7%) of 14 histologically diagnosed parathyroid carcinoma in 21 years showed recurrence and rest showed a benign behavior. The question still remains—What are reliable features (clinical, biochemical, histological, or molecular) that can diagnose a parathyroid neoplasm as malignant which actually behave in a malignant fashion?

Conclusion

The present study showed direct association of worrisome histological features (capsular invasion, adjacent tissue invasion and diffuse solid sheet-like pattern) in parathyroid neoplasms with malignant IHC markers like PF loss and overexpression of Gal-3 and PGP9.5. These findings suggest that the current histological criteria for diagnosing malignant behavior in parathyroid neoplasms may be substantiated by hypercalcemic crisis (biochemical), and predominant oncocytic cell type (histological) to formulate a comprehensive criterion for diagnosing malignant potential or aggressive tumor behavior in parathyroid neoplasms. However, there are no gold standard features either clinical, biochemical, or histological in parathyroid neoplasms to predict the risk of malignancy except for distant metastasis or histological recurrence.

References

1. DeLellis RA. Parathyroid tumors and related disorders. Mod Pathol 2011;24:S78–S93. DOI: 10.1038/modpathol.2010.132.
2. Shane E. Clinical review 122. Parathyroid carcinoma. J Clin Endocrinol Metab 2001;86:485–493. DOI: 10.1210/jcem.86.2.7207.
3. Harinarayan CV, Gupta N, et al. Vitamin D status in primary hyperparathyroidism in India. Clin Endocrinol 1995;43:351–358.
4. Agarwal G, Prasad KK, et al. Indian primary hyperpara-thyroidism patients with parathyroid carcinoma do not differ in clinicoinvestigative characteristics from those with benign parathyroid pathology. World J Surg 2006;30:732–742. DOI: 10.1007/s00268-005-0366-5.
5. Soin AS, Gupta S, et al. Primary hyperparathyroidism- an Indian study. Indian J Cancer 1994;31:72–77.
6. Diaconescu MR, Glod M, et al. Clinicopathological phenotype of parathyroid carcinoma: therapeutic and prognostic aftermaths. Chirurgia 2015;110:66–71.
7. Chan JKC. Tumors of thyroid and parathyroid glands. In: Fletcher CDM, ed. Diagnostic Histopathology of Tumors. 4th ed. China: Elsevier Saunders, 2013; pp.1273–1293, vol. 2.
8. Chan JK, Tsang WY. Endocrine malignancies that may mimic benign lesions. Semin Diagn Pathol 1995;12:45–63.
9. Bondeson L, Grimelius L, et al. Parathyroid carcinoma. In: DeLellis RA, Lloyd RV, Heitz PU, Eng C, ed. World Health Organization Classification of Tumors, Pathology & Genetics Tumors of Endocrine Organs. Lyon: IARC Press, 2004; pp.124–127.
10. Sandelin K, Tullgren O, et al. Clinical course of metastatic parathyroid cancer. World J Surg 1994;18:594–598.
11. Marsh DJ, Hahn MA, et al. Molecular diagnosis of primary hyperparathyroidism in familial cancer syndromes. Expert Opin Med Diagn 2007;1:377–392. DOI: 10.1517/17530059.1.3.377.
12. Howell VM, Gill A, et al. Accuracy of combined gene product 9.5 and parafibromin markers for immunohistochemical diagnosis of parathyroid carcinoma. J Clin Endocrinol Metab 2009;94:434–441. DOI: 10.1210/jc.2008-1740.
13. Kim HK, Oh YL, et al. Parafibromin immunohistochemical staining to differentiate parathyroid carcinoma from parathyroid adenoma. Head Neck 2012;34:201–206.
14. Erickson LA, Jin L, et al. Parathyroid hyperplasia, adenomas, and carcinomas: differential expression of p27Kip1 protein. Am J Surg Pathol 1999;23:288–295.
15. Farnebo F, Auer G, et al. Evaluation of retinoblastoma and Ki-67 immunostaining as diagnostic markers of benign and malignant parathyroid disease. World J Surg 1999;23:68–74.
16. Vargas MP, Vargas HI, et al. The role of prognostic markers (MiB-1, RB, and bcl-2) in the diagnosis of parathyroid tumors. Mod Pathol 1997;10:12–17.
17. Vasef MA, Brynes RK, et al. Expression of cyclin D1 in parathyroid carcinomas, adenomas, and hyperplasias: a paraffin immunohistochemical study. Mod Pathol 1999;12:412–416.
18. Juhlin CC, Hooq A. Parafibromin as a diagnostic instrument for parathyroid carcinoma-lone ranger or part of the posse? Int J Endocrinol 2010;324964. DOI: 10.1155/2010/324964.
19. Wang O, Wang CY, et al. Expression of Ki-67, galectin-3, fragile histidine triad, and parafibromin in malignant and benign parathyroid tumors. Chin Med J 2012;125:2895–2901.
20. Lin L, Zhang JH, et al. The parafibromin tumor suppressor protein inhibits cell proliferation by repression of the c-myc proto-oncogene. Proc Natl Acad Sci U S A 2008;105:17420–17425. DOI: 10.1073/pnas.0710725105.

21. Cui C, Lal P, et al. Expression of parafibromin in major renal cell tumors. Eur J Histochem 2012;56:e39. DOI: 10.4081/ejh.2012.e39.
22. Fernandez-Ranvier GG, Khanafshar E, et al. Defining a Molecular Phenotype for Benign and Malignant Parathyroid tumors. Cancer 2009;115:334–344. DOI: 10.1002/cncr.24037.
23. Krzeslak A, Lipinska A. Galectin-3 as a multifunctional protein. Cell Mol Biol Lett 2004;9:305–328.
24. Song L, Tang JW, et al. Galectin-3 in cancer. Clin Chim Acta 2014;431: 185–191. DOI: 10.1016/j.cca.2014.01.019.
25. Tokumaru Y, Yamashita K, et al. The role of PGP9.5 as a tumor suppressor gene in human cancer. Int J Cancer 2008;123:753–759. DOI: 10.1002/ijc.23354.
26. Tezel E, Hibi K, et al. PGP9.5 as a prognostic factor in pancreatic cancer. Clin Cancer Res 2000;6:4764–4767.
27. Yamazaki T, Hibi K, et al. PGP 9.5 as a marker for invasive colorectal cancer. Clin Cancer Res 2002;8:192–195.
28. Hibi K, Westra WH, et al. PGP9.5 As a Candidate Tumor Marker for Non-Small-Cell Lung Cancer. Am J Pathol 1999;155:711–715. DOI: 10.1016/S0002-9440(10)65169-3.
29. Campbell LK, Thomas JR, et al. Protein gene product (PGP 9.5) is not a specific marker of neural and nerve sheath tumors: an immunohistochemical study of 95 mesenchymal neoplasms. Mod Pathol 2003;16:963–969. DOI: 10.1097/01.MP.0000087088.88280.B0.
30. Bergero N, De Pompa R, et al. Galectin-3 expression in parathyroid carcinoma: immunohistochemical study of 26 cases. Hum Pathol 2005;36:908–914. DOI: 10.1016/j.humpath.2005.06.020.
31. Saggiorato E, Bergero N, et al. Galectin-3 and Ki67 expression in multiglandular parathyroid lesions. Am J Clin Pathol 2006;126:59–66. DOI: 10.1309/9NXP-7FRF-87MU-2PCK.
32. Tan MH, Morrison C, et al. Loss of parafibromin immunoreactivity is a distinguishing feature of parathyroid carcinoma. Clin Cancer Res 2004;10:6629–6637. DOI: 10.1158/1078-0432.CCR-04-0493.
33. Gill AJ, Clarkson A, et al. Loss of nuclear expression of parafibromin distinguishes parathyroid carcinomas and hyperparathyroidism-jaw tumor (HPT-JT) syndrome-related adenomas from sporadic parathyroid adenomas and hyperplasias. Am J Surg Pathol 2006;30:1140–1149. DOI: 10.1097/01.pas.0000209827.39477.4f.
34. Juhlin CC, Villablanca A, et al. Parafibromin immunoreactivity: its use as an additional diagnostic marker for parathyroid tumor classification. Endocr-Relat Cancer 2007;14:501–512. DOI: 10.1677/ERC-07-0021.
35. Juhlin CC, Haglund F, et al. Loss of expression for the Wnt pathway components adenomatous polyposis coli and glycogen synthase kinase 3-beta in parathyroid carcinomas. Int J Oncol 2009;34: 481–492.
36. Juhlin CC, Nillson IL, et al. Parafibromin and APC as screening markers for malignant potential in atypical parathyroid adenomas. Endocr Pathol 2010;21:166–177. DOI: 10.1007/s12022-010-9121-z.
37. Truran PP, Johnson SJ, et al. Parafibromin, Galectin-3, PGP9.5, Ki67, and Cyclin D1: Using an immunohistochemical panel to aid in the diagnosis of parathyroid cancer. World J Surg 2014;38:2845–2854. DOI: 10.1007/s00268-014-2700-2.
38. LiVolsi VA, Baloch Z, et al. Oncocytic lesions of the neuro-endocrine system. Adv Anat Pathol 2014;21:69–82. DOI: 10.1097/PAP.0000000000000011.
39. Kumari N, Chaudhary N, et al. Role of histological criteria and immunohistochemical markers in predicting risk of malignancy in parathyroid neoplasms. Endocr Pathol 2016;27:87–96. DOI: 10.1007/s12022-016-9426-7.
40. Brown S, O'Neill C, et al. Parathyroid carcinoma: increasing incidence and changing presentation. ANZ J Surg 2011;81:528–532.
41. Kruijff S, Sidhu SB, et al. Negative parafibromin staining predicts malignant behavior in atypical parathyroid adenomas. Ann Surg Oncol 2014;2:426–433. DOI: 10.1245/s10434-013-3288-8.