Association of PTP4A3 expression and tumour size in functioning pituitary adenoma: a descriptive study

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Received 6 May 2020
Revised 9 June 2020
Accepted 10 June 2020
Published Online First 2 July 2020

To cite: Moyano Crespo GD, Cecenarro LA, Perez P, *et al*. *J Clin Pathol* 2021;**74**:190–193.

ABSTRACT

Background PTP4A3 is a subclass of a protein tyrosine phosphatase super family and is expressed in a range of epithelial neoplasms. We evaluated PTP4A3 expression and its association with clinicopathological parameters in different types of functioning pituitary adenomas.

Methods A total of 34 functioning pituitary adenomas samples were evaluated in this observational study. PTP4A3 expression was examined by immunohistochemical staining, and, possible correlations between PTP4A3 and some clinicopathological variables were investigated.

Results PTP4A3 was expressed in 19 out of 34 tumours (55%), at the cytoplasmic level of tumorous cells. Moreover, there was significant association (p=0.042) between PTP4A3 expression and tumorous size.

Conclusions PTP4A3 was expressed in more than half of the tumours analysed, with there being a significant association with the tumorous size of functioning adenomas. This allows to speculate that PTP4A3 may regulate tumour growth, although further investigations are necessary to determine if this phosphatase can serve as a biomarker or used as a therapeutic target in pituitary macroadenomas.

BACKGROUND

The pituitary adenomas are one of the most frequent intracranial tumours, accounting for 10% of these and also 90% of intrasellar lesions with a prevalence of 1/1500, with approximately half of these tumours being functioning (46%–64%).[1–3] Although pituitary adenomas are histologically benign, some may grow and become locally aggressive, resulting in a shortened lifespan.[4 5] Different types of functioning pituitary adenomas are currently treated by medical therapies such as dopamine and somatostatin agonists, surgery or radiotherapy. However, these treatments are not entirely satisfactory and patients can fail to respond to traditional approaches.[6]

Protein tyrosine phosphatases (PTPs) are a large family of enzymes that catalyse the removal of phosphate groups attached to the tyrosine residues on their substrates, and are essential for regulating a myriad of cellular processes.[7] In the tumorous pituitary gland, classical PTPs are considered to be cell growth regulators.[8] Protein tyrosine phosphatase 4A3 (PTP4A3, also known as PRL-3) belongs to this group and has been implicated in the control of cell cycle, survival, angiogenesis, adhesion, cytoskeleton remodelling, epithelial-mesenchimal transition, motility and invasion.[9] Moreover, PTP4A3 is a growth-associated protein in some tumorous types, with many studies having identified PTP4A3 to be a marker of tumour progression in different neoplasms.[10–13] Although an increased PTP4A3 expression, both at the mRNA and protein level, has been reported in several solid tumours, its expression in functioning pituitary adenomas has not yet been investigated.

Recently, a humanised antibody, PRL-3-zumab, was shown to inhibit PTP4A3-positive cancer cells in vivo, and thus this represents a feasible approach for antitumour immunotherapy.[14] Taking into consideration this finding and that new therapeutic targets in functioning pituitary adenomas are required for improved endocrinological treatments, we performed an observational descriptive study of PTP4A3 expression in 34 functioning pituitary adenomas and investigated possible associations with some clinicopathological parameters.

METHODS

Patients and samples

A transsphenoidal surgery was performed on all sellar tumours, and the inclusion and exclusion criteria are shown in box 1.

An observational, descriptive and retrospective study of normal pituitary glands (n:2; 30 min postmortem, aged 25–45 years, patients with no evidence of endocrine or histological abnormalities) and functioning pituitary adenomas of 34 adults (aged 20–64 years) was conducted at the Centro de Microscopía Electrónica-INICSA-Conicet.

Patients had undergone surgery at Hospital Córdoba, Sanatorio Allende or Clínica Reina Fabiola, Córdoba, Argentina between the years 2004 and 2015. These institutions performed the clinical diagnosis and reticulin staining, hormone analysis and Ki67 immunohistochemistry characterisation, and also provided the samples in paraffin blocks.

The following data were collected: sex, age, tumorous size (macroadenoma/microadenoma), invasion, previous medical treatment, histopathology, hormone staining and Ki67 labelling index (table 1).

Immunohistochemical staining

Sections 3 µm thick were obtained from 34 functional pituitary adenomas and processed for immunohistochemistry. Briefly, the samples were

Box 1 Patient inclusion and exclusion criteria

Patient inclusion criteria
- Female and male patients older than 18 years of age.
- Functioning pituitary adenomas.
- Availability of clinical data (previous medical treatment, tumorous size, tumorous invasion).
- Availability of immunolabelling report of adenohypophyseal hormones and Ki67.

Patient exclusion criteria
- Sellar non-pituitary adenomas (neuronal and paraneuronal tumours; mesenchymal tumours; germ cell tumours; haematological tumours; secondary tumours).
- Non-functioning pituitary adenomas.

deparaffinised in xylene and hydrated in alcohols, and the sections were heated in a microwave for 15 min in a citrate buffer (pH 6.0). Next, the sections were incubated first with hydrogen peroxide 0.5% solution in methanol to block endogenous peroxidase, and then with PTP4A3 rabbit polyclonal antibody (Abcam to anti-PTP4A3 ab82568, USA, 1/70) overnight at 4°C, and finally with biotinylated antirabbit antibody with ABC complex (Vector Laboratories, Burlingame, California, USA). The reaction was detected using DAB as chromogen (Sigma, St. Louis, Missouri, USA). Positive controls for PTP4A3 were performed in human colon adenocarcinoma, with negative controls carried out by omitting the primary antibody incubation. The number of positively stained cells in the human adenomas was determined in 10 random fields at ×400 magnification. The total number of positive cells as a proportion of the number of cells in the field of vision was used to assign positive or negative values. If the percentage of positive cells was ≤10%, this was recorded as '0'; a percentage of between 11% and 25% was assigned '+'; a value of 26%–50% was recorded as '++'; a percentage of 51%–75% was assigned '+++' and finally, a value >76% was recorded as '++++'.

Western blot analysis

Protein extracts (50 µg) from two normal pituitary and three functioning pituitary adenomas (non-invasive corticotroph maroadenoma, invasive somatrotroph macroadenoma and invasive lactotroph macroadenoma) were separated in a polyacrylamide gel (Sigma-Aldrich), transferred to a nitrocellulose

Table 1 Clinical variables and pathological parameters

Case	Gender	Age	Size	Invasion	Previous medical treatment	Histopathology	Hormone IHC	Ki67 %
1	M	59	M	Yes	Naïve to treatment	Sparsely granulated lactotroph adenoma	PRL	8
2	M	51	M	Yes	Naïve to treatment	Sparsely granulated lactotroph adenoma	PRL	1
3	F	30	M	NA	Naïve to treatment	Sparsely granulated lactotroph adenoma	PRL	1
4	M	27	M	NA	Naïve to treatment	Sparsely granulated lactotroph adenoma	PRL	10
5	M	35	M	Yes	Naïve to treatment	Sparsely granulated lactotroph adenoma	PRL	5
6	M	60	M	NA	Naïve to treatment	Sparsely granulated lactotroph adenoma	PRL	5
7	F	20	m	NA	Resistant	Sparsely granulated lactotroph adenoma	PRL	1
8	F	38	m	NA	Resistant	Sparsely granulated lactotroph adenoma	PRL	1
9	F	31	m	NA	Resistant	Sparsely granulated lactotroph adenoma	PRL	1
10	F	26	m	NA	Resistant	Sparsely granulated lactotroph adenoma	PRL	1
11	M	57	M	NA	Naïve to treatment	Densely granulated somatotroph adenoma	STH	2
12	F	33	M	Yes	Naïve to treatment	Densely granulated somatotroph adenoma	STH	1
13	F	64	M	NA	Naïve to treatment	Sparsely granulated somatotroph adenoma	STH	0
14	F	49	M	NA	Naïve to treatment	Sparsely granulated somatotroph adenoma	STH	1
15	M	29	M	NA	Naïve to treatment	Sparsely granulated somatotroph adenoma	STH	3
16	F	58	m	NA	Naïve to treatment	Densely granulated somatotroph adenoma	STH	3
17	M	37	m	NA	Naïve to treatment	Sparsely granulated somatotroph adenoma	STH	2
18	F	35	M	NA	Naïve to treatment	Mammosomatotroph adenoma	STH/PRL	4
19	M	28	M	NA	Naïve to treatment	Mammosomatotroph adenoma	SHT/PRL	2
20	M	53	m	NA	Naïve to treatment	Mammosomatotroph adenoma	SHT/PRL	5
21	F	41	m	NA	Naïve to treatment	Mammosomatotroph adenoma	STH/PRL	1
22	M	32	m	No	Naïve to treatment	Thyrotroph adenoma	TSH	5
23	M	35	M	Yes	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	1
24	F	32	M	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	5
25	F	62	M	Yes	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	2
26	F	51	M	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	3
27	F	31	M	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	2
28	F	41	M	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	2
29	F	55	m	No	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	0
30	F	23	m	No	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	3
31	M	30	m	No	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	3
32	F	21	m	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	1
33	F	25	m	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	7
34	F	38	m	NA	Naïve to treatment	Densely granulated corticotroph adenoma	ACTH	1

Gender: M: male, F: female; size: M: macroadenoma, m: microadenoma.
ACTH, adrenocorticotropic hormone; IHC, immunohistochemistry; NA, not available; PRL, prolactin; STH, somatotroph hormone; TSH, tirotroph hormone.

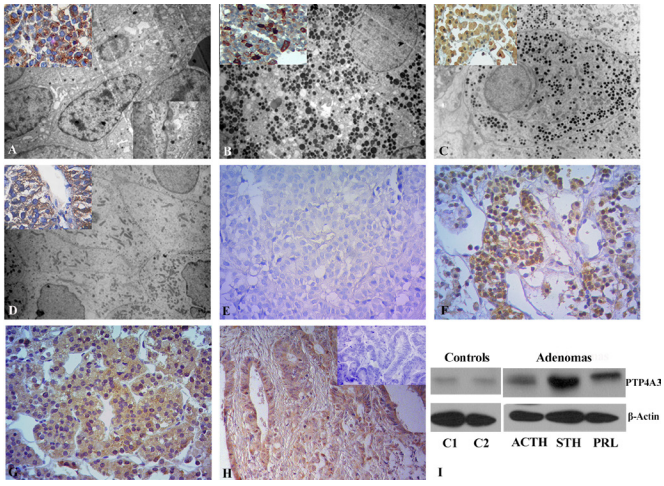

Figure 1 Ultrastructure and hormone immunolabelling in lactotroph (A), somatrotroph (B), coticotroph (C) and tirotroph (D) adenomas. (E) Negative control of protein tyrosine phosphatase 4A3 (PTP4A3) immunolabelling in pituitary adenomas; (F) PTP4A3 expression, mainly localised in the cytoplasm (×400, magnification). (G) +++PTP4A3 expression, (H) positive PTP4A3 control in colon cancer sample. Inset: negative control omitting primary antibody. (I) PTP4A3 semi-quantitative analysis in normal gland and different pituitary adenomas. ACTH, adrenocorticotropic hormone; PRL, prolactin; PTP4A3, protein tyrosine phosphatase 4A3; STH, somatotroph hormone.

membrane (Amersham International) and the non-specific binding was blocked with phosphate-buffered saline 5% non-fat dried milk at room temperature. The membranes were then rinsed and incubated overnight with anti-PTP4A3 (Abcam to anti-PTP4A3 ab82568, USA) (1:166) or anti-β-actin (1:4000; Sigma-Aldrich). The blots were incubated with HPRT-conjugated bovine antigoat (1:2500; Santa Cruz Biotechnology), goat anti-mouse (1:2500, Jackson ImmunoResearch) or goat antirabbit secondary antibodies (1:5000, BioRad). The membranes were thoroughly rinsed in Tris-buffered saline 0.1% Tween 20, and the HPRT-coupled secondary antibody was revealed using enhanced chemiluminescence western blot analysis detection reagents (GE Healthcare), with the emitted light captured on Hyperfilm (GE Healthcare).

Statistical analyses

Data were presented as mean (SD) for continuous data or for frequency and percentages (categorical data). We estimated the 95% CI for the main quantitative variables. The T-test was used to compare PTP4A3 and the Ki67 labelling index between tumorous sizes (macroadenoma vs microadenoma). The Fisher's exact test was used to assess an association between invasion and tumour size, a p value <0.05 was considered to be statistically significant for all the analyses. The statistical analysis was performed using the Stata 15.1 statistical package.[15]

RESULTS

PTP4A3 expression in functional pituitary adenomas

Pituitary adenomas were characterised by the H&E stain, immunohistochemistry hormone determination and transmission electron microscopy (figure 1A–D). In this cohort, 59% of patients were female, with the mean (SD) age being 39 (12.96) years.

The clinicopathological findings revealed that there were 29% lactotroph cell adenomas, 20% somatotroph adenomas, 11% mammosomatotropic adenomas, 35% corticotroph cell adenomas and 3% tirotroph cell adenomas (figure 1). Of the above, 56% were macroadenomas and 44% microadenomas. As four of the lactotroph cell adenomas were resistant to previous medical treatment (dopamine agonist), these were treated surgically.

Table 2 PTP4A3 expression in pituitary adenomas

Adenoma	n	Neg	1+	2+	3+	4+
PRL	10	5	2	2	0	1
GH	11	4	3	4	0	1
TSH	1	1	0	0	0	0
ACTH	12	5	0	5	0	2

ACTH, adrenocorticotropic hormone; GH, growth hormone; PRL, prolactin; PTP4A3, protein tyrosine phosphatase 4A3; TSH, tirotroph hormone.

The mean (SD) of the Ki67 index was 2.68 (0.39); 95% CI 11.88 to 3.48. Sixty-eight per cent of the adenomas revealed a Ki67 index ≤3%, with the Ki67 index being independent of tumour size ($p>0.05$). Unfortunately, the presence or absence of invasion could only be determined in 10 out of 34 functional pituitary adenomas.

The PTP4A3 expression in 34 functioning pituitary adenomas was determined by immunohistochemistry, with the mean (SD) of PTP4A3 being 23.02857 (4.72); 95% CI 13.42 to 32.62. As shown in figure 1F-G, PRL-3 protein was mainly localised in the cytoplasm, which sometimes occurred as granulated loci in the strongly positive samples. According to the criteria, the PTP4A3-positive expression rate was 64% in the adenoma group (table 2). The semi-quantitative phosphatase analysis showed that there was a higher expression of PTP4A3 in proliferative pituitary lesions than in normal glands (figure 1I). However, there was only a significant and greater difference found for PTP4A3 between pituitary macroadenomas and microadenomas ($p=0.042$) (table 3). Finally, for the adenoma pituitary linage cells, the PTP4A3 expression was similar in the PRL-somatotroph hormone-tirotroph hormone (54%) linage and adrenocorticotropic hormone linage (58%).

Table 3 Relationship of PTP4A3-positive expression and pituitary adenomas clinicopathological features

		PRL-3-positive expression	
Clinical data	n	Positive (cases)	Percentage (%)
Age (years)			
>55	9	3	33.33
<55	25	16	64
Gender			
Men	13	8	61.53
Women	21	11	52.38
Adenoma			
PRL	10	5	50
GH	11	7	63.63
TSH	1	0	0
ACTH	12	7	58.33
Tumorous size			
Macro	19	13	68.42
Micro	15	6	40

ACTH, adrenocorticotropic hormone; GH, growth hormone; PTP4A3, protein tyrosine phosphatase 4A3; TSH, tirotroph hormone.

DISCUSSION

Functioning pituitary tumours are benign proliferations with different biological characteristics and behaviours, especially with regard to tumour size and invasion.[4] Similar to other series reported, in our cohort 59% of patients were female, but the mean age was about 39 years lower than for other studies.[3] Women were predominant and at an earlier age and most markedly for corticotroph tumours, as was previously reported.[3]

We conducted an observational descriptive study to evaluate PTP4A3 expression in the normal pituitary gland and in a functioning adenoma cohort. This phosphatase is in normal tissue mainly expressed in the heart, skeletal muscle and to a lesser extent in the prostate.[16] PTP4A3 also shows low basal levels in the human normal pituitary gland in agreement with the fact that phosphatase is expressed at low levels in other normal human epithelial tissue.[16] However, this PTP has largely unknown physiological cellular functions, although it has been associated with terminal cell differentiation as well as appearing to be important in ensuring cell cycle progression by facilitating G1/S transition.[16 17]

In our investigation, 19 out of 34 functioning pituitary adenomas exhibited detectable immunolabelling of PTP4A3, with 26%–50% of the immunopositive cells occurring in more than half of PTP4A3-positive adenomas. One important point was that 63.63% of somatotroph adenomas, 58.33% of corticotroph adenomas and 50% of lactotroph adenomas were PTP4A3-positive tumours. To date, few pituitary investigations have reported PTPs' expression. It has been proposed that the 'classical PTPs', namely SHP-1, SHP-2, and DEP-1/PTPn, play a pivotal role in SRIF/SRL-mediated control of cell growth, and seem to be activated by ligand-binding to all the different SST subtypes. Cells transfected with the individual somatostatin receptor (SSTR) subtypes have demonstrated that all the five SSTRs are able to induce PTP activity.[8 18] However, we report for first time a PTP4A3 increased expression in a group of different cell linage pituitary adenomas. Although no substrate has yet been clearly identified for the PTPs, a few have been suggested for PTP4A3, such as the PI3K-Akt pathway, although the direct mechanism involved still remains unclear.[19]

Monsalves *et al* suggested that the pituitary adenoma growth rate is influenced by various patient-specific and tumour-specific characteristics, such as age, sex, specific subtype, hormonal activity and immunohistochemical profile, including the mind-bomb homolog 1 labelling index status.[20] Here, we demonstrated that there was significant difference between PTP4A3 expression and pituitary adenoma size, thereby revealing a possible new molecule that may regulate tumour growth, at least in some adenomas. We also consider that our findings have identified a new concept in pituitary pathology, in which a group of PTP4A3-positive macroadenomas could be a potential therapy target for new treatments to reduce the tumorous size, and may be also used as a postoperative adjuvant therapy to prevent recurrence.[14] Although we cannot extrapolate the results to fit the entire population (inclusive bias), a further multicentre study with a large sample size should be conducted, to determine if PTP4A3 immunostaining could also be used to identify potential patients as a PRL-3-zumab target.

Take home messages

- Protein tyrosine phosphatase 4A3 (PTP4A3) is expressed in the normal pituitary gland.
- Overexpression of PTP4A3 was observed in a series of 19 patients with pituitary adenomas, with phosphatase expression occurring in the PRL-growth hormone-tirotroph hormone linage and adrenocorticotropic hormone linage cells.
- There was a significant difference between PTP4A3 expression in pituitary macroadenomas versus microadenomas.

Handling editor Runjan Chetty.

Acknowledgements The authors would like to thank native speaker Dr Paul Hobson for editing the English language of the manuscript.

Contributors All authors contributed to the design of the study, writing or critical review of the manuscript, analysis and interpretation of data. All agreed to submission of the manuscript.

Funding This work was supported by La Agencia Nacional de Promoción Científica y Tecnológica, Fondo Nacional de Ciencia y Tecnología (ANPCyT-FONCYT-PICT 2014-2555), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET- PIP-Res #154/2014) and Secretaria de Ciencia y Tecnología de la Universidad Nacional de Cordoba (SECyT–UNC Res # 313/2016).

Competing interests None declared.

Patient consent for publication Obtained.

Ethics approval CIEIS del Hospital Córdoba (Registro Nacional de Investigaciones en Salud N° CO000152 (RENIS)).

Provenance and peer review Not commissioned; internally peer reviewed.

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