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Overexpression of prothymosin alpha is related to pituitary adenoma recurrence but not to adenoma invasiveness and proliferation

Nadekspresja protymozyny alfa w gruczolakach przysadki jest związana z nawrotowością a nie z proliferacją komórek i inwazyjnością guza

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Abstract

Introduction: Prothymosin alpha (ProTα) is a peptide initially considered as a thymic hormone, but further studies have shown its wide distribution in different tissues and organs. It has a prevalent nuclear localisation and is thought to be involved in the control of proliferation and apoptosis. In earlier studies, the overexpression of ProTα was found in several human tumours, including pituitary adenomas. The present study deals with the relations of ProTα to the pituitary adenoma hormonal phenotype, proliferation, recurrence and invasiveness.
Material and methods: Sixty two pituitary adenomas were included in the study. The invasiveness of the tumours was estimated before surgery by means of magnetic resonance imaging. The paraffin sections of the tumours were immunostained with an antibody against the C-terminal fragment (101-109) of ProTα and with anti-Ki-67 antibody. The hormonal phenotype of the investigated pituitary adenomas had been established previously by means of immunostaining with antibodies to pituitary hormones (GH, PRL, FSH, LH, TSH, ACTH and α-subunit).
Results: Strong immunostaining with anti-ProTα antibody occurred in the subpopulation of cell nuclei and the walls of intratumoural blood vessels. ProTα index is higher in clinically non-functioning pituitary adenomas (CNFPA) compared to any type of functioning adenomas. There was no difference in the percentage of ProTα- positive cell nuclei in non-invasive *vs.* invasive adenomas, but it was significantly more frequent in recurrent than in primary tumours. Moreover, the decrease of ProTα index was found in somatotroph tumours treated with somatostatin analogues *vs.* untreated ones. The percentage of ProTα nuclei did not correlate with Ki-67 index.
Conclusions: The overexpression of nuclear ProTα in pituitary adenomas is related to tumour recurrence, but not to proliferation or invasiveness. **(Endokrynol Pol 2014; 65 (5): 382–386)**

Key words: *prothymosin alpha; pituitary adenoma; recurrence; invasiveness; proliferation*

Streszczenie

Wstęp: Protymozyna alfa (ProTα) jest peptydem początkowo uważanym za hormon grasicy, jednak późniejsze badania wykazały jej występowanie w różnych tkankach i narządach. Dominująca lokalizacja wewnątrzjądrowa ProTα sugeruje jej udział w kontroli proliferacji komórkowej i apoptozy, a najnowsze badania wykazały jej nadekspresję w wielu ludzkich nowotworach, w tym gruczolakach przysadki. Celem prezentowanej pracy było ustalenie związku pomiędzy ProTα a fenotypem hormonalnym, proliferacją, nawrotowością i inwazyjnością gruczolaków przysadki.
Materiały i metody: Zbadano 62 gruczolaki przysadki. Inwazyjność guzów oceniono na podstawie przedoperacyjnego badania rezonansu magnetycznego. Skrawki parafinowe gruczolaków przysadki zbadano immunohistochemicznie, stosując przeciwciała skierowane przeciwko C-końcowemu fragmentowi (101-109) ludzkiej ProTα i przeciwciała przeciw antygenowi Ki-67. Fenotyp hormonalny gruczolaków został ustalony w oparciu o badanie immunohistochemiczne z użyciem przeciwciał przeciw hormonom przysadkowym (GH, PRL, FSH, LH, TSH, ACTH i α podjednostce).
Wyniki: Silny dodatni odczyn immunohistochemiczny dla ProTα stwierdzono w jądrach komórkowych gruczolaków i w ścianie wewnątrzguzowych naczyń krwionośnych. Indeks ProTα był wyższy w klinicznie nieczynnych gruczolakach przysadki niż w guzach wykazujących klinicznie czynność hormonalną. Stwierdzono znamienne nasilenie odczynu jądrowego dla ProTα w nawrotowych gruczolakach w porównaniu z guzami pierwotnymi, natomiast nie obserwowano różnic w nasileniu odczynu dla ProTα w guzach nieinwazyjnych i inwazyjnych. Ponadto w gruczolakach somatotropowych leczonych analogami somatostatyny obserwowano niższy indeks ProTα niż w guzach nieleczonych. Pozytywny odczyn jądrowy dla ProTα nie korelował jednakże ze wskaźnikiem proliferacji komórkowej Ki-67.
Wnioski: Nadekspresja jądrowa ProTα w gruczolakach przysadki jest związana z nawrotowością guzów, a nie z nasileniem ich proliferacji komórkowej i inwazyjnością. **(Endokrynol Pol 2014; 65 (5): 382–386)**

Słowa kluczowe: *protymozyna alfa; gruczolak przysadki; nawrotowość; inwazyjność; proliferacja komórkowa*

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Introduction

Prothymosin alpha (ProTα) is a peptide consisting of 111 amino acid residues. It was initially considered as a thymic hormone but further studies showed its wide distribution in different tissues and organs [1]. It has been found that ProTα exerts both intracellular and extracellular biological activities [2]. Outside the cell, ProTα and its fragment, thymosin α-1, act as immunostimulators and are useful in the treatment of immunodeficiency and malignances [2]. Within the cell, the role of ProTα in the control of cell proliferation and / or apoptosis has been suggested [1–3]. In some recent studies, the overexpression of ProTα has been found in malignant tumours such as gastric [4], prostate [5, 6] and thyroid cancers [7, 8]. The quoted authors suggest that ProTα may be useful as a marker and prognostic factor of malignancy. ProTα has also been detected in pituitary adenomas [9–11] and suggested to be a marker of adenoma recurrence [11]. The present paper reports on ProTα expressed in relation to tumour recurrence, invasiveness, proliferation and hormonal phenotype.

Material and methods

Tumour samples

Sixty two pituitary adenomas, removed by transsphenoidal adenectomy, were included in the study. On the basis of the examination before surgery, 12 patients were diagnosed with acromegaly, seven with Cushing's disease, three with hyperprolactinaemia, and 40 with clinically nonfunctioning adenomas (NFPA). The invasiveness of the tumours was estimated before surgery by means of magnetic resonance imaging. Ten tumours were classified as non-invasive and 25 as invasive. The study was approved by the Local Bioethical Committee, decision number RNN/16/07/KE.

Immunohistochemistry

The paraffin sections of the tumours were immunostained with an antibody against the C-terminal fragment (101-109) of prothymosin alpha (kindly received from Dr Evangelia Livaniou, Institute of Radioisotopes and Radiodiagnostic products, National Science for Scientific Research 'Demokritos', Athens, Greece) or with anti-Ki-67 antigen (MIB-1) antibody (Dako-Cytomation, Denmark). The primary antibodies were applied in the working solution 1:100. The control slides were stained with the omission of the primary antibody. The hormonal phenotype of the investigated pituitary adenomas had been established previously by means of immunostaining with antibodies to pituitary hormones (GH, PRL, FSH, LH, TSH, ACTH and α-subunit). The visualisation of reactions was done by means of the streptavidin-biotin-peroxidase technique with the use of StreptABC/HRP kit (Dako-Cytomation) and 3,3'-diaminobenzidine as chromogen. The number of PTα-immunopositive and Ki-67-immunopositive cell nuclei was estimated in 500 randomly counted cell nuclei and expressed as a percentage (%). The numerical data was analysed statistically using the program Statistica 10.

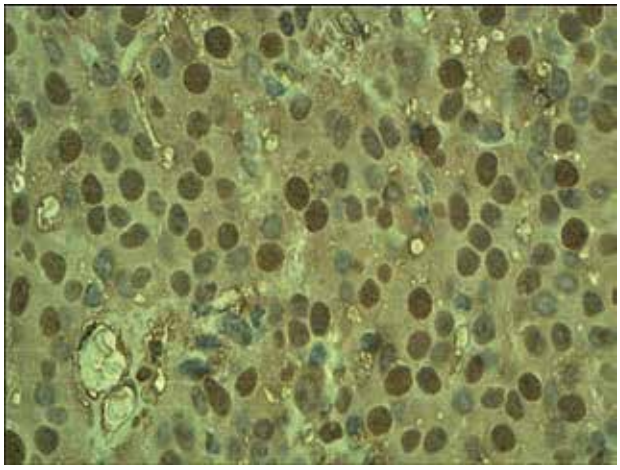

Figure 1. *Immunopositive (dark brown) and immunonegative (blue) cell nuclei in pituitary adenoma immunostained with anti-ProTα antibody. Original magnification 400×*

Rycina 1. *Immunopozytywne (ciemno-brązowe) i immunonegatywne (niebieskie) jądra komórkowe w gruczolaku przysadki barwionym immunohistochemicznie z przeciwciałem anty-ProTα. Powiększenie oryginalne 400×*

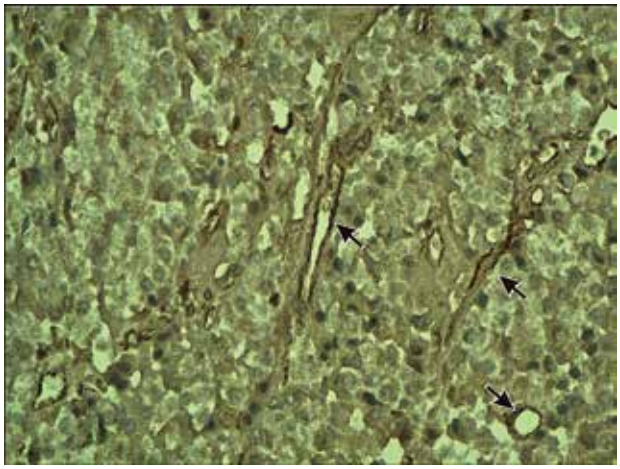

Figure 2. *Immunostaining of intratumoural blood vessels (arrows) with anti-ProTα antibody in pituitary adenoma. Original magnification 200×*

Rycina 2. *Odczyn immunohistochemiczny w wewnątrzguzowych naczyniach krwionośnych (strzałki) z przeciwciałem anty-ProTα gruczolaku przysadki. Powiększenie oryginalne 200×*

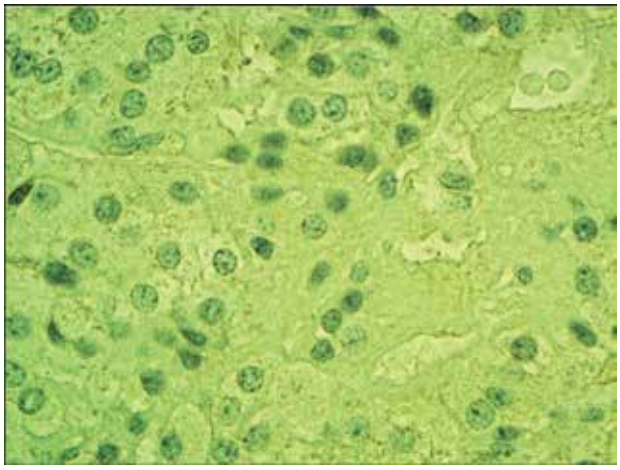

Figure 3. *The same tumour as in Figure 2 stained with omission of anti-ProTα antibody. Original magnification 400×*

Rycina 3. *Ten sam guz co na rycinie 2 barwiony z pominięciem przeciwciała anty-ProTα. Powiększenie oryginalne 400×*

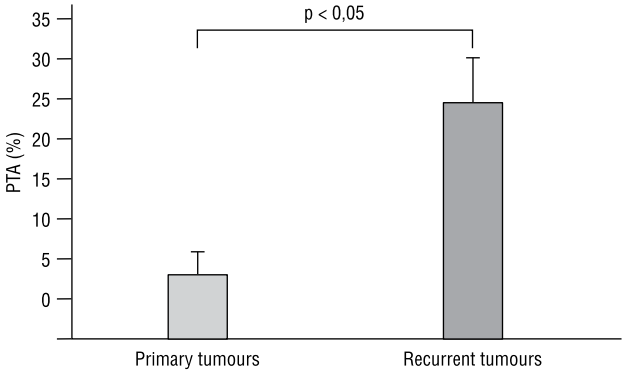

Figure 4. *Percentage of ProTα-immunopositive nuclei in primary and recurrent pituitary adenomas*

Rycina 4. *Odsetek ProTα-immunopozytywnych jąder w pierwotnych i nawrotowych gruczolakach przysadki*

Results

Strong immunostaining with anti-ProTα antibody occurred in the subpopulation of cell nuclei (Fig. 1) and the walls of intratumoural blood vessels (Fig. 2). Moreover, a weak-to-moderate immunostaining could also be observed in tumoural cell cytoplasm. No staining was found in the control slides, where the primary antibody was omitted (Fig. 3).

There was no difference in the percentage of ProTα-positive cell nuclei in non-invasive *vs.* invasive adenomas (18.1 ± 7.3% *vs.* 19.5 ± 4.0%. mean ± SEM, respectively). On the other hand, the ProTα-positive cell nuclei were significantly more frequent in recurrent than in primary tumours (25.9 ± 4.7% *vs.* 7.15 ± 2.4%, $p < 0.05$, Fig. 4). Similarly, ProTα index was significantly higher in primary tumours which later recurred within a five year period compared to those without documented recurrence within this period (23.8 ± 7.1% *vs.* 6.1 ± 1.1%, $p < 0.05$). Some tendency towards a negative correlation (albeit not significant) was observed between ProTα indices and the time period which elapsed before the tumour recurrence (Fig. 5). However, the percentage of ProTα nuclei does not correlate with Ki-67 index (Fig. 6). Considering the abundance of ProTα nuclei in the relation to the adenoma phenotype, it is evident that ProTα index is higher in clinically non-functioning pituitary adenomas (NFPA) compared to any type of functioning adenomas. For instance, the ProTα-positive nuclei are more abundant in ACTH-expressing NFPA without manifestation of Cushing's disease ('silent corticotropinoma') than in corticotropinomas manifesting

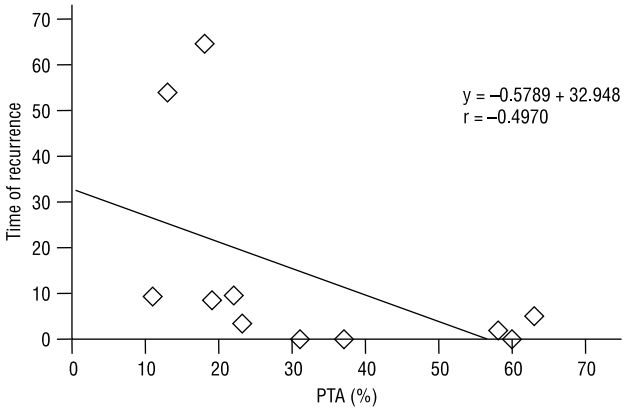

Figure 5. *Study of correlation between ProTα index and the time (months) elapsed to the second surgical intervention*

Rycina 5. *Korelacja miedzy indeksem ProTα a czasem (w miesiącach), który upłynął do drugiej interwencji chirurgicznej*

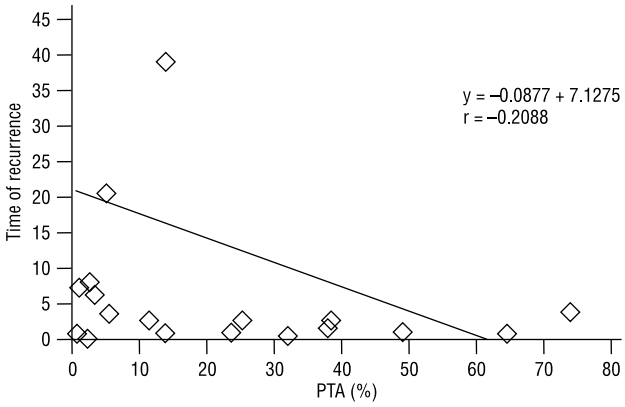

Figure 6. *Study of correlation between ProTα and Ki-67 indices in pituitary adenomas*

Rycina 6. *Badanie korelacji między indeksami ProTα i Ki-67 w gruczolakach przysadki*

with Cushing's disease (19.4 ± 6.6% *vs.* 2.2 ± 0.6%). Similarly, 'silent somatotropinomas' reveal a higher ProTα index compared to pharmacologically untreated acromegaly (32.8 ± 9.9% *vs.* 16.7 ± 7.7% respectively). Interestingly, the nuclear ProTα index is significantly lower in samples taken from patients with acromegaly treated before surgery with long-acting somatostatin analogues (octreotide or lanreotide) than from untreated patients (1.9 ± 0.7% *vs.* 16.7 ± 7.7% respectively, $p < 0.05$, Fig. 7). The low value of ProTα index (1%) was also observed in one case of acromegaly treated before surgery with bromocriptine. Another localisation of the strong expression of ProTα is in the walls of intratumoural blood vessels. The ProTα overexpression occurred in all vascular profiles within all the tumours examined, irrespective of their hormonal phenotype, invasiveness or recurrence.

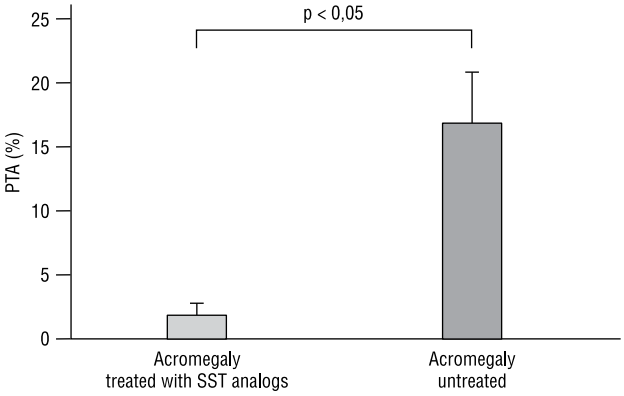

Figure 7. *Percentage of ProTα-immunopositive nuclei in somatotropinomas treated and untreated with somatostatin (SST) analogues*

Rycina 7. *Odsetek ProTα-immunopozytywnych jąder w gruczolakach somatotropowych leczonych i nie leczonych analogami somatostatyny (SST)*

Discussion

The main finding of our paper was the relation of ProTα nuclear overexpression with pituitary adenoma recurrence. In contrast, ProTα nuclear index is not related to tumour invasiveness and tumour proliferation. There is no correlation between ProTα and Ki-67 indices. Moreover, Ki-67 index does not differ in primary and recurrent pituitary tumours [10]. Certainly, tumour invasiveness – estimated as the ability to infiltrate adjacent structures by tumoural tissue – contributes to tumour recurrence, mostly because it implies a higher risk of non-radical excision of adenoma. High levels of proliferation markers (mostly of Ki-67) are also considered as prognostic factors for tumour progression, but opinions on them are divided [11–17]. Some authors believe the value of the Ki-67 index in the prediction of tumour progress is limited or even absent [14, 16–18]. It is of interest that another putative marker of pituitary adenoma aggressiveness described by us — the ectopic expression of follicle stimulating hormone (FSH) receptor — correlates well with the adenoma invasiveness and Ki-67 index, but not with the adenoma recurrence risk [19, 20]. The question of molecular role of ProTα in pituitary tumour progression remains unclear. Its involvement in cell proliferation control is less probable, because of the lack of correlation between ProTα and Ki-67. The inhibition of apoptosis by ProTα seems more probable. If this is true, the impairment of apoptosis is more important in tumour recurrence than proliferation. The anti-apoptotic action of ProTα has been repeatedly demonstrated in different tissues [21, 22] but further studies concerning pituitary adenomas are needed. Another finding worth underlining is the strong expression of ProTα in the vascular walls, mostly in the endothelium. Since ProTα expression concerns all the vessels observed, the immunostaining with anti-ProTα antibody may serve for the study of intratumoural vascularisation.

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