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Expression of follicle stimulating hormone receptors in pituitary adenomas — a marker of tumour aggressiveness?

Ekspresja receptorów folitropiny w gruczolakach przysadki — marker agresywności guza?

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Abstract

Introduction: In our earlier study, we found that pituitary adenomas, like other human tumours, express ectopically follicle stimulating hormone receptors (FSHR) in intratumoural blood vessels endothelia and/or tumoural cells. The aim of the present paper was to provide more detailed data on FSHR expression in different subtypes of pituitary adenomas and to evaluate its possible role as a prognostic and/or predictive biomarker in these tumours.

Material and methods: Forty two pituitary adenomas, surgically removed, were immunostained with antibodies against the pituitary hormones, antigen Ki-67 and 1–190 fragment of FSHR.

Results: The positive FSHR immunostaining was found in blood vessels endothelia of 88% of adenomas and in tumoural cells of 40% adenomas. In tumoural cells, the incidence of at least moderate FSHR immunostaining is significantly higher in invasive tumours (68%) compared to non-invasive (12%) ones, and higher (albeit not statistically significantly) in invasive-proliferating adenomas (Ki-67 > 3%, grade 2b) compared to invasive but non-proliferating (Ki-67 < 3%, grade 2a) ones.

Conclusions: The present study confirms that pituitary adenomas ectopically express FSHR in intratumoural blood vessels endothelia and tumoural cells. Moreover, the expression in tumoural cells is prevalent in invasive and proliferating adenomas *vs.* non-invasive and non-proliferating tumours. **(Endokrynol Pol 2014; 65 (6): 469–471)**

Key words: *follicle stimulating hormone receptor (FSHR); pituitary adenoma; marker of aggressiveness*

Streszczenie

Wstęp: W poprzednich naszych badaniach stwierdziliśmy, że gruczolaki przysadki, podobnie jak inne ludzkie nowotwory, wykazują ektopową ekspresję receptorów folitropiny (FSHR) w śródbłonkach wewnątrzguzowych naczyń krwionośnych i/lub komórkach guza. Celem obecnej pracy jest bardziej szczegółowa ocena ekspresji FSHR w różnych subtypach gruczolaków przysadki i ocena jej roli jako biomarkera prognostycznego w tych guzach.

Materiał i metody: Czterdzieści dwa gruczolaki przysadki, usunięte chirurgicznie, zbadano immunohistochemicznie z użyciem przeciwciał przeciw hormonom przysadkowym, antygenowi Ki 67 i fragmentowi 1–190 ludzkiego FSHR.

Wyniki: Dodatni odczyn immunohistochemiczny na FSHR stwierdzono w wewnątrzguzowych naczyniach krwionośnych 88% badanych gruczolaków i w komórkach guzowych 40% badanych gruczolaków. W komórkach guzowych występowanie odczynu na FSHR o nasileniu co najmniej umiarkowanym było znamiennie wyższe w guzach inwazyjnych (68%) w porównaniu z guzami nieinwazyjnymi (12%) i wyższe (jakkolwiek nie znamienne statystycznie) w gruczolakach inwazyjnych proliferujących (Ki-67 > 3%, grade 2b) w porównaniu z guzami inwazyjnymi o niskiej proliferacji (Ki-67 < 3%, grade 2a).

Wnioski: Obecna praca potwierdza, że gruczolaki przysadki wykazują ektopową ekspresję FSHR w wewnątrzguzowych naczyniach krwionośnych i komórkach guzów. Ekspresja w komórkach guzów przeważa w gruczolakach inwazyjnych i proliferujących w porównaniu z nieinwazyjnymi i o słabej proliferacji. **(Endokrynol Pol 2014; 65 (6): 469–471)**

Słowa kluczowe: *receptor folitropiny (FSHR); gruczolaki przysadki; marker agresywności*

Introduction

Recent studies have revealed the ectopic expression of follicle stimulating hormone receptors (FSHR) in the endothelium of intra- and peritumoural blood vessels of several human neoplasms [1–4]. Moreover, in some tumours, like adrenal tumours [2], thyroid cancers [4], neuroendocrine tumours [3, 5], and soft tissue sarcomas [6], the expression of FSHR occurs also in tumoural cells. In our earlier study, we found also FSHR expression in the majority of pituitary adenomas [2].

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The aim of the present paper was to provide more detailed data on FSHR expression in different subtypes of pituitary adenomas and to evaluate its possible role as a prognostic and/or predictive biomarker in these tumours.

Material and methods

Tumour samples

Forty two pituitary adenomas, removed by transsphenoidal adenectomy, were included in the study. On the basis of a clinical examination, six cases were diagnosed as acromegaly, seven as Cushing's disease, two as prolactinoma, and 27 as 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/537/11/KB.

Immunohistochemistry

The samples were fixed in 10% buffered formalin or Bouin-Hollande fixative. Paraffin sections (approx. 5 μm thick) were immunostained using the rabbit anti-human FSH-R polyclonal antibody sc-13935 purchased from Santa Cruz Biotechnology Inc. This antibody was raised against 1–190 amino acid sequence from the human FSH-R. In order to estimate the hormonal phenotype of the pituitary adenomas, immunohistochemical detection of pituitary hormones or their subunits was performed (for details see [2]) samples. Additionally, 33 pituitary adenomas were also immunostained with anti-Ki-67 (MIB-1) antibody (Dako Cytomation) to estimate the proliferative status. The primary antibodies were applied in a working dilution of 1:100. The visualisation of primary antibodies was done using either the StreptABComplex/HRP Duet (pituitary hormones and Ki-67) or the Dako REAL EnVision Detection System (FSHR). The immunoreaction intensity for FSHR protein in the tumoural cells was scored semiquantitatively using a descriptive scale as follows: negative staining (score: 0), weak staining (score: 1), moderate staining (score: 2) and strong staining (score: 3). Only strong (score 3) or moderate (score 2) immunostaining was considered to be meaningful.

The statistical comparison of the data was performed by means of χ^2 test corrected for small samples. As a positive control, a biopsy sample of the human testis was immunostained. As a negative control, the primary antibody was omitted in the staining procedure.

Results

FSHR expression in tumoural cells was found in 17/42 (40%) adenoma samples (Figs. 1 and 2). The expression of FSHR in tumoural cells was found in 4/6 cases of acromegaly and in 13/27 cases of NFPA. Clinically nonfunctioning adenomas are very heterogeneous considering their immunohistochemical phenotype (mainly they express gonadotropins). However, the incidence of FSHR is roughly similar in particular phenotype subgroups, perhaps with the exception of silent GH/PRL adenomas in which FSHR incidence is higher (5/6). The incidence of FSHR expression was significantly higher in invasive (68%) than in non-invasive tumours (12%;

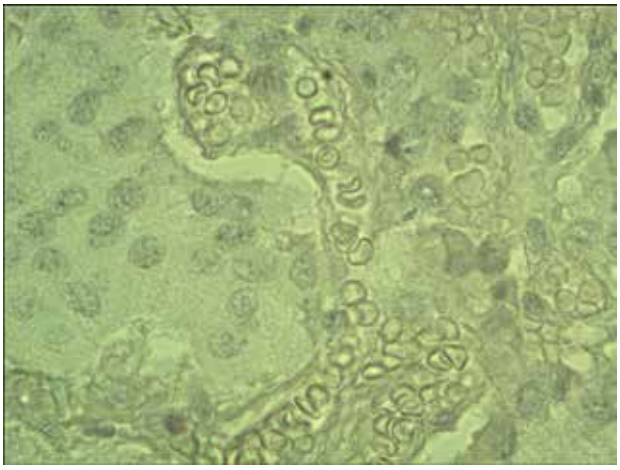

Figure 1. *The same tumour as in Figure 2 stained without the primary antibody. Original magnification 400×*

Rycina 1. *Ten sam guz jak na rycinie 2 barwienie bez pierwotnego przeciwciała. Oryginalne powiększenie 400×*

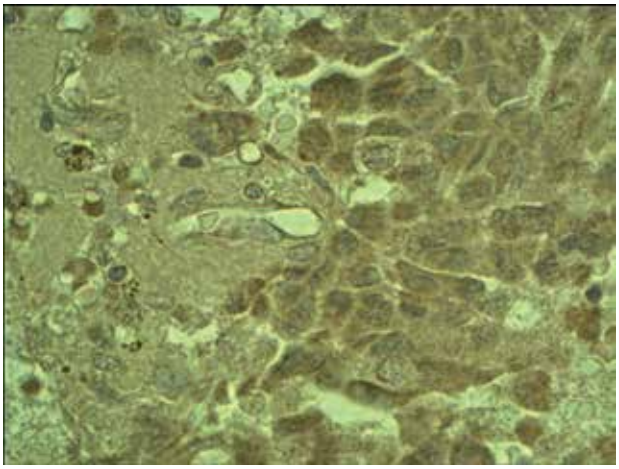

Figure 2. *Non-functioning pituitary adenoma (silent somatotropinoma) in a 71-year-old man. The positive FSHR immunostaining in tumoural cells and vascular endothelia (arrows) can be seen. Original magnification 400×*

Rycina 2. *Nieczynny hormonalne gruczolak przysadki (cichy somatotropinoma) u 71-letniego mężczyzny. Widoczny dodatni odczyn immunohistochemiczny FSHR w komórkach nowotworowych i komórkach śródbłonka naczyń (strzałki). Oryginalne powiększenie 400×*

$p < 0.001$). The incidence of FSHR immunostaining was also higher (albeit not statistically significantly) in invasive proliferating tumours (80%; Ki-67 > 3) grade 2b according the classification of Trouillas et al. [7]) than in invasive but non proliferating (55%; Ki-67 < 3%, grade 2a) ones.

In contrast, no difference between primary (38%) and recurrent tumours (36%) was observed. The incidence of at least moderate FSHR immunostaining is also about two-fold higher in primary tumours which later recurred within a 5-year period compared to those without documented recurrence within this period (6/8; 75% *vs.* 5/14; 36%). As in other tumours, pituitary adenomas exhibit also ectopic FSHR expression in the endothelium of intratumoural blood vessels (Fig. 2). This expression was observed in 78% of tumours including all cases of acromegaly, all but one case of Cushing's disease, and 70% of NFPA. There was no significant difference between recurrent and non-recurrent adenomas (73% *vs.* 81%, respectively). A tendency towards a higher incidence, albeit statistically not significant, could be observed in invasive *vs.* non-invasive tumours (71% *vs.* 60%) and in proliferating (Ki-67 > 3%) *vs.* non-proliferating (Ki-67 < 3%) adenomas (85% *vs.* 64%).

Discussion

The present study confirms that pituitary adenomas ectopically express FSHR in intratumoural blood vessels endothelia and tumoural cells. Moreover, the expression in tumoural cells is prevalent in invasive and proliferating adenomas *vs.* non-invasive and non-proliferating adenomas. A similar tendency, although less evident, can be observed in respect of vascular endothelia.

These findings corroborate with earlier observations concerning other human tumours. For instance, in liposarcomas, FSHR expression can be observed in more cases of dedifferentiated compared to well-diffentiated liposarcomas. The benign lipomas and non-neoplastic fat is FSHR negative [6]. In the thyroid, FSHR is strongly expressed in thyroid cancers (both differentiated and anaplastic), scarce in benign adenomas, and absent in normal thyroidal epithelium [4]. FSHR is also expressed in cultured androgen-independent prostate cancer cells, which are considered as less differentiated than androgen-dependent [8].

Thus, FSHR expression in pituitary adenomas, as in other tumours, may be considered as a marker of aggressiveness. However, its prognostic value as an exclusive marker seems to be limited. On the other hand, the overexpression of FSHR in pituitary adenomas may constitute a novel therapeutic target in these tumours. It is known that in the normal ovary, as well as in ovarian cancer, FSH acting via its receptors leads to enhanced cell proliferation, decreased apoptosis and increased angiogenesis [9–11].

It may be hypothesised that ectopically expressed FSHR plays the same role in pituitary adenomas.

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