

Molecular biology investigation of somatostatin and estrogen receptors in clinically non-functioning pituitary adenomas

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SUMMARY

Objective: Non-functioning pituitary adenomas (NFA) comprise about 25–30% of all pituitary tumors. Transsphenoidal neurosurgery, the treatment of choice, is often unsuccessful and frequently leaves tumor remnants. The effect of subsequent biological treatment is mediated by transmembrane receptors in the adenoma cells. Our aim was to determine the somatostatin (SSTR1–5) and estrogen receptor 1 (ER1) expression profile in 69 specimens of NFA.

Material and Methods: Adenoma samples were submerged in RNAlater Tissue Protect and transported frozen to the laboratory. The RNA was isolated by Trizol Reagent and transcribed to cDNA by SuperScript III. The expression of the receptors was determined by quantitative real time PCR. Results were normalized to the beta-glucuronidase housekeeping gene.

Results: With exceptions of SSTR4 and SSTR5, all other subtypes of receptors were expressed in the examined tumor specimens. The median relative quantification values were: 63.8% for SSTR1, 55.4% for SSTR2, 19.6% for SSTR3, 2.6% for SSTR4, 9.2% for SSTR5, and 75.4% for ER1.

Conclusion: Variable expression of somatostatin and estrogen receptors could influence the effectiveness of the biological treatment. Therefore, the receptors expression profile should be determined before the NFA therapy by somatostatin analogs or estrogen receptor modulators.

Keywords: clinically non-functioning pituitary adenomas, somatostatin receptor, estrogen receptor, somatostatin analogs, estrogen receptor modulators.

SOUHRN

Drastíková M., Gabalec F., Čáp J., Beránek M.: Molekulárně biologické vyšetření estrogenového a somatostatinných receptorů u klinicky afunkčních adenomů hypofýzy.

Cíl studie: Klinicky afunkční adenomy (KAA) představují zhruba 25–30% všech tumorů hypofýzy. Neurochirurgická operace nádoru bývá často neúspěšná a zůstává po ní nádorové reziduum. Účinek následné biologické léčby je závislý na přítomnosti transmembránových receptorů v nádorových buňkách. Naším cílem bylo určit expresní profil somatostatinných receptorů (SSTR1–5) a estrogenového receptoru 1 (ER1) u 69 vzorků KAA.

Materiál a metody: Vzorky adenomů se uchovávaly v RNAlater Tissue Protect až do doby zpracování v laboratoři. RNA jsme izolovali Trizolem a přepsali do cDNA pomocí SuperScriptu III. Expres jednotlivých receptorů byla stanovena metodou kvantitativní real time PCR. Získané výsledky jsme přepočítali na housekeepingový gen pro beta-glukuronidázu.

Výsledky: U vyšetřovaného souboru 69 afunkčních adenomů byly kromě SSTR4 a SSTR5 exprimovány všechny zbývající vyšetřované receptory. Mediány relativních hodnot byly: 63,8% pro SSTR1, 55,4% pro SSTR2, 19,6% pro SSTR3, 2,6% pro SSTR4, 9,2% pro SSTR5 a 75,4% pro ER1.

Závěr: Variabilní exprese jednotlivých podtypů SSTR a ER1 může ovlivnit účinnost biologické léčby KAA. Proto by určení expresního profilu sledovaných receptorů mělo předcházet zahájení biologické léčby KAA somatostatinnými analogy či modulátory estrogenových receptorů.

Klíčová slova: klinicky afunkční adenomy hypofýzy, somatostatinný receptor, estrogenový receptor, somatostatinná analogy, modulátory estrogenových receptorů.

Introduction

The overwhelming majority of pituitary tumors are benign and 25–30% of them represent clinically non-functioning pituitary adenomas (NFA) [1]. The major part of NFA (up to 90%) produces either a low, non-significant amount of hormones or defective hormones [2]. Only 5–10% of NFA are immunohistochemically negative, “null cell” adenomas [3]. In view of the fact that the hormone oversecretion is typically absent, the NFA diagnosis is often delayed until macroadenoma is

formed [4]. In this stage, NFA are manifested by mass effects of the tumor on surrounding tissue, resulting in headache, visual field defects and hypopituitarism [5].

The treatment of choice of NFA is transsphenoidal neurosurgery [6]. However, because of supra- and especially parasellar tumor extension, the operation is often not completely successful and frequently leaves tumor remnants which can regrow during long-term follow-up [7]. This fact has led to the development of new therapy strategies using pharmacological treatment: somatostatin analogs (SA) and estrogen receptor modulators. The ef-

fects of these drugs are mediated by transmembrane receptors in the adenoma cells [8]. The aim of the study was to determine the somatostatin (SSTR subtypes 1–5) and estrogen receptor 1 (ER1) expression profile and describe biological variability of the receptors in 69 NFA specimens.

Materials and Methods

The group of patients was made up of 41 men (37–87 years old; median 65.5) and 28 women (36–83 years old; median 65.5). Adenoma tissue samples were obtained from patients during transsphenoidal surgery at the Neurosurgery Clinic, Central Military Hospital in Prague and University Hospital in Hradec Kralove. Sample collection and laboratory analysis were conducted with the informed consent of the patients in accordance with the requirements of the Clinical Research Ethics Committees.

A portion of the tumor was used for pathological examination and the remaining part was submerged in the nucleic acid stabilizing solution (RNAlater Tissue Protect, Qiagen, Germany) and frozen at -80°C until RNA extraction. All adenomas were immunohistochemically analyzed for adenohipophyseal hormones. In 75.9% of the samples, we detected follicle-stimulating and/or luteinizing hormone, or their alpha subunit. In a minor part, silent corticotroph adenomas (8.4%), plurihormonal adenomas (8.4%), and “null cell” adenomas (7.2%) were diagnosed.

The RNA was isolated by Trizol Reagent (Invitrogen, USA) following manufacturer’s instructions and transcribed to cDNA by SuperScript III First-Strand Synthesis (Invitrogen). Real-time PCR master mix (25 μl) contained 12.5 μl TaqMan Universal PCR Master Mix (Applied Biosystems, USA), 5 μl of cDNA, 300 nM of each primer and 200 nM of hydrolysis fluorescent probe. The sequences of primers and probes for SSTR1, 2, 3 and 5 were published previously [9]; SSTR4 and ER1 analyses were performed using Taqman Gene Expression Assays Hs01566620_s1 and Hs00174860_m1 (Invitrogen). The expression of the receptors was determined by quantitative real time PCR in the RotorGene 6000 thermal cycler (Corbett Research, Australia) under following conditions: 50°C 2 min, 95°C 10 min, then 50 cycles consisting of 95°C 15 s for denaturation and 60°C 1 min for both annealing and extension. For calibration, serial diluted plasmids pCR4 (Invitrogen) with SSTR1–5 and ER1 inserts (Generi Biotech, Czech Republic) were used. Results were normalized to the beta-glucuronidase (GUS) housekeeping gene and expressed in percentages.

Statistical analyses were carried out by MedCalc statistical software, version 5.0. The Pearson correlation coefficient was employed for statistical analysis of SSTR and ER1 expressions. Obtained r values were tested using the equation published by Nishioka et al. [8]. P values less than 0.05 were considered statistically significant.

Results

SSTR1–3 and ER1 receptors were expressed in all the 69 examined adenomas. SSTR4 and SSTR5 were

detectable in only 82% and 58% of them, respectively. Absolute median values of mRNA expression were: 3283 copies/ μl for SSTR1 (range 910–242070), 3600 copies/ μl for SSTR2 (1413–148681), 746 copies/ μl for SSTR3 (63–46914), 172 copies/ μl for SSTR4 (0–9923), 454 copies/ μl for SSTR5 (0–43777), and 3106 copies/ μl for ER1 (611–460786). Also, normalized data on SSTR4 and SSTR5 were lower compared with all other SSTRs. The median relative quantification values were: 63.8% (for SSTR1), 55.4% (SSTR2), 19.6% (SSTR3), 2.6% (SSTR4), 9.2% (SSTR5), and 75.4% (ER1). The relative expression data on each receptor are displayed in Fig. 1.

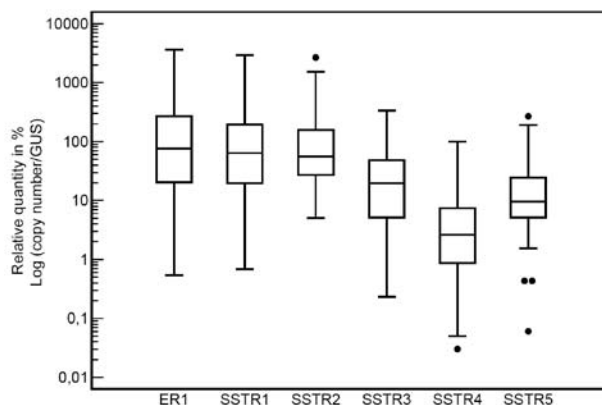


Fig. 1. The box-and-whisker diagram of SSTR and ER1 relative expression levels. The bottom and top of the box are the first and third quartiles, and the band inside the box is the second quartile (median). The whiskers extend to the minimum and maximum data values and dots represent outliers. The abbreviation GUS means beta-glucuronidase housekeeping gene.

We found significant correlations among SSTR1, SSTR2, and SSTR3. The highest one was between SSTR1 and SSTR2 ($r=0.94$; $P<0.001$; shown in Fig. 2), followed by the correlation between SSTR1 and SSTR3 ($r=0.87$; $P<0.001$), and SSTR2 and SSTR3 ($r=0.87$; $P<0.001$). The expression of ER1 did not significantly correlate with the expression of any somatostatin receptor subtype.

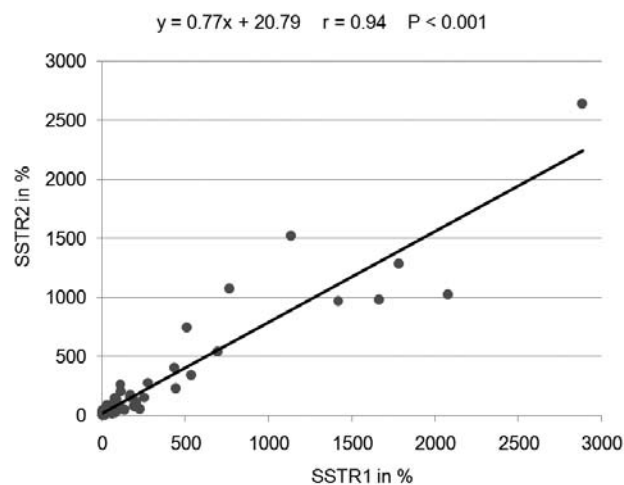


Fig. 2. Correlation between relative quantities of SSTR1 and SSTR2 mRNA transcripts in non-functioning pituitary adenomas.

Discussion

Despite the fact that SA are commonly used in the treatment of acromegaly, neuroendocrine tumors and Cushing's disease patients who cannot undergo surgery, or have postoperative residues [10, 11], transsphenoidal neurosurgery is still the first therapeutic approach to NFA. In most cases, the operation is followed by rapid improvement in headache and visual disturbances caused by tumor mass [5]. However, the extensive NFA surgery is very dangerous as very important nerve fibers and vessels are found near the pituitary gland.

Growing tumor remnants after the transsphenoidal adenectomy are a frequent indication for radiotherapy. From an Italian retrospective study it is evident that patients with adjuvant radiotherapy have a similar risk of tumor recurrence or regrow as patients without postoperative residue [12]. The contribution of postoperative radiotherapy is still controversial because it can be associated with long-term side effects: increased incidence of pituitary deficiencies, optic nerve atrophy and visual deterioration and two-fold raised cumulative risk of brain tumors up to 20 years after the operation [4, 5].

New biological drugs including synthetic somatostatin agonists (lanreotide, octreotide, pasireotide, etc.) could help with tumor shrinkage. The growth of remnants occurs at a faster rate in patients younger than the age of 61 [7].

Currently, several different techniques are able to provide important information about the number and functions of somatostatin receptors in the pituitary adenoma. Immunohistochemical analysis detects the presence of somatostatin receptor protein on adenoma cells *in vitro* using the standardized scoring system [13, 14]. Static positron emission tomography enables diagnostic imaging and preoperative semi-quantitative prediction of SSTR density in the pituitary [15]. Real-time PCR technology shows results of the quantitative analysis of the receptor gene expression in the adenoma normalized to housekeeping genes.

Our molecular biology data on SSTR and ER1 revealed individual expression profiles in NFA which showed a large biological variability with the SSTR1, SSTR2, and ER1 expression dominance. This fact makes the SA treatment of NFA difficult and in many cases could decrease the effectiveness of the SA monotherapy [4]. Also, if we take into account that ER1 mRNA relative quantity in NFA is higher than SSTR and that estrogen therapy increases SSTR2 and SSTR3 expressions [16], combination of SA and ER modulators could improve the NFA treatment effectiveness. Therefore, the SSTR and ER1 expression profile should be determined before initiating NFA therapy by SA and/or estrogen receptor modulators.

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