ORIGINAL ARTICLE

Expression of somatostatin receptor subtypes in primary and recurrent gonadotropinomas: are somatostatin receptors involved in pituitary adenoma recurrence?

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Abstract

OBJECTIVE: Surgical treatment of pituitary macroadenomas often fails because of tumor recurrence after the operation. The causes of tumor recurrence are complex, but one of them may be the high growth potential of the adenoma. As somatostatin receptors mediate antiproliferative, anti-angiogenic and pro-apoptotic actions, it seemed reasonable to investigate their expression in dependence on the adenoma recurrence.

METHODS: Samples of primary and recurrent gonadotropinomas excised surgically from patients were examined. This type of pituitary adenomas was chosen because of its relatively high recurrence rate. The adenoma phenotype and expression of somatostatin receptor subtypes 1–5 (SSTR 1–5) were investigated by immunohistochemistry, and the level of SSTR expression was semiquantitatively scored.

RESULTS: It was found that the adenomas undergoing the early recurrence have lower expression of SSTR 2A and 3 in comparison to those which did not recur during 5 years lasting observation. On the other hand, the recurrent tumors show higher expression of SSTR 1, 2A, 3 and 5 subtypes than their primary counterparts.

CONCLUSIONS: It is hypothesized that SSTR may, at least in part, counteract adenoma recurrence. On the other hand, it can be also presumed that the recurrent gonadotropinomas may be more sensitive to somatostatin analog treatment than primary ones. These hypotheses need to be confirmed in further studies.

INTRODUCTION

Surgical treatment of pituitary macroadenomas often fails because of the tumor recurrence after the operation. This complication is caused by incomplete excision of the tumor. The latter may be due to the large size of the tumor, its invasiveness resulting in the infiltration of the adjacent structures and the re-growth of the remnant tumoral mass. Gonadotropinomas, the adenomas expressing follicle-stimulating hormone (FSH), luteinizing hormone (LH) or their free α and/or β-subunits (α, β-SU), present a relatively high recurrence rate of nearly 30 % (Chanson & Brochier 2005). In our material, the percentage of the recurrent gonadotropinomas was even nearly 40% (Pawlikowski *et al.* 2010). Therefore, this type of pituitary adenoma was chosen for the present study. These tumors are a frequently-occurring subtype of pituitary adenomas, constituting about 30% of all operated pituitary macroadenomas (Pawlikowski *et al.* 2000) and the vast majority of clinically non-functioning pituitary adenomas (Chanson & Brochier 2005; Kunert-Radek *et al.* 2004). The main therapeutic management of this kind of tumor until today is the transphenoidal surgery (Chaidarun & Klibanski 2002; Jaffe 2006). The possibility of medical therapy is considered including the application of somatostatin (SST) analogs, dopamine agonists or combination of both (for review see: Colao *et al.* 2008). The majority of gonadotropinomas were shown to express the different somatostatin receptor (SSTR) subtypes (Pawlikowski *et al.* 2003; Pisarek *et al.* 2009). Our recent data show the following expression pattern: SSTR 3>SSTR 2B>SSTR 1=SSTR 2A>SSTR 5 (27.3%, 22.7%, 18.2 % and 13.6% respectively) (Pisarek *et al.* 2009). Since somatostatin receptors mediate the antiproliferative (Schally 1988; Pawlikowski *et al.* 1989; Lamberts *et al.* 1991; Florio 2008), anti-angiogenic and pro-apoptotic (Sharma *et al.* 1996; Voltering 2003; Hu *et al.* 2004) action of somatostatin and its analogs and their expression is a mandatory (although not always sufficient) condition of the positive effects of SST analogs therapy, it seemed reasonable to investigate the link between SSTR and the adenoma recurrence. The aim of the present study was to answer the question, whether the expression of SSTR in these tumors are linked with the risk of recurrence and whether primary and recurrent gonadotropinomas differ in SSTR expression level.

MATERIAL AND METHODS

This project received the approval of the Ethics Committee of Medical University of Łódź, nr: RNN/97/06/KE. Twenty-seven samples of gonadotroph pituitary adenomas were surgically excised from 17 patients (11 males and 6 females, aged 39–73 years, mean age 52.6 years). The mean age of patients with primary gonadotropinomas and with recurrent tumors was 51.6 years and 53.4 years, respectively. The tissue samples were fixed in 10% formalin or Bouin-Hollande fixative and paraffin embedded. The material was divided into 4 groups. The first group included 5 primary gonadotropinomas from patients in whom the recurrent tumors developed during 1–5 years of observation and re-operated (Group I; Gr I). As recurrent we considered the cases needing the second surgical intervention because the tumor re-growth. The second group consisted of 5 samples of the recurrent tumors removed from the same patients who belonged to the 1st group (Group II; Gr II). The third group was composed of 7 samples of primary gonadotropinomas without relapse for at least 5 years after surgery (Group III; Gr III) and the last one included all recurrent gonadotropinomas investigated (10 samples, Group IV; Gr IV).

The paraffin sections of all tumor samples were immunostained using poly- or monoclonal antisera against pituitary hormones to reveal the expression of FSH, LH and their αSU and/or β-SU. Immunohistochemical examination of somatostatin receptor subtypes was performed as described by Schulz *et al.* (1998) and Pawlikowski *et al.* (2003). In brief, 4–5 μm sections were first dewaxed in xylene and rehydrated in ethanol. The antigen retrieval procedure performed prior to primary antibody incubation consisted of two 5-min microwave treatments in 0.01 M citric acid buffer (pH 6.0) for at 720 W and one time at 420 W. To quench endogenous peroxidase activity, sections were incubated in methanol containing 1.5% H_2O_2 for 10 min at room temperature. Any non-specific binding was blocked by preincubation with 3% normal goat serum for 30 min at room temperature. The working dilution of antibodies (commercially-available rabbit polyclonal antisera raised against carboxyl-terminal fragments of specific human somatostatin receptor subtypes SSTR 1–5 including SSTR 2A and SSTR 2B isoforms from GRAMSCH Laboratories, Schwabhausen, Germany) was 1:1 000 (diluted in 0.05 M TRIS buffer, pH 7.6 containing 2% goat serum). Following overnight incubation in 4 °C in humidified chamber with primary antibodies, the tissues were treated with anti-rabbit IgG biotinylated goat antibody (1:800, DAKO, Denmark) and streptavidin complex (Strept ABC/HRP, DAKO, Denmark). The immunoreaction was visualized with 3.3'-diaminobenzidine (DAB, DAKO, Denmark) solution. For a negative control, the primary antibody was omitted and normal goat serum was used. At least six sections were examined per one tumor. The intensity of immunoreaction for specific receptor proteins was scored semiquantitatively using a descriptive scale as follows: strong staining (score: 3), moderate staining (score: 2), weak staining (score: 1) and trace staining (score: 0.5) and by two independent observers. The mean scores ± SEM were calculated and analysed statistically.

Statistical analyses

Statistical analyses were performed using a paired t-test for groups (I + II), Student's t-test for groups (III + IV)

and (I + III). A statistical estimation of the frequency of somatostatin receptor subtypes SSTR 1–5 expression, with at least moderate intensity was performed using a McNemar's test. In all analyses, a $p<0.05$ was considered as significant.

RESULTS

The results presented in the figures below are the mean scores of immunoreaction intensity. As can be seen there, the gonadotropinomas with later recurrences (Gr I) have lower scores of expression of the SSTR 1–5 in comparison to those which did not recur (Gr III). In the cases of SSTR 2A and SSTR 3 these differences were statistically significant (Figure 1). Moreover, the expression of SSTR 1–5 in the primary gonadotropinomas with later recurrences (Gr I) have significantly lower scores of SSTR subtypes as compared to their recurrent counterparts (Gr II). The differences were statistically significant for SSTR 1, 2A, 3, 5 (Figure 2). Visible differences, albeit not statistically significant, were observed between the group of primary gonadotroph pituitary tumors without recurrences (Gr III) and the group of all the recurrent gonadotropinomas (Gr IV) (Figure 3). Table 1 shows the frequency of incidence of the particular SSTR subtypes in the investigated groups of tumors. Only a moderate (score: 2) or stronger immunostaining was considered as significant SSTR expression. The differences in particular receptor subtype frequency were observed between the tumors groups. Significantly (as estimated by means of the McNemar's test) higher frequency of SSTR 1 and SSTR 2B incidence in Group II as compared to Group I was shown. Moreover, the SSTR 2A frequency was significantly higher in Group II than in Groups I and III. A similar tendency in frequency of SSTR 3 and SSTR 5 expression was also observed. Thus, SSTR 1, 2A, 2B, 3 and 5 are more frequent in recurrent tumors than in their primary counterparts. Additionally, in cases of SSTR 2A, 3 and 5, this tendency is also observed in comparison to the group of primary adenomas which did not recur (see also Figure 4, a–c).

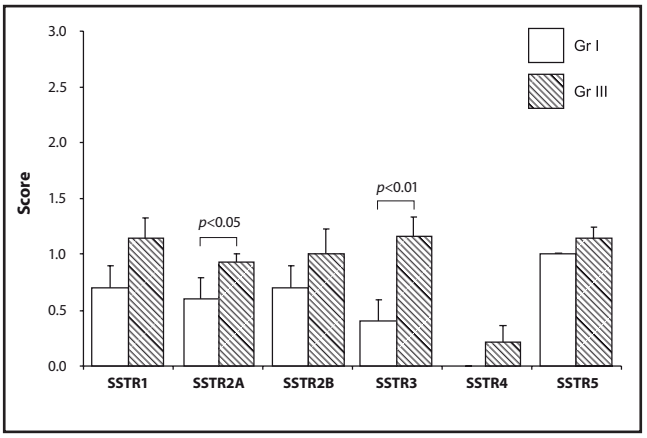

Fig. 1. Expression of the somatostatin receptor subtypes SSTR 1–5 in the group of primary gonadotropinomas which later recurred (Gr I) and in the group of primary gonadotropinomas which did not recur (Gr III). The immunoreaction intensity for specific receptor proteins was scored semiquantitatively using a descriptive scale as follows: strong staining (score: 3), moderate staining (score: 2), weak staining (score: 1) and trace staining (score: 0.5). The mean scores of reactions ± SEM were calculated. Statistical analysis was performed using Student's t-test, a $p<0.05$ was considered significant.

DISCUSSION

The data presented above show that the primary gonadotropinomas undergoing the early recurrence have a lower expression of SSTR 2A and 3 in comparison to those which did not recur during 5 years lasting observation. Although the present study uses a semiquantitative method to estimate the level of SSTR expression, earlier studies using the same approach demonstrated a positive correlation between immunostaining inten-

Tab. 1. The incidence of somatostatin receptor subtypes SSTR 1-5 with at least moderate (score: 2) intensity of immunostaining (IHC).

No	The groups of gonadotropinomas	SSTR 1	SSTR 2A	SSTR 2B	SSTR 3	SSTR 4	SSTR 5
1.	**Group I** primary gonadotropinomas which have later recurrences	0/5	0/5	0/5	0/5	0/5	0/5
2.	**Group II** the recurrences of the same primary tumors	3/5 *	3/5 **	3/5 *	4/5	0/5	3/5
3.	**Group III** primary gonadotropinomas which did not recur	1/7	0/7	1/7	1/6	0/7	0/7
4.	**Group IV** all recurrent tumors	3/10	4/10	4/10	5/10	0/10	3/10

Statistical analysis was made using McNemar's test, $p<0.05$ was considered significant.
* significant vs Group I; ** significant vs Group I and III.

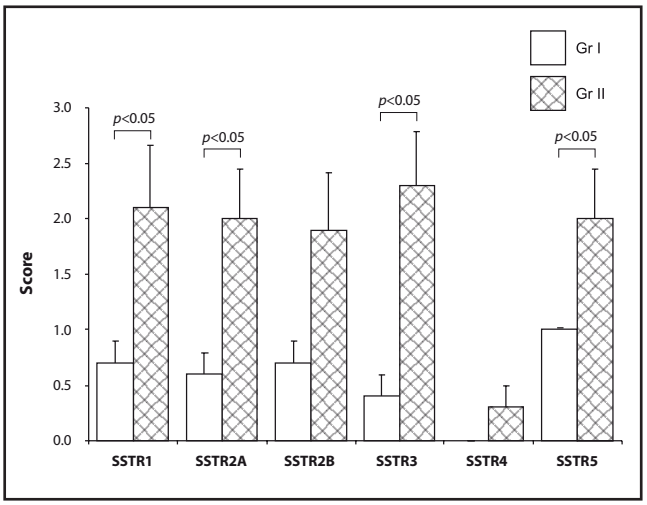

Fig. 2. Expression of the somatostatin receptor subtypes SSTR 1–5 in primary gonadotropinomas which have later recurrences (Gr I) and in the group of recurrences of the same primary tumors (Gr II). The immunoreaction intensity for specific receptor proteins was scored semiquantitatively using a descriptive scale as follows: strong staining (score: 3), moderate staining (score: 2), weak staining (score: 1) and trace staining (score: 0.5). The mean scores of reactions ± SEM were calculated. Statistical analysis was made using a paired t-test, a $p<0.05$ was considered significant.

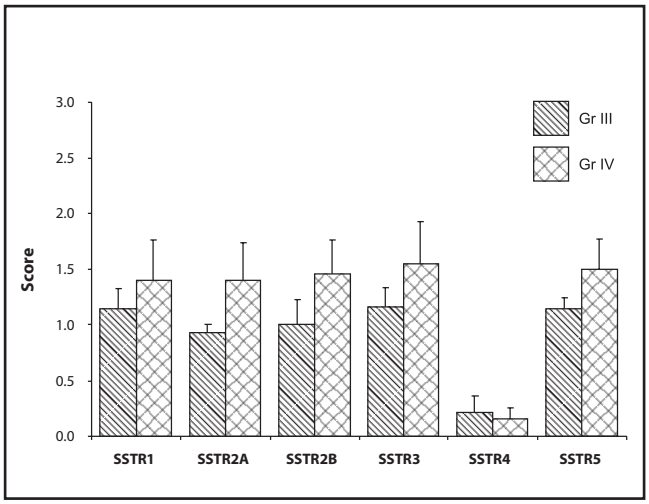

Fig. 3. Expression of the somatostatin receptor subtypes SSTR 1–5 in the group of primary gonadotropinomas which did not recur (Gr III) and in the group of all recurrent tumors (Gr IV). The immunoreaction intensity for specific receptor proteins was scored semiquantitatively using a descriptive scale as follows: strong staining (score: 3), moderate staining (score: 2), weak staining (score: 1) and trace staining (score: 0.5). The mean scores of reactions ± SEM were calculated. Statistical analysis was performed using Student's t-test, a $p<0.05$ was considered significant.

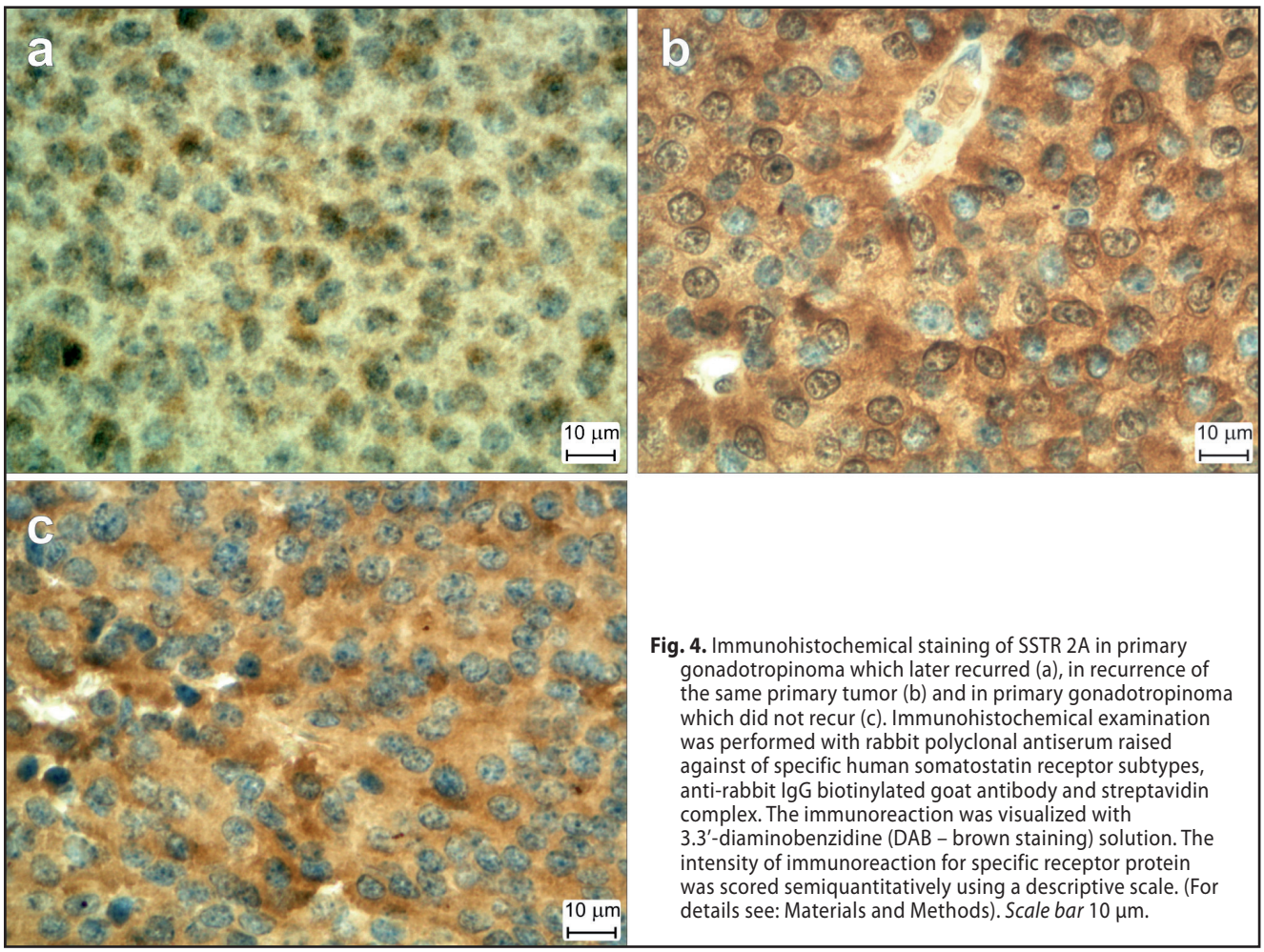

Fig. 4. Immunohistochemical staining of SSTR 2A in primary gonadotropinoma which later recurred (a), in recurrence of the same primary tumor (b) and in primary gonadotropinoma which did not recur (c). Immunohistochemical examination was performed with rabbit polyclonal antiserum raised against of specific human somatostatin receptor subtypes, anti-rabbit IgG biotinylated goat antibody and streptavidin complex. The immunoreaction was visualized with 3.3′-diaminobenzidine (DAB – brown staining) solution. The intensity of immunoreaction for specific receptor protein was scored semiquantitatively using a descriptive scale. (For details see: Materials and Methods). *Scale bar* 10 µm.

sity and biological response to somatostatin analogs (Gruszka *et al.* 2006; Pawlikowski *et al.* 2007; Pawlikowski *et al.* 2008). Papotti *et al.* (2002) showed that the expression of SSTR in gastroenteropancreatic neuroendocrine tumors (GEP/NET) decreases with increasing malignancy of tumors. A question arises whether the same regularity concerns also the pituitary adenomas. Taking into consideration that SSTR mediates the antiproliferative and pro-apoptotic effects in different tissues including the anterior pituitary (see the data cited in the Introduction), we hypothesize that high or low SSTR expression may contribute inversely (at least in part) to pituitary adenoma re-growth. Possibly that adenoma recurrence is favoured by low SSTR expression and counteracted by high SSTR expression. However, this presumption needs to be confirmed in further studies based on larger sample sizes, including also pituitary adenoma types other than gonadotropinomas.

Another novel observation made in the present study is the higher expression of SSTR 1, 2A, 3 and 5 subtypes in the recurrent gonadotropinomas than that in their primary counterparts. This finding is rather unexpected and the mechanism of the observed upregulation remains unknown. However, this observation may suggest that the recurrent tumors could be more sensitive to treatment with somatostatin analogs than primary tumors, because SSTR expression is a mandatory (although not always sufficient) condition of the positive effects of SST analogs therapy. However, this presumption needs the confirmation in the clinical studies comparing the effectiveness of SST analogs treatment of primary and recurrent gonadotropinomas. So far, the data concerning the treatment of clinically nonfunctioning adenomas (a group of adenomas which includes in majority gonadotropin-expressing adenomas) with currently applied SST analogs is not encouraging (Warnet *et al.* 1997; Colao *et al.* 2003; Colao *et al.* 2008). On the other hand, in vitro studies have shown that the receptor-selective somatostatin analogs significantly suppressed cell viability in the majority of cultures of this adenoma type (Zatelli *et al.* 2004; Gruszka *et al.* 2006) and the effect was correlated with SSTR immunostaining intensity score (Gruszka *et al.* 2006).

CONCLUSIONS

It is hypothesized that somatostatin receptors may, at least in part, counteract adenoma recurrence. On the other hand, it can be also presumed that the recurrent gonadotropinomas may be more sensitive to somatostatin analog treatment than primary ones. These hypotheses need to be confirmed in further studies.

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Conflict of Interest Statement

The authors declare that they have no competing interests.

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