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Evaluation of expression of somatostatin receptor 1, 2, 3, 5 and dopamine D2 receptor in spindle cell oncocytomas of posterior pituitary

Jiri Soukup[1] · Petra Kasparova[1] · Ales Kohout[1] · Boris Rychly[2] · Veronika Skarkova[3] · Martin Syrucek[4] · Filip Gabalec[5]

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Abstract

Purpose Spindle cell oncocytomas (SCOs) are very rare tumors of the posterior pituitary with potential for locally aggressive behaviour. Their treatment includes surgery and possibly radiotherapy, however other options are lacking. Somatostatin receptors (SSTs) are a possible therapeutic target for somatostatin analogues and their expression has been demonstrated recently in closely related pituicytomas, but there are no data about their presence in SCOs.

Methods We collected five cases of SCO from four patients including one recurrent case. Immunohistochemical detection of TTF1, GFAP, CD68, SST_1, SST_2, SST_3, SST_5 and D2 dopamine receptor (D2DR) was performed. Intensity, percentage of positive cells and pattern of expression was evaluated in semiquantitative fashion. Protein expression of SST_{1-5} and D2DR was further evaluated by western blot.

Results Mean patient age was 61.8 years (range 47–71 years) with male to female ratio 1:1. In one patient, samples from the original tumor and its recurrence 16 years later were assessed. TTF1 was positive in all five cases, no expression of GFAP and CD68 was seen. Immunohistochemical expression of SST_1 was noted in 1/5 cases, SST_2 in 2/5 cases, including recurrent case but not the original case. SST_3 was expressed in 3/5 tumors and D2 dopamine receptor in 4/5 cases. Western blot was successfully performed in four samples. SST_2, SST_3 and D2DR expression was identified in all the samples, including two cases originally negative for SST_2 and one case negative for SST_3 by immunohistochemistry. The number of positive cells and level of expression varied among different areas of the same tumors. No expression of SST_5 was observed. In the patient with the recurrent tumor, intensity of SST_2, SST_3 and D2DR expression varied between original tumor and its recurrence.

Conclusions We demonstrated presence of different SST subtypes and D2DR in spindle cell oncocytomas. The most commonly expressed subtype was SST_2 and SST_3, while no expression of SST_5 was observed. Expression showed spatial heterogeneity and temporal changes as seen in the recurrent case. The biological meaning of SSTs expression in SCOs is unclear as well as whether it may be exploited in treatment of selected cases.

Keywords Spindle cell oncocytoma · Posterior pituitary · Somatostatin receptors · Predictive markers

✉ Jiri Soukup
2jiri.soukup@gmail.com

[1] The Fingerland Department of Pathology, University Hospital and Faculty of Medicine Hradec Kralove, Charles University, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

[2] Cytopathos s.r.o, Kutuzovova 255/23, 831 03 Bratislava, Slovakia

[3] Department of Medical Biology and Genetics, Faculty of Medicine Hradec Kralove, Charles University, Simkova 870, 500 03 Hradec Kralove, Czech Republic

[4] Pathology Department, Na Homolce Hospital, Roentgenova 2, 150 30 Prague 5, Prague, Czech Republic

[5] 4th Department of Internal Medicine, University Hospital and Faculty of Medicine Hradec Kralove, Charles University, Sokolska 581, 500 05 Hradec Kralove, Czech Republic

Introduction

Spindle cell oncocytomas (SCO) are very rare tumors arising in posterior lobe of the pituitary gland [1]. In contrast to adenomas, they are supposed to arise from pituicytes and share immunophenotypic features, such as nuclear expression of TTF1 with closely related pituicytoma and granular cell tumor of posterior pituitary [1]. Although originally considered indolent, these lesions may repeatedly recur and contribute to significant morbidity [2]. The current treatment of choice is a complete surgery and radiotherapy in selected cases. Compared to the pituitary adenomas in which treatment with somatostatin analogues and dopamine agonists is an option in selected cases, no targeted therapy for SCOs is available so far.

Somatostatin receptors (SSTs) are transmembrane receptors belonging to the G-protein coupled receptor superfamily. There are five genes encoding closely related proteins SST_1, SST_2, SST_3, SST_4 and SST_5 [3]. These are variably expressed in normal neuroendocrine tissues and tumors including pituitary adenomas [3–5]. Recently, extensive review about SST including extensive description of signalling pathways and therapeutic implications was published [6]. We recommend going to that article for more details.

Dopamine receptors are transmembrane receptors belonging also to the G-protein coupled receptor superfamily. There are five genes encoding closely related proteins D1–D5. Among them, dopamine D2 receptor (D2DR) is the most important target for dopamine agonist treatment in pituitary adenomas [7].

Recently, expression of somatostatin receptors (SSTs) has been reported in some cases of pituicytomas [8] revealing possible novel therapeutic options in these rare tumors. However, the data about expression of neither SSTs, nor D2DR in SCOs are available so far. Since SSTs and D2DR are a possible therapeutic target, expression of both in SCOs may represent a rationale for targeted therapy in patients with tumors unamenable to standard therapeutic approaches.

Materials and methods

The archives of three pathology institutes were searched for cases diagnosed as a spindle cell oncocytoma. The consent for tissue collection was obtained from the patients before the surgery. The clinical data were retrieved from patients' files of the institutions when available. All samples have been formalin fixed and paraffin embedded. 4-µm-thick sections were cut for routine H&E staining and additional immunohistochemical studies. The list of antibodies and relevant details of immunohistochemistry protocols used are

Table 1 Antibodies used in the study

Antibody	Clone	Dilution	Manufacturer	Pretreatment	Incubation time	Visualisation
TTF1	8G7G3/1	1:100	Dako, Glostrup, Denmark A/S	Ventana CC1, 64 min	32 min	Ventana ultraView
GFAP	EP672Y	Prediluted	Ventana, Basel, Switzerland	Ventana CC1, 32 min	16 min	Ventana ultraView
CD68	PG-M1	1:50	Dako, Glostrup, Denmark A/S	Ventana CC1, 64 min	32 min	Ventana ultraView
Ki67	30-9	Prediluted	Ventana, Basel, Switzerland	Ventana CC1, 32 min	16 min	Ventana OptiView
SSTR1	UMB7	1:100	Abcam, Cambridge, MA, USA	Tris–EDTA pH9, 97 °C, 20 min	30 min	DAKO EnVision FLEX
SSTR2	Polyclonal, Atlas antibodies	1:75	Sigma Aldrich s.r.o. CR	Ventana CC1, 64 min	64 min	Ventana OptiView
SSTR2	UMB1	1:1500	Abcam, Cambridge, MA, USA	Ventana CC1, 36 min	32 min	Ventana ultraView
SSTR3	UMB5	1:750	Abcam, Cambridge, MA, USA	Ventana CC1, 20 min	36 min	Ventana ultraView
SSTR5	UMB4	1:750	Abcam, Cambridge, MA, USA	Ventana CC1, 32 min	32 min	Ventana OptiView
D2DR	Polyclonal	1:200	Origene, Rockville, MD, USA	Ventana CC1, 16 min	32 min	Ventana OptiView
BRAF V600E	VE1	Prediluted	Ventana, Basel, Switzerland	Ventana CC1, 64 min	32 min	Ventana OptiView

summarized in Table 1. Normal pancreas was used as an on-slide positive control for $SST_{2,3}$ and SST_5 whereas a normal pituitary was used as a control tissue for SST_1 and dopamine receptor D_2. Ventana Cell Conditioning solution (CC1) was used for pretreatment in detection of majority of antibodies, employing heat induced epitope retrieval (HIER) in high pH. The sections staining was carried out on Benchmark Ultra stainer from Ventana/Roche with either Ventana ultraView Universal DAB detection kit or Ventana OptiView DAB IHC detection kit: both methods use avidin–biotin complex method with horseradish peroxidase as an enzyme and DAB (3,3′-diaminobenzidine) as chromogen. Sections staining for SST_1 was performed in Agilent/Dako Autostainer 48, using PT-Link pretreatment system and EnVision Flex detection kit for visualisation. All slides were subsequently counterstained with haematoxylin.

All cases were reviewed by a single pathologist (J.S.). The tested markers have been evaluated in semiquantitative fashion for the intensity of the stainings (0—no positivity, 1—mild, 2—moderate, 3—strong) and the percentual number of positive cell. Patterns of positivity (i.e. presence of membranous or cytoplasmic staining) were noted. Ki67 count was performed on 200 cells in hot-spots.

For a western blot assay, three 15 µm thick paraffin sections were cut from each sample. A limited size of sample in case 1 did not allow to obtain enough material for the analysis. Cases 2, 3 and 5 were composed entirely of tumor tissue, in case 4, a small area of normal pituitary was identified comprising around 10% of overall cellularity of the sample. Due to the location among the tissue fragments, we were unable to perform successful microdissection and the tissue was processed altogether. New H&E stained sections were made after collection of material for western blot to evaluate possible presence of normal pituitary tissue in deeper levels that might confound western blot results. Tissue of a healthy pituitary gland from an autopsy was used as a positive control and processed the same way. Protein extractions was performed using Qproteome FFPE Tissue Kit (Qiagen) according to the recommendations described in the kit. The sections were deparaffinised and then transferred into Qproteome FFPE Tissue Kit buffer. Approximately 1.5 mm^3 tissue was processed in 100 µl of buffer. Proteins was extracted according to the protocol provided and stored frozen at − 20 °C [9]. Protein concentrations were determined using the QuantiPro BCA protein assay kit, according to the instructions provided by the supplier (Sigma Aldrich, Czech Republic). Sizing, quantitation, and purity assessments for all tested samples were provided using Agilent 2100 Bioanalyzer system. Samples were diluted to the same concentration and then boiled for 5 min/95 °C in SDS sample buffer (Tris–HCl pH 6.8, 40% glycerol, 6% SDS, 0.2 M DTT, 0.1 g bromphenol blue). Thereafter 30 µg of sample were loaded onto SDS/polyacrylamide gel and separated proteins were transferred to a PVDF membrane (100 V, 90 min). Membranes were incubated at 25 °C for 1.5 h with TBST—milk solution [10 mM Tris–HCl (pH 8.0), 150 mM sodium chloride, and 0.1% Tween 20, 5% non-fat dry milk (TBST)]. Primary antibodies were diluted according to the manufacturer's protocols in TBST containing 2% of milk or 2% of BSA at 4 °C overnight followed by six 5 min washes in TBST. Following primary antibodies were used: SST_1, clone UMB7, 1:1500, Abcam; SST_2, clone UMB1, 1:5000, Abcam; SST_3, clone UMB5, 1:1500, Abcam and D2 Dopamine receptor, polyclonal, 1:2000, Merck-Millipore, cat. no. AB5084P. Beta-actin (clone AC-15, 1:10 000; Sigma-Aldrich) was used in the assay as a housekeeping protein to demonstrate successful protein extraction. After washing, the membranes were incubated with secondary peroxidase-conjugated antibodies (1:20 000, 2 h, 25 °C) and repeatedly washed with TBST. The signal was developed with a chemiluminescence ECL Prime Western Blotting Detection Reagent (Amersham, GE Healthcare Life Science) and quantity of chemiluminescence was detected using Imaging System (Gel Logic 2200 Pro). Relative intensity of chemiluminescence was assessed, using control tissue as a reference.

Results

Patients and samples

In total, five tumours from four patients were retrieved (Table 2). Two samples (case 4 and 5) from the same patient represented specimens from the first surgery and the recurrence that occured 16 years later. The mean age of patients at the time of initial diagnosis was 61.8 years with male to female ratio 1:1. Clinical details and follow-up data for cases 1 and 2 were not available. In case 3, patient presented with visual field disturbances. At the time of the surgery, tumour measured 28 mm in a largest diameter and only small reduction of its volume was reached due to the perioperational bleeding, leaving the patient with large tumour residue. Six months later at the time of writing of this paper, the patient is alive and well, with improvement of visual parameters. Patient 4 presented also with visual field disturbances. The tumour measured initially 25 mm in diameter and the first surgery in 1998 left a residue measuring 8 mm in diameter. Until 2004 (6 years), the residue had showed no progression during regular MRI scans. At that time, however, the patient was lost for follow-up and returned only 10 years later in 2014 with recurrence measuring 25 mm in diameter invading right carotic sinus. After the second surgery no subsequent clinical follow-up in our institution was available.

Table 2 Patients data and immunohistochemistry results

	Sex	Age	Ki67 (%)	SSTR1	SSTR2 (UMB1 clone)	SSTR3	SSTR5	D2DR
Tumour 1	Male	71	7	Negative	Tumour negative, focal endothelial positivity	Tumour negative, focal endothelial positivity	Negative	Negative
Tumour 2	Male	62	3	1 + < 1%, membranous	Tumour negative, focal endothelial positivity	1+, 10%, cytoplasmic, focal endothelial positivity	Negative	1+, 90% cytoplasmic
Tumour 3	Female	67	7	Negative	1–2+, 10% membranous; 1+, 90% cytoplasmic, endothelium negative	Tumour negative, focal endothelial positivity	Negative	1–2+, 80%, cytoplasmic
Tumour 4	Female	47	4	Negative	Tumour negative, focal endothelial positivity	1–2+, 10%, cytoplasmic, focal endothelial positivity	Negative	1+, 30%, cytoplasmic
Tumour 5—recurrence of tumour 4	female	63	9	Negative	1–2+, 20% membranous, focal endothelial positivity	1–2+, 30%, cytoplasmic, focal endothelial positivity	Negative	2+, 30%, cytoplasmic

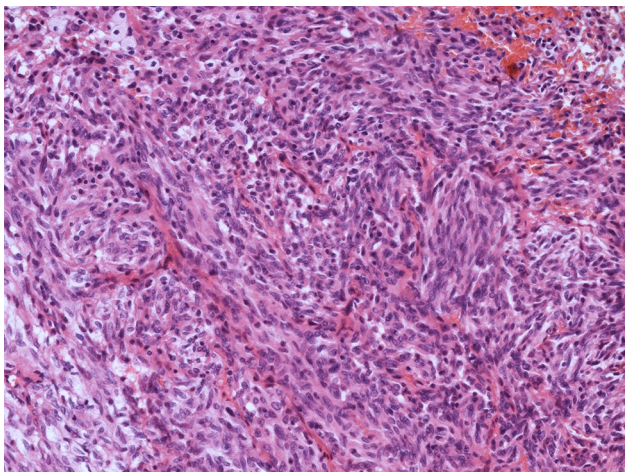

Fig. 1 The tumors were composed of spindle to oval, eosinophilic cells (case 4, × 100 H&E)

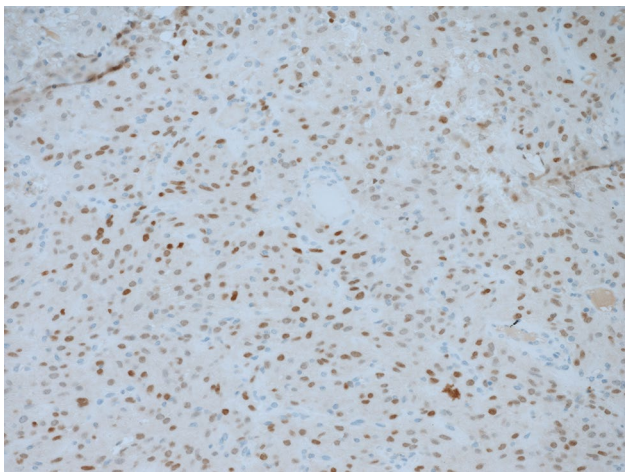

Fig. 2 Nuclear expression of TTF1 in tumor cells (case 2, × 100, TTF1 immunohistochemistry)

Microscopic and immunohistochemical findings

Histologically, all tumors were composed of spindled to ovoid cells growing in short fascicles or whorls. Majority of the cells showed eosinophilic cytoplasm with fine granularity, although there were areas, where the cells showed rather clear cytoplasm with limited granularity, or the cytoplasm had rather homogenous than truly granular character. The nuclei were mostly uniform and regular, oval-shaped, with scattered cells showing mild to moderate cytologic atypia, and often with presence of nucleoli (Fig. 1). Mitotic activity was noted only in case 4 and its recurrence (case 5), reaching 1 mitosis/10 HPF in both. The tumours stained uniformly for TTF1 (Fig. 2), albeit with variable intensity from case to case. CD68 was positive in the small fraction of the cells dispersed in all tumours. GFAP was uniformly negative. Ki67 stained in average 6% of cells. Immunohistochemical detection of mutated form of BRAF V600E was negative in all cases. $SST_{1–5}$ and D2DR expression pattern is summarized in Table 2. Only faint membranous positivity of SST_1 was observed in isolated cells (< 1%) in case 2 (Fig. 3). SST_2 expression was detected using two different antibodies (Abcam monoclonal UMB1 and polyclonal antibody). Using polyclonal antibody, no expression was observed. Monoclonal antibody showed weak to moderate membranous expression in cases 3 and 5 (Fig. 4). In addition in case 3, widespread weak cytoplasmic positivity was observed. In all but one (case 3) focal positivity of staining in endothelium of tumour vessels was observed. In contrast, no positivity was observed in endothelium in normal pituitary tissue contained in case 4 nor in vessels of the positive control. SST_3 was regularly expressed in endothelium of

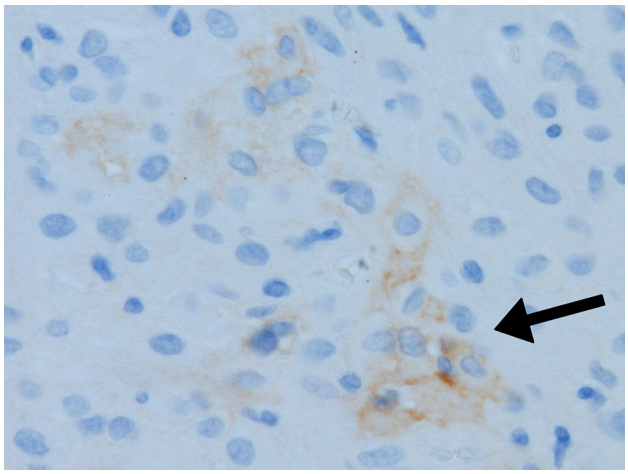

Fig. 3 Only scarce tumor cells in case 2 expressed SST_1 (original magnification × 400, detail, SST_1 immunohistochemistry, arrow: positive cells)

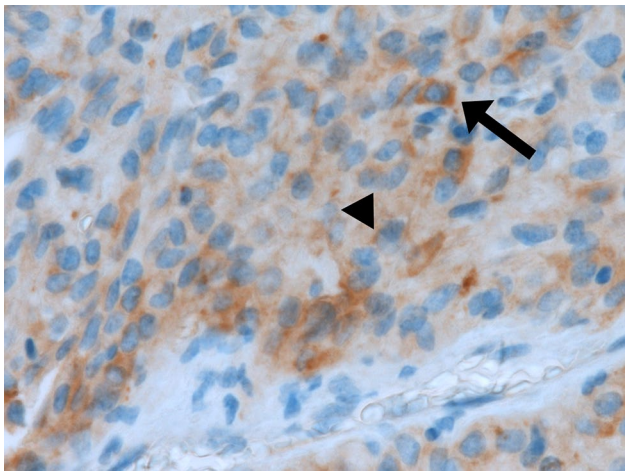

Fig. 5 Moderate SST_3 positivity in case 5 (original magnification × 400, detail, SST_3 immunohistochemistry, arrow: moderate positivity, arrowhead: mild positivity)

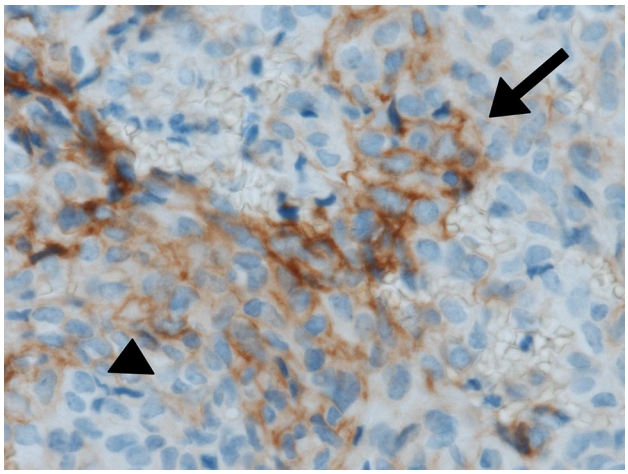

Fig. 4 Mild to moderate membranous positivity of SST_2 in case 5 (original magnification × 400, detail, SST_2 immunohistochemistry, arrow: moderate positivity, arrowhead: mild positivity)

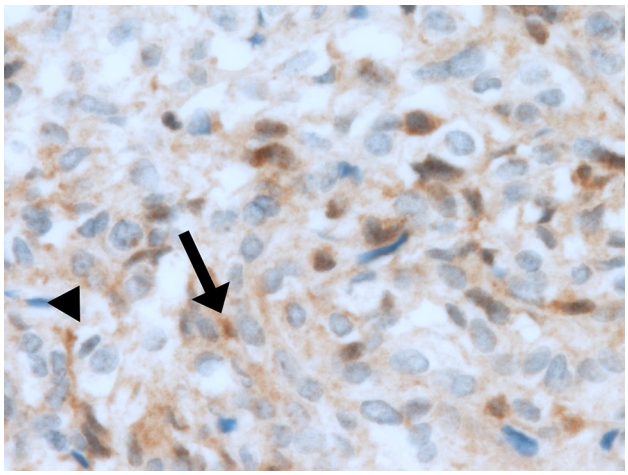

Fig. 6 Mild to moderate positivity of D2DR in case 5 (original magnification × 400, detail, D2DR immunohistochemistry, arrow: moderate positivity, arrowhead: mild positivity)

tumoral vessels. Mild to moderate cytoplasmic positivity of SST_3 was noted in three cases (2, 4 and 5—Fig. 5) including recurrent case and membranous positivity of isolated cells in case 4 was noted. SST_5 staining was negative in all tested cases. D2DR was expressed in all but one case (1), showing weak to moderate cytoplasmic intensity in tumor cells (Fig. 6). Positive controls for SST_2, SST_3, SST_5 and D2DR are showed in Fig. 7.

Western blot results

In order to validate the results, western blot analysis was performed. Due to the insufficient amount of material, case 1 was not analysed. Protein isolation was successful in all four remaining samples (case 2–5) and the control tissue (normal pituitary) as demonstrated by a positive band of beta-actin of predicted size (Fig. 8). In all four cases, expression of SST_2, SST_3 and D2DR was detected, resulting in bands of predicted sizes (75 kDa for SST_2, 75 kDa for SST_3 and 50 kDa for D2DR). Anti-SST_1 antibody did not stain positively control sample; although weak bands of predicted size were observed in cases 2 and 4, these results were disregarded. Relative quantification was performed, and the control tissue was used as a reference value (1.0). Mean SST_2 level of expression in the cohort was 1.24; the strongest expression was observed in

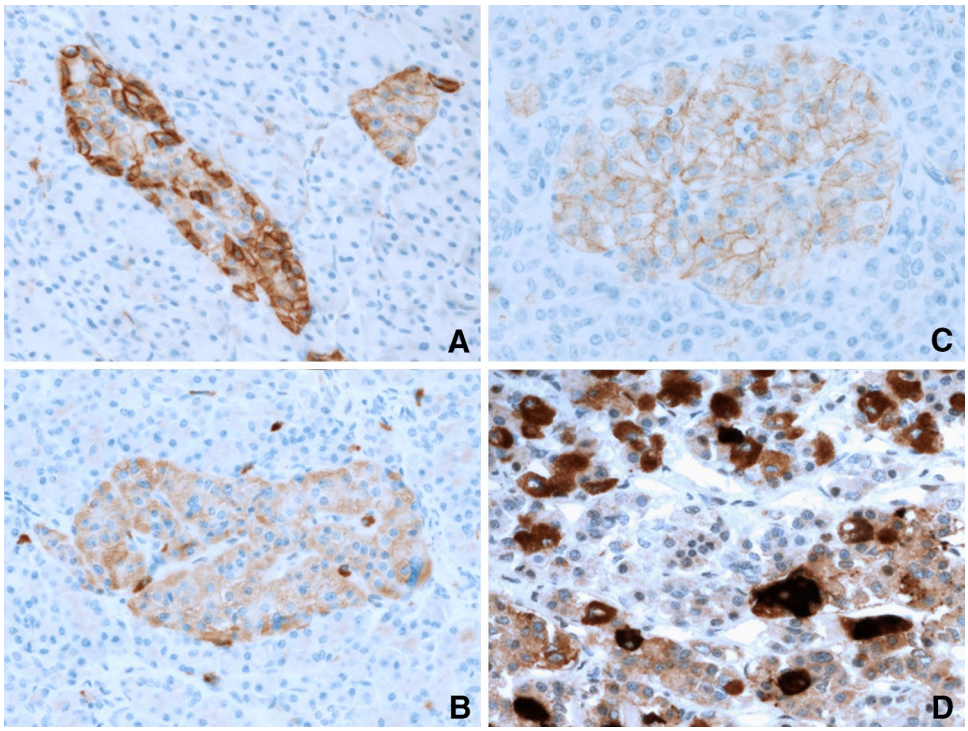

Fig. 7 Result of staining of control tissues—**a** SST_2, pancreatic islets, original magnification × 200, detail; **b** SST_3, pancreatic islets, original magnification × 200, detail; **c** SST_5, pancreatic islets, original magnification × 200, detail; **d** D2DR, normal pituitary, original magnification × 200, detail

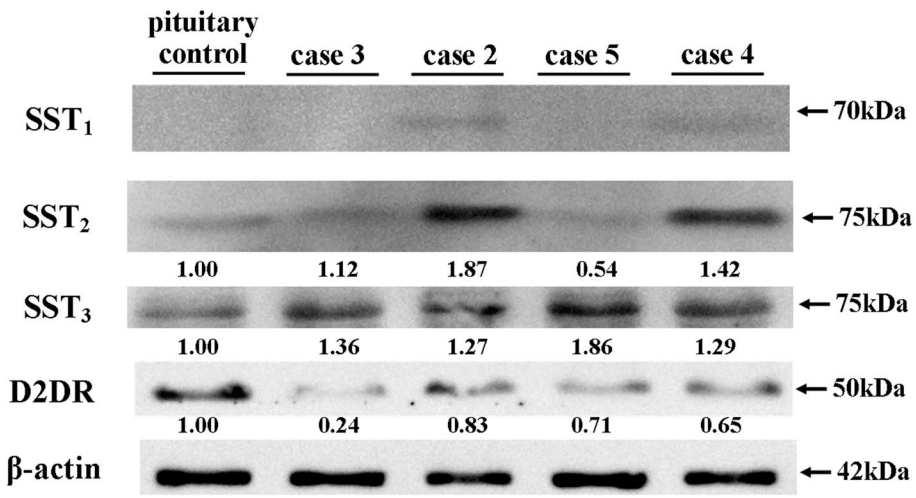

Fig. 8 Comparison of SST_1, SST_2, SST_3 and D2DR protein expression in cases 2, 3, 4 and 5 using western blot analysis. Beta-actin was used as a loading control

case 2 (1.87). Mean SST_3 level of expression in the cohort was 1.45; the strongest expression was observed in case 5 (1.86). Mean D2DR level of expression in the cohort was 0.61; the strongest expression was observed in case 2 (0.83). The expression data are shown in Fig. 8. New H&E slides made after the sampling for the blot showed no appearance of any normal pituitary tissue in cases 2, 3 and 5. In case 4, a small proportion of a normal pituitary comprising < 5% was still present.

Discussion

Spindle cell oncocytomas (SCOs) are very rare tumors arising in posterior lobe of pituitary gland. The tumors consist of spindled cells arranged in short fascicles or whorls. The cells usually have strongly eosinophilic and delicately granular cytoplasm containing multiple mitochondria [10]. SCOs are characterised by expression of a

TTF1 transcription factor, a common feature shared with another two other tumors of posterior pituitary—namely pituicytoma (PC) and granular cell tumor (GCT). TTF1 is expressed in specialised glial cells (pituicytes) which represent the main cellular population of the posterior pituitary lobe. Due to the shared ultrastructural characteristics and TTF1 expression, pituicytes were implied as a cell of origin for all three of primary posterior pituitary tumours (SCO, PC, GCT) [1]. Although all three tumors are considered grade I in the current WHO classification, particularly SCOs have been well known for a more aggressive behaviour characterised by repeated tumor recurrences, especially if removed incompletely [2, 11]. Due to the local anatomical conditions, surgical treatment is limited and patients with unresectable tumors are usually subjected to local radiation treatment with Leksell gamma knife.

SSTs are targets for pharmacologically active somatostatin analogues: octreotide, lanreotide and pasireotide [12]. Octreotide and lanreotide show the highest affinity towards SST_2 and SST_5, while pasireotide binds with higher affinity to SST_1 and SST_3 as well [13]. Although SSTs are expressed in many different types of cancers (i.e. gliomas, renal cell carcinomas, colorectal cancer), treatment with somatostatin analogues proved to be so far of clinical significance only in pituitary adenomas and neuroendocrine tumors. The data and previous experience in other solid tumors thus implicate that response to the somatostatin analogues treatment cannot be predicted solely on the SSTs expression [14].

SSTs expression was reported in pituicytomas recently. Mende et al. evaluated expression of $SST_{2,3}$ and SST_5 with immunohistochemistry in 10 cases from 9 patients. Their study showed variable levels of expression of SST_3 and SST_5 in 7/10 of cases and weak positivity of SST_2 in 3/10 of cases. In our study, SST_2 expression was not detected by the polyclonal antibody. Immunohistochemistry with monoclonal antibody UMB1 however detected weak to moderate membranous and cytoplasmic expression in 2/5 cases. These data are in line with previous study [15], where increased sensitivity of UMB1 clone compared to other polyclonal antibodies has been demonstrated. The recurrent SCO (case 5) showed SST_2 expression, whereas the original tumor removed during the first surgery (case 4) was negative. This could suggest either temporal variation in SSTs expression during tumor development and progression or a selection of SST expressing subclone due to previous surgery. This idea would not be unsound, given the fact that SSTs were expressed only in a fraction of cells in the tumors in our series. From the practical standpoint, such a finding would warrant further reevaluation of SSTs expression after repeated surgeries, if it proved to be of predictive value in the future. Unexpectedly, endothelial positivity of SST_2 in intratumoral vessels was observed in 4/5 tumours but not in positive controls nor in normal pituitary tissue available in case 4. Endothelial expression of SST_2 was described previously in schwannomas [16] and in normal endothelium of human cornea [17] and its presence may reflect different biological features of newly formed intratumoral vessels. Immunohistochemical expression of SST_3 was detected in 3/5 of cases, including the recurrent case that showed increase in expression compared to the primary tumour. The expression was predominantly cytoplasmic with rare membranous positivity in case 4. Such a pattern of positivity might reflect increased rate of receptor internalisation [18, 19] with low level of subsequent redistribution into cellular membrane [20]. The patients in our study were not treated with somatostatin analogues prior to the surgery, thus such a finding imply a role of endogenous somatostatin in the process. SST_1 expression was generally absent, with only a few cells in case 2 showing weak expression. SST_5 was not detected in any of the cases and none of the tumours in our file stained positive for V600E mutated form of BRAF, this finding is consistent with previously published data [1].

Using western blot assay, expression of SST_2 and SST_3 was identified in 4/4 analysed cases including two cases negative for SST_2 (case 2 and case 4) and 1 case negative for SST_3 (case 3) by immunohistochemistry. Western blot quantification furthermore did not correlate with immunohistochemical quantification. There are several possible explanations of these phenomena. First, in all negative tumors, there was at least some expression of SST_2/SST_3 in tumor vessels that might contribute to the positive blot results. Given the ratio of the tumor cells and the endothelia, this would probably make only a small contribution, however the exact quantification of SSTs expression in endothelium was beyond the scope and methodical background of this study. Second, standard paraffin sections for immunohistochemistry were 4 µm thick, however, the protein lysate for western blot was isolated from three 15 µm thick tissue sections, meaning that almost 11 times larger number of tumor cells was examined in the blot assay. Since there was a clear spatial variability of expression among the tumor cells, blot results may be simply more representative due to the higher number of evaluated cells. Third, there could have been loss of some protein material during the protein isolation phase: since the initial amount of tissue in the cases 2 and 4 was significantly higher (due to the amount of the tissue in the blocks) compared to the case 3 and 5, even an equal absolute loss of proteins during the isolation would lead to more pronounced relative reduction of expression in the western blot results. Fourth, western blot provides more sensitive and exact method of detection compared to the immunohistochemistry: immunohistochemical reaction may not detect small concentrations of the protein dispersed on the slide and thus lead to false negative results while the blot technique

basically evaluates "protein concentrate". In this situation, potential clinical importance of such low expression levels undetectable by immunohistochemistry would be questionable. Lastly, in case 4, there were small fragments of normal pituitary gland that we were unable to dissect before the blot assay. Cells of normal pituitary comprised around 10% of overall cellularity and thus they could contribute to the expression of SST_2 and SST_3 in this case. However, SST_3 was detected in tumor cells by immunohistochemistry and it is questionable, whether such a small fraction of cells would justify higher SST_2 expression than that seen in the control sample composed entirely of the normal pituitary. In cases 2, 3 and 5, no normal pituitary tissue was identified in serial H&E sections made before and after sampling for the western blot.

Due to the small size of the cohort, no tendency was possible to identify in the relationship between blot results and immunohistochemistry.

Mean expression levels of both SST_2 and SST_3 in the blot assay were higher in the tumors compared to the normal pituitary tissue. Blot results demonstrated quantitative differences in SST_2 and SST_3 between primary tumor (case 4) and its recurrence (case 5), with similar results for SST_3 compared to the immunohistochemistry but different for SST_2.

Expression of D2 receptor has never been assessed in $TTF1^+$ tumours of posterior pituitary. D2DR is the main receptor expressed in pituitary tissues and main pharmacological target of dopamine antagonists, used commonly for treatment of prolactinomas and less frequently also for treatment of other pituitary adenomas. In our file, 4/5 cases showed expression of D2DR, ranging from 30 to 90% of cells. Intensity was low to moderate, with cytoplasmic pattern of expression. This pattern of positivity has been reported repeatedly in the literature in other tumors [21–23] and the expression was shown to correlate with results of western blot assay in previously published literature [23]. These data correspond with results of more detailed studies dealing with subcellular kinetics of D2R distribution [24]. D2DR expression was positively verified by the western blot in all four cases with positive immunohistochemistry results. Mean level of expression was lower compared to the normal pituitary. The levels of expression differed between blot and immunohistochemistry, although with lesser variability than levels of SST_2 and SST_3.

Most pituitary adenomas express D2DR, but the expression is quite heterogeneous. The predictive value of immunohistochemical or quantitative PCR analysis of D2DR expression and their potential use for further management of the patients with pituitary adenomas is still debated [25]. Thus the use of dopamine agonists in the treatment of adenomas, except prolactinomas, is still a matter of controversy.

The other morphological and immunohistochemical findings in our cohort supported previous accounts on spindle cell oncocytomas, with respect to their morphology, consistent positivity of TTF1 and lack of GFAP expression.

Conclusion

This is the first study in the literature addressing the expression of SSTs and D2R in spindle cell oncocytomas. Since these tumours are extremely rare but potentially locally aggressive, it is important to explore targets for possible novel therapeutic strategies. By immunohistochemistry and western blot, we have demonstrated expression of D2DR, SST_2 and SST_3 in several cases of SCOs. The results of both methods have not fully correlate. In one patient with recurrent disease, differences in expression implied possible temporal dynamics of SSTs expression during the evolution of the lesion. However, given the complexity of SSTs signalling system and previous experience with somatostatin analogues treatment in other solid tumours, it is difficult to estimate the precise biological and predictive value of our findings. Therefore, more biological and clinical studies are needed for further validation of our findings and their possible clinical use.

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