ORIGINAL ARTICLE

Mapping of somatostatin receptor types in GH or/and PRL producing pituitary adenomas

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Background: Somatostatin is a tetradecapeptide exerting inhibitory action on endocrine and exocrine cell secretion and proliferation. Somatostatin receptors (SST) are widely expressed in various neoplasms including endocrine tumours. Using immunohistochemistry, the expression of SST_1, SST_{2A}, SST_{2B}, SST_3, SST_4, and SST_5 was studied in tissue microarrays (TMAs), using a series of 90 human pituitary adenomas producing growth hormone and/or prolactin, including 30 of each somatotroph, lactotroph, and mixed somatotroph/lactotroph adenoma type.

Methods: For immunohistochemistry, the standard avidin biotin complex method enhanced by tyramide was used, using polyclonal antisera for all SST types. A four point scoring system was used to assess the membranous immunopositivity.

Results: All SST types were positive in all tumour types, showing varying immunoreactivity scores. SST_5 and SST_{2A} were the predominant receptors, showing strong expression in high frequency in all three adenoma types. Strong expression of SST_1 was higher in lactotroph adenomas than in other tumour types.

Conclusions: The immunohistochemical results of SST expression are in agreement with most findings of previous molecular studies. The fact that SST_{2A} expression is predominant suggests that pharmaceutical octapeptide somatostatin analogues may act through this receptor, while the role of SST_{2B} may be merely synergistic.

Somatostatin imhibits cell secretion and proliferation via five specific G-protein coupled membrane receptors.[1–3] Somatostatin receptors (SST) 1, 3, 4, and 5 are encoded by intronless genes, whereas SST_{2A} and SST_{2B} are generated through alternate mRNA splicing of the SST_2 gene.[4] All SST types have been detected by reverse transcriptase PCR mRNA analysis or binding assays in human endocrine tumours; pituitary adenomas, gastroenteropancreatic neuroendocrine tumours, medullary thyroid carcinomas, lung small cell carcinomas, and pheochromocytomas.[5–10] Since the introduction of SST immunohistochemistry in 1998,[11] a few studies on endocrine tumours have appeared in the literature.[6 12–19]

Tissue microarrays (TMAs) are collections of many tissue samples in a single paraffin block. The technique enables rapid, inexpensive, and large scale screening of archival material with histochemistry, immunohistochemistry in situ hybridisation, and fluorescent in situ hybridisation; .[20–23]

The aim of this study was to investigate using immunohistochemistry and TMAs the expression of SST types in a large number of pituitary adenomas producing growth hormone (GH) and/or prolactin (PRL). This study is the first immunohistochemical investigation of all SST types in functioning pituitary adenomas and in correlation with the histological type.

MATERIALS AND METHODS

In total, 30 unselected cases of somatotroph, 30 lactotroph, and 30 mixed somatotroph–lactotroph adenomas, from the last 5 years, were retrieved from the files of the Pituitary Tumor Reference Center, Department of Pathology, G. Gennimatas Athens General Hospital. All cases were classified by histology, histochemistry, and immunohistochemistry for all adenohypophysial hormones (GH, PRL, adrenocorticotropic hormone, β-thyroid stimulating hormone, β-follicle stimulating hormone, β-luteinising hormone, and the α-subunit of glycoprotein hormones).

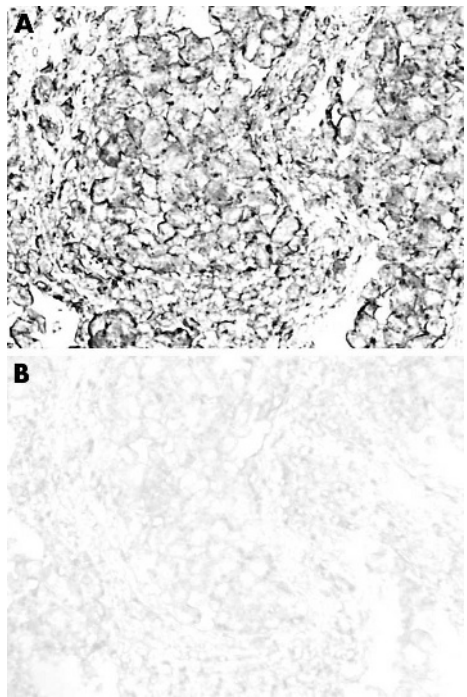

Figure 1 Positive (A) and negative (B) control for SST_1 in breast carcinoma (original magnification ×20).

Three TMAs were constructed, one for each adenoma type. For the TMAs, two samples of each tumour were selected from well preserved areas of the original paraffin block, after

Abbreviations: GH, growth hormone; PRL, prolactin, SST, somatostatin receptors; TMA, tissue microarray

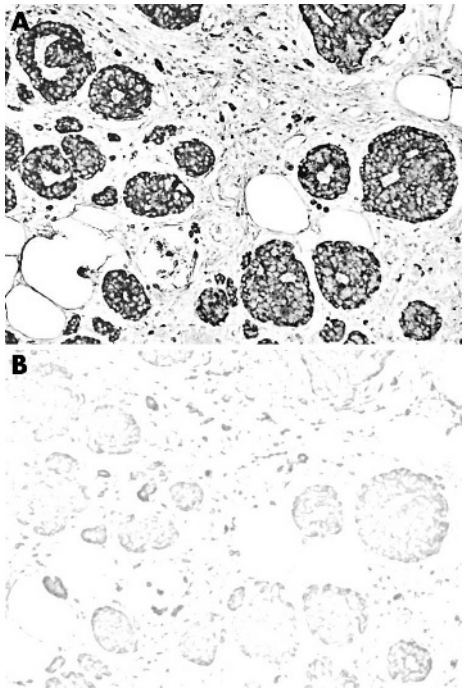

Figure 2 Positive (A) and negative (B) control for SST_3 in breast carcinoma (original magnification ×20).

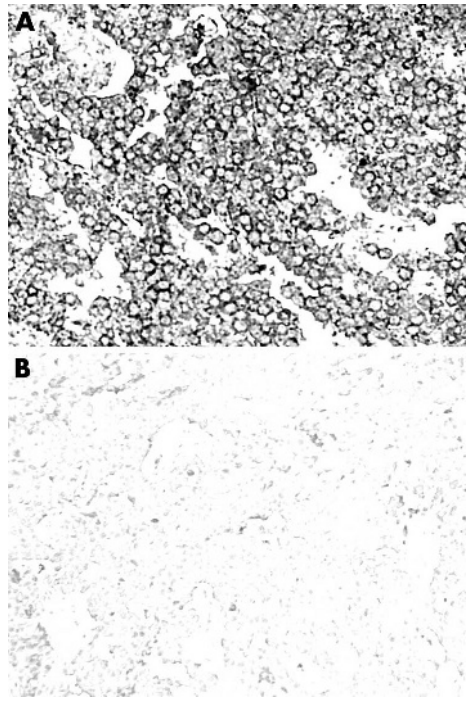

Figure 3 Positive (A) and negative (B) control for SST_4 in medullary thyroid carcinoma (original magnification ×20).

review of the haematoxylin and eosin stained diagnostic slides. For immunohistochemistry, polyclonal antisera against all SST types (1, 2A, 2B, 3, 4, and 5) were used (dilution 1:3000; Gramsch Lab, Schwabhausen, Germany). The standard ABC method using the Elite Vectastain detection system (Vector Laboratories Inc., Burlingame, CA, USA) was applied, with tyramide amplification, as described previously.[24] Sections, microwaved in citrate buffer pH 6.0 for 20 min, were then incubated with the primary antibodies overnight at 4°C. A second series of sections, where the antiserum was substituted by citrate buffer, served as negative control. All SST antisera used in our study have been repeatedly tested by other investigators in various tumours.[14 16 17 19 24] GH producing pituitary adenomas have been proved to be the most well established tumours for SST_2 and SST_5 expression by clinical and in vitro techniques, and also by octreoscan for SST_2.[6 10 25–32] Additional tissues from invasive duct carcinomas of the breast were used as controls for SST_1 and SST_3 and medullary thyroid carcinomas for SST_4[15 24] (figs 1–3). For the assessment of SST membranous immunopositivity, a four point scoring system was used, similar to that used for Her-2/neu in breast carcinomas (table 1).

RESULTS

All SST types were positive in all tumour types showing varying immunoreactivity scores. In somatotroph adenomas, SST_5 and SST_{2A} predominated, positive in 100% and 96.5% of the tumours respectively, followed by SST_{2B}, SST_4, SST_3, and SST_1 (figs 4 and 5). SST_5 and SST_{2A} also showed the highest frequency in score 3+ (56.5% and 49% respectively). In mixed somatotroph lactotroph adenomas, the predominant receptors were also SST_5 and SST_{2A} (95.6%), followed by SST_4, SST_1, SST_3, and SST_{2B} (figs 6, 7). The highest numbers of 3+ scores were found in SST_5 and SST_{2A} (53.3% and 43.2% respectively). Lactotroph adenomas showed the highest expression of SST_5 overall (85.3%) and expression with a score of 3+ (58.8%). Scores of 3+ occurred at lower frequency for SST_{2A} and SST_1, followed by SST_3 and SST_4, with an absence of SST_{2B} (fig 8).

Heterogeneity of SST expression was detected among the various histological types and among tumours of the same type. SST_5 was the predominant receptor in all three adenoma types (fig 9). Strong SST_{2A} expression was mostly found in somatotroph and mixed somatotroph–lactotroph adenomas. SST_{2A} expression exceeded that of SST_{2B} in all adenoma types (figs 10, 11). Strong expression of SST_1 was the highest in lactotroph adenomas (fig 12).

Cytoplasmic immunoreactivity was noted in the majority of tumours, occasionally making it difficult to detect incomplete or moderate membranous stain scores (1+ and 2+).

For further investigation of antigen heterogeneity, SST immunohistochemistry was performed on unselected cases using the original paraffin blocks. All SST types showed varying immunopositivity throughout the tissue section. However, comparison with the tissue microarray cores revealed no major differences in the immunohistochemical scoring. During sectioning of the microarray blocks, loss of one sample of the duplicate was detected in 15% of cases, while both samples were lost in about 4%.

Table 1 Scoring system for the evaluation of SST immunopositivity, similar to Her-2/neu in breast carcinoma

Score	Staining results	% of cells stained	Membrane staining pattern
0	+/−	<10%	–
1+	+	>10%	Faint or incomplete
2+	+	>10%	Weak to moderate, complete
3+	+	>10%	Strong, complete

DISCUSSION

Octapeptide somatostatin analogues are particularly effective in regulating hormonal hypersecretion in somatotroph and thyrotroph adenomas.[25] Given that responsiveness to therapy is directly related to the receptor status of the adenoma, in vitro techniques for reliable SST detection on tumour cells are of clinical importance.[26] Detection of SST can be achieved by: (*a*) receptor mRNA analysis using reverse transcriptase PCR or in situ hybridisation, and (*b*) receptor protein analysis using radioligand binding studies. These commonly used techniques are demanding in time and skill, involve radioactivity, and may produce results with poor cellular resolution. It should also be noted that tumour mRNA levels may not necessarily reflect the protein receptor levels.[4 26] Therefore, morphological localisation of the receptor protein on cell membranes is necessary for precise evaluation of the tumour receptor status.[26] Immunohistochemistry on formalin fixed paraffin embedded tissues enables the localisation of all receptor protein types with high cellular resolution and correlation with tissue morphology. However, careful optimisation is required before it can be applied to routine pathology.

In our study, TMAs enabled screening of all SST in a large series of adenomas. Duplicate samples from each donor tumour considerably decreased antigen heterogeneity and risk of complete tissue loss during sectioning.

In somatotroph adenomas, SST_5 and SST_{2A} predominated. The expression of SST_5 and SST_{2A} in somatotroph adenomas has also been frequently reported in previous studies with mRNA receptor analysis and receptor autoradiography.[6 10 27–32] To our knowledge, this study is the first time that SST_{2B} has been detected in somatotroph adenomas, because most molecular and binding studies have not discriminated between the two subtypes of receptor 2. In a single report of SST mRNA analysis in three somatotroph adenomas, the results were negative for SST_{2B}, while SST_{2A} was present.[29] In our study, SST_{2B} was expressed in the great majority of somatotroph adenomas, however, the level of 3+ expression was considerably less that that for SST_{2A}. SST_1 and SST_3 were detected in a considerable number of cases in our series, although scores of 3+ were infrequent. Our results are keeping with previous SST mRNA studies, in which SST_1 was also detected in GH secreting adenomas, whereas SST_3 was more variably expressed.[6 30 33–35] We also noted SST_4 in many somatotroph adenomas, but scores of 3+ were infrequent. Previous mRNA studies in somatotroph adenomas reported SST_4 in only a few sporadic cases.[29 30] As emphasised previously, tumour mRNA levels may not necessarily reflect the protein receptor levels.[4 26] Indeed, recent comparative studies of SST mRNA analysis and immunohistochemistry reported conflicting results in some tumours.[14 17]

Results from mixed somatotroph–lactotroph adenomas were similar to somatotroph adenomas. Mixed somatotroph–lactotroph adenomas in the literature are usually classified in the GH producing tumours. In a unique molecular study, which discriminated pure GH secreting adenomas from mixed GH/PRL secreting tumours,[31] SST_2 and SST_5 were the predominant receptors in both adenoma groups. In addition, quantitative analysis showed no significant differences in the mRNA levels of these receptors between the two adenoma types. Analogous results with no major differences in SST_{2A} and SST_5 overall expression and scores of 3+ were observed in our study. Furthermore,

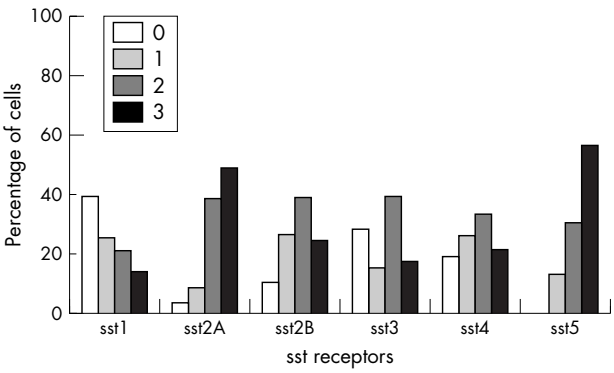

Figure 4 This histogram presents the frequency of each SST receptor in somatotroph adenomas. The vertical axis shows the percentage of positive tumours for each receptor according to the four point scoring system. The grey scale is immunopositivity.

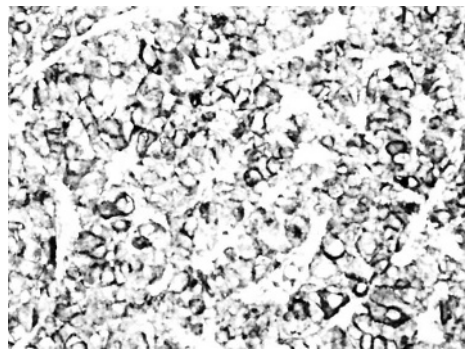

Figure 5 Score of 3+ for SST_3 in a somatotroph adenoma. Complete and strong membranous immunopositivity was seen in the majority of adenoma cells (original magnification ×40).

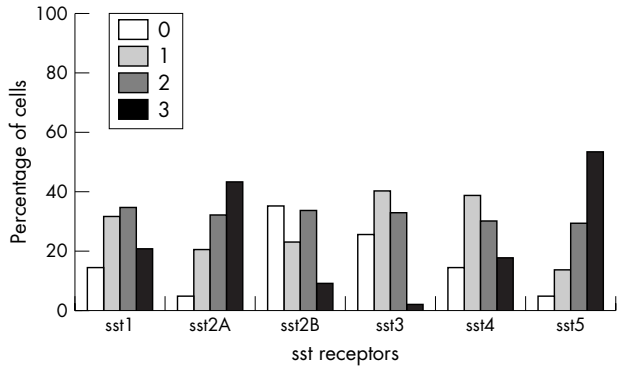

Figure 6 SST type frequency in mixed somatotroph–lactotroph adenomas.

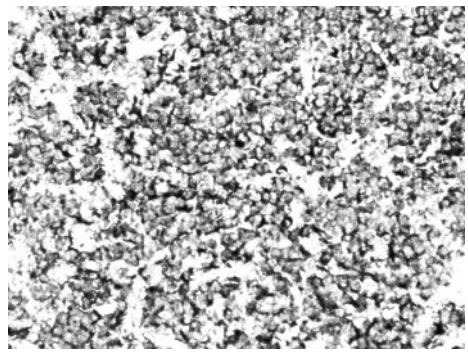

Figure 7 Moderate immunoreactivity for SST_4 in a mixed somatotroph–lactotroph adenoma (original magnification ×20).

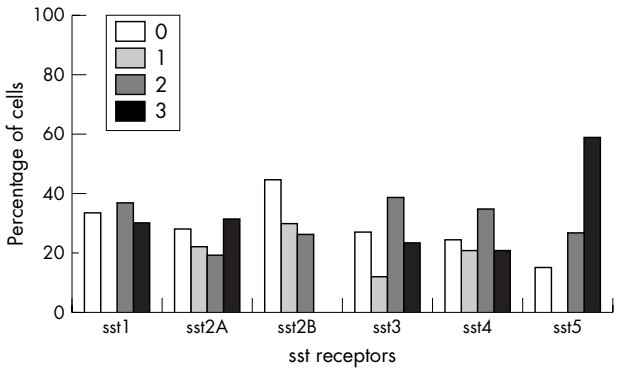

Figure 8 SST type frequency in lactotroph adenomas.

Jaquet[31] reported average SST_5 mRNA levels 10 fold higher than SST_2 levels, showing a predilection over SST_5 expression in both types of tumours. This was comparable with our findings, where SST_5 showed the highest frequency in both somatotroph and mixed somatotroph–lactotroph adenomas.

In lactotroph adenomas, the predominant receptor was also SST_5, followed by SST_{2A}. The stronger expression of SST_1 compared with the acromegaly related tumours seems to be a characteristic of prolactinomas. According to mRNA studies the most frequently expressed receptors in human prolactinomas were SST_1,[27 29 34 36 37] SST_5,[28 34 37] and SST_2.[29 30 33 34] Jaquet[37] showed by quantitative mRNA analysis that SST_2 levels were magnitudes lower compared with SST_5 and SST_1, which predominated. Our observations showing frequent and strong expression of SST_5, SST_{2A}, and SST_1 are compatible with these results.

We detected cytoplasmic immunostaining of varying degree in several adenomas, an observation previously reported for other neuroendocrine tumours.[12 17–19] Intracellular SST localisation is not an artefact, and it can be attributed to agonist induced receptor internalisation,[26] a main mechanism for tumour tachyphylaxis, during the therapeutic management with somatostatin analogues.[38] Given that internalised receptors are considered inactive for agonist binding, only membranous immunopositivity reflects the functional receptor status of the tumour.

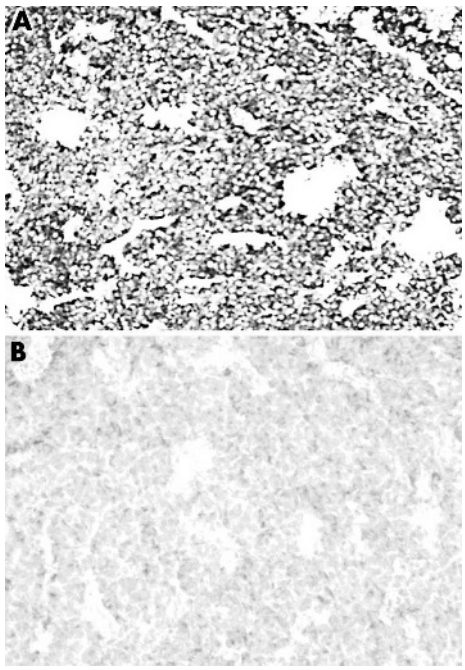

Figure 9 Strong immunoreactivity for SST_5 in (A) a somatotroph adenoma; (B) the respective negative control (original magnification ×10).

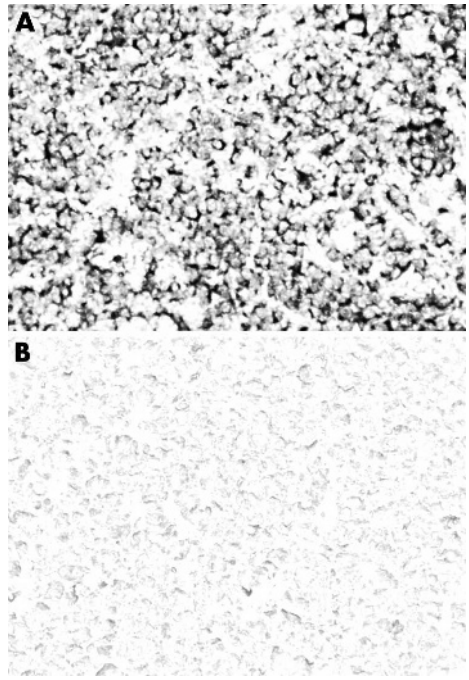

Figure 10 Strong immunoreactivity for SST_{2A} in (A) a mixed somatotroph–lactotroph adenoma; (B) the respective negative control (original magnification ×20).

The efficacy of octapeptide somatostatin analogues in acromegaly treatment is attributed to their high binding affinity, mainly to SST_2, expressed in GH secreting adenomas.[25 39] Our findings for the first time provide clear and direct morphological evidence for this assumption, demonstrating the presence of the SST_2 protein on the cytoplasmic membrane of tumour cells. SST_{2A} expression exceeded that of SST_{2B} in all tumour types examined, suggesting that somatostatin analogues may exert their pharmaceutical action mainly through SST_{2A}, while the role of SST_{2B} may be merely synergistic.

In tissue culture studies, the SST_5 preferential compound BIM-23268, or the SST_2 and SST_5 bispecific compound BIM-23244, achieved a more potent GH inhibition compared with octreotide in some adenoma experiments.[40] Our results, showing high SST_5 density in the majority of GH producing tumours, provide morphological support to these experimental observations. Assessment of SST tumour profile by immunohistochemistry may clarify the in vivo resistance of some adenomas from acromegalic patients to octreotide therapy. Furthermore, it may contribute to the application of novel therapeutic modalities with bispecific[40] or universal somatostatin analogues, such as SOM 230,[41] in the management of both GH and GH/PRL secreting adenomas.

Clinical experience has shown that octapeptide somatostatin analogues are ineffective in regulating hyperprolactinemia in patients with lactotroph adenomas.[42] In our study, strong expression of SST_{2A} was detected in one third of the tumours, suggesting that the receptor although present, may not play an active role in the regulation of PRL secretion. There is evidence from cultures of human pituitary adenomas that SST_5 exclusively mediates PRL release, unlike GH, which is regulated by both SST_2 and SST_5.[43] Besides the receptor density, other

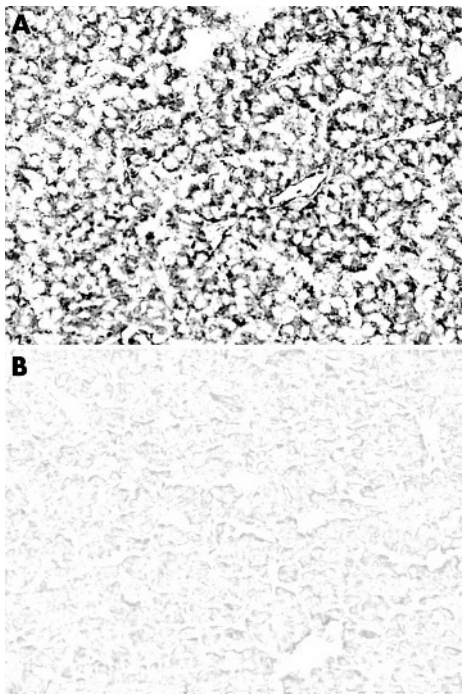

Figure 11 Strong immunoreactivity for SST_{2B} in (A) a pituitary somatotroph adenoma; (B) the respective negative control (original magnification ×20).

mechanisms such as formation of homodimers and heterodimers regulate the receptor signalling system and determine the response of hormone secretion to somatostatin analogues. In vitro studies have shown that SST_5 and SST_1 form heterodimers, resulting in increased ligand affinity and modified SST functionality.[44] The investigators suggested that upregulation of certain receptors such as SST_1 in heterodimers may lead to desensitisation of the other SST types, in order to maintain an overall normal somatostatin responsiveness in pituitary cells.[44] Formation of such heterodimers with upregulated function could be an explanation for the possible desensitisation of SST_{2A} in prolactinomas.

Immunohistochemistry, useful for SST characterisation of gastroenteropancreatic and lung endocrine tumours, generally has a good correlation with reverse transcriptase PCR results.[14 17] In the present study, we showed that immunohistochemistry can serve as a tool for detecting SST expression in pituitary adenomas. Strong SST_2 immunoreactivity was observed in a single insulinoma case that was very sensitive to octreotide treatment, indicating that the efficacy of somatostatin analogues depends on SST density on tumour cells.[18] Based on this observation, we suggest that scores of 3+, corresponding to high receptor density, may indicate tumours that are more susceptible to therapy. However, extensive clinicopathological studies are necessary to correlate the SST immunohistochemical profile of pituitary adenomas with their responsiveness to somatostatin analogues.

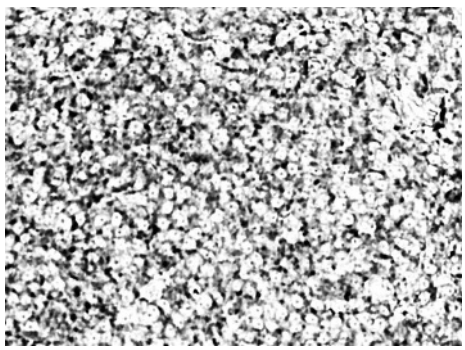

Figure 12 Moderate to strong membranous distribution of SST_1 immunoreactivity in a lactotroph adenoma (original magnification ×20).

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- Somatostatin is a tetradecapeptide exerting inhibitory action on endocrine and exocrine cell secretion and proliferation.
- Somatostatin receptors (SST) are widely expressed in various neoplasms including endocrine tumours.
- The expression of six SST types was examined in a tissue microarray series of 90 human pituitary adenomas producing growth hormone and/or prolactin, using polyclonal antibodies.
- All SST types were positive in all tumour types, but SST_5 and SST_{2A} were the predominant receptors. Strong expression of SST_1 was higher in lactotroph adenomas than in other tumour types.
- The immunohistochemical results agree with most previous studies.
- The study suggests that pharmaceutical octapeptide somatostatin analogues may act through the SST_{2A} receptor, while the role of SST_{2B} may be merely synergistic.

In conclusion, we provide morphological evidence for the predominance and high membrane density of SST_{2A} and SST_5 in GH producing adenomas, a result in keeping with the action of octapeptide somatostatin analogues through these receptor types in acromegaly. The higher SST_{2A} expression compared with SST_{2B} further suggests that SST_{2A} may play a more critical role in the efficacy of these analogues. SST immunohistochemistry, applied in pituitary adenoma pathology, may become a useful tool for the selection of patients for complementary treatment with the present or new somatostatin analogues. The immunohistochemical demonstration of all SST types in pituitary adenomas may contribute to the design and validation of new SST type selective or consensus somatostatin analogues.

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