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JNS

LABORATORY INVESTIGATION

Octreotide therapy in meningiomas: in vitro study, clinical correlation, and literature review

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OBJECTIVE Meningiomas express somatostatin receptor subtype 2 (SST2), which is targeted by the somatostatin analog octreotide. However, to date, using somatostatin analog therapy for the treatment of these tumors in clinical practice has been debated. This study aims to clarify the in vitro effects of octreotide on meningiomas for precise clinical applications.

METHODS The effects of octreotide were analyzed in a large series of 80 meningiomas, including 31 World Health Organization (WHO) Grade II and 4 WHO Grade III tumors, using fresh primary cell cultures to study the impact on cell viability, apoptosis, and signal transduction pathways.

RESULTS SST2 mRNA was detected in 100% of the tested meningiomas at levels similar to those observed in other SST2-expressing tumors, neuroendocrine tumors, or pituitary adenomas. Octreotide significantly decreased cell proliferation in 88% of meningiomas but did not induce cell death. On average, cell proliferation was more inhibited in the meningioma group expressing a high level of SST2 than in the low-SST2 group. Moreover, octreotide response was positively correlated to the level of merlin protein and inversely correlated to the level of phosphorylated p70-S6 kinase, a downstream effector of the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway. Octreotide inhibited Akt phosphorylation and activated tyrosine phosphatase without impacting the extracellular regulated kinase (ERK) pathway.

CONCLUSIONS Octreotide acts exclusively as an antiproliferative agent and does not promote apoptosis in meningioma in vitro. Therefore, in vivo, octreotide is likely to limit tumor growth rather than induce tumor shrinkage. A meta-analysis of the literature reveals an interest in octreotide for the treatment of WHO Grade I tumors, particularly those in the skull base for which the 6-month progression-free survival level reached 92%. Moreover, somatostatin analogs, which are well-tolerated drugs, could be of interest for use as co-targeting therapies for aggressive meningiomas.

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KEY WORDS meningioma; therapy; octreotide; somatostatin; merlin; SST2

Surgery is the primary course of treatment for patients with meningioma, and radiotherapy is used when tumors are inoperable. As of now, there is no consensus in favor of chemotherapy;[9] hence, it is rarely used in these patients' care.

Meningiomas express somatostatin receptor (SST) subtype 2 (SST2).[14,15,43] This molecular characteristic is targeted in clinical practice when performing SPECT imaging (with radiolabeled octreotide) for the differential diagnosis of skull base tumors.[30]

Octreotide and lanreotide, both SST2 agonists, are pivotal therapeutic drugs for the treatment of somatotroph adenomas and gastroenteropancreatic neuroendocrine tumors (GEP-NETs), which are slow-growing tumors similar to meningiomas. These somatostatin analogs are used not only to suppress hormonal hypersecretion but also to

ABBREVIATIONS βGus = β-glucuronidase; BrdU = bromodeoxyuridine; ERK = extracellular regulated kinase; GEP-NET = gastroenteropancreatic neuroendocrine tumor; mTOR = mammalian target of rapamycin; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; PFS6 = 6-month progression-free survival; SST = somatostatin receptor; SST2 = SST subtype 2; VEGF = vascular endothelial growth factor; WHO = World Health Organization.

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control tumor growth. However, in meningiomas, the use of somatostatin analogs remains incidental.

Data from preclinical studies in numerous models have provided evidence for direct and indirect mechanisms by which somatostatin analogs exert antitumor effects.[7] Direct antitumor activity is mediated through the SST expressed in tumor cells and results from blocking cell division or inducing apoptosis, depending on the SST subtype and cell type. SST2 prevents cell growth by activating specific tyrosine phosphatases (SHP1, SHP2) and inhibiting the Ras/extracellular regulated kinase (ERK) and PI3K/Akt pathways, leading to the induction of cyclin-dependent kinase inhibitors and cell cycle arrest.[16,37]

Somatostatin analogs also exert numerous indirect antitumor effects, including 1) inhibition of growth factors and hormone secretion that drive tumor growth; 2) induction of antiangiogenic effects that reduce tumor blood flow, particularly by inhibiting vascular endothelial growth factor (VEGF) secretion; and 3) promotion of immunomodulatory effects to stimulate the body's natural antitumor mechanism.[37]

The somatostatin analogs have demonstrated antineoplastic activities in slow-growing neuroendocrine tumors and have good tolerability and safety profiles. Given these characteristics, they are attractive candidates for the treatment of patients with meningioma, particularly when long-term treatment is required. Some clinical studies have analyzed octreotide efficacy in patients with meningioma, yet their conclusions remain disputed and are sometimes undefined.[10,17,18,21,23,38,40,42,45] Moreover, the results of octreotide treatment in meningioma cells in vitro have been contradictory.[4,26] Therefore, should we reject octreotide for meningioma therapy?

To clarify the direct antitumor effects of octreotide, we conducted an in vitro study using a large set of human meningiomas that included all histological subtypes and World Health Organization (WHO) Grade I, II, and III tumors. Moreover, we analyzed the signal transduction pathways triggered by octreotide and correlated the inhibition of cell proliferation to cellular markers. We also performed a meta-analytic review of all clinical data from the literature.

Methods

Materials

Octreotide was obtained from Novartis International AG.

Primary Cell Culture of Fresh Human Meningiomas

The study was performed on human meningiomas from 80 patients (Supplementary Table). The WHO grade (using 2007 criteria) for each tumor was determined by neuropathological review: 45 WHO Grade I tumors, 31 WHO Grade II , and 4 WHO Grade III. The present study was approved by the ethics committee of Aix-Marseille University and was conducted after obtaining informed consent from each patient. Briefly, freshly harvested tumor fragments were minced into small pieces (< 1 mm^3) and disaggregated into single cells by exposure to 0.37% type I collagenase (ThermoFisher Scientific Inc.) for 2 hours. Cells were resuspended in complete medium (1:1 ratio of DMEM high glucose [4.5 g/L] and F12 media, supplemented with 10% fetal bovine serum and 100 U/ml each of penicillin, streptomycin, and glutamine).[20,36] The experiments were performed within the first 2 weeks after surgery and before the third subculture. Throughout this time period, the tumor cells in primary culture maintained their SST2 expression levels and their response to octreotide (Fig. 1).[20] Experiments were performed on randomly selected tumors based on the quantity of tumor cells available after tumor dissociation (Supplementary Table).

Cell Viability

Cell viability was assessed by luminescent cell viability assay (Cell Titer-Glo, Promega Corp.) performed in triplicate on 24-well plates containing 2×10^4 meningioma cells per well. Twenty-four hours after seeding, the cells were incubated in low-serum media (5%) and treated with octreotide (10^{-10} to 10^{-8} M) for 3 days. All cell viability studies were performed on Day 3 because the cells were still proliferating and had not yet reached confluence. Cell viability results in treated versus untreated cells were expressed as a mean percent of the control. Direct cell counting was also performed on 3 tumors using a Scepter Automated Cell Counter (EMD Millipore Corp.).

BrdU Incorporation Assay

A total of 4×10^3 cells were seeded into each well of a 96-well plate. After 24 hours, cells were incubated in low-serum media and treated with octreotide (10^{-9} M) for 2 days. On the 3rd day, bromodeoxyuridine (BrdU) was added to a final concentration of 1 μM. After incubation of the cells for 16 hours, DNA synthesis was assayed using the Cell Proliferation ELISA BrdU Kit (Roche Diagnostics), and newly synthesized BrdU-DNA was determined using a microplate reader (Berthold Technologies).

TUNEL Assay

DNA fragmentation was detected by TUNEL using the ApopTag Red In Situ Apoptosis Detection Kit (EMD Millipore Corp.). A 10^{-8} M dose of octreotide and a 10^{-10} M dose of staurosporine (Sigma Aldrich; positive control) were applied to meningioma cells that were previously seeded on 14-mm cover glass. After 2 days, the cells were fixed with paraformaldehyde for 15 minutes. Each experimental condition was assayed in quadruplicate. Apoptotic cells were then viewed and scored manually using a Leica/Leitz DMRB microscope with a PL Fluotar ×100 objective. The percentage of apoptotic cells was evaluated based on > 2000 counted cells in 70–160 successive fields.

Determination of Caspase Activity

The activity of caspase-3 and -7 was measured by luminescent Caspase-Glo assay (Promega Corp.). Twenty-four hours after seeding 2×10^4 meningioma cells into each well of a 24-well plate, the cells were incubated in low-serum media (5%) and then treated with octreotide (10^{-8} M) for 3 days. The assay was performed in triplicate. Results were expressed as a mean percentage of caspase activity in treated versus untreated cells.

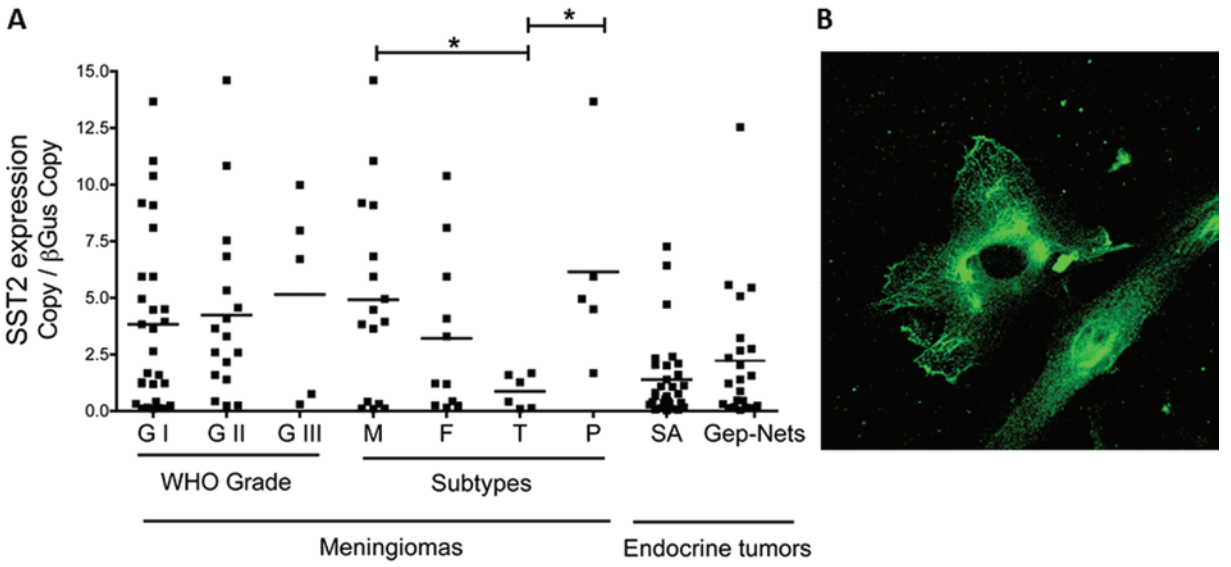


FIG. 1. SST2 expression in meningiomas. **A:** SST2 mRNA levels were quantified by real-time PCR in a large series of 50 meningiomas, 30 human somatotroph adenomas (SA), and 20 human GEP-NETs. Meningiomas were classified according to WHO grades (G) or histological subtypes: meningothelial (M), fibrous (F), transitional (T), or psammous (P). The level of SST2 mRNA was significantly lower in the transitional subtype than in meningothelial and psammous subtypes (*$p < 0.05$). SST2 mRNA levels, whatever the category of meningioma considered, were similar to those observed in human somatotroph adenomas or GEP-NETs. **B:** Immunostaining of SST2 in a meningioma cell showed membrane and cytosolic expression. Original magnification ×100. Figure is available in color online only.

Western Blot Analysis

Twenty-hours after seeding, cells were incubated in low-serum media for 16 hours and then treated with octreotide (10^{-9} M) for 3 or 16 hours, depending on the experiment. Meningioma lysates were obtained by mechanical homogenization in lysis buffer.[12] The denatured proteins (25 μg) were separated on 10% or 15% SDS-PAGE gels and transferred to polyvinyl difluoride membrane (Perkin Elmer). After blocking, the membrane was incubated with primary antibody overnight at 4°C, followed by incubation with horseradish peroxidase–conjugated secondary antibody. The proteins were detected using Lumina Forte Western HRP substrate (EMD Millipore Corp.) in a G:BOX (Ozyme Corp.). Primary antibodies were mouse monoclonal antibodies against merlin, SHP1, cyclin D1, S6 ribosomal protein (S6), phospho-S6 ribosomal protein Ser235/Ser236 (p-S6), Akt, phospho-Akt Ser473 (p-Akt), ERK (1/2), phospho-ERK Thr202/Tyr204 (p-ERK), IRS1, phospho-IRS1 Ser636/Ser639 (p-IRS1), and β-actin. All antibodies were purchased from Cell Signaling Technology Inc.

SHP1-proteins were immunoprecipitated using the Protein G Immunoprecipitation Kit (Sigma-Aldrich). The immunoprecipitated proteins were analyzed by Western blotting.

Detection of SST2 mRNA

SST2 mRNA expression was assessed using real-time polymerase chain reaction (PCR). Fifty meningiomas were analyzed. Total RNA was extracted from 2.5×10^5 cells and reverse-transcribed into complementary DNA (cDNA) using Superscript II Reverse Transcriptase (ThermoFisher Scientific Inc.). The 5′ exonuclease (Taq man) assay was used for quantifying SST2 mRNA, and SST2 mRNA levels were normalized to those of β-glucuronidase (βGus).[41] A series of 30 human somatotroph adenomas and 20 GEP-NETs (the usual targets of octreotide) were analyzed in parallel.

Immunocytochemistry

The expression and localization of SST2 were assessed by immunocytochemistry. Meningioma cells were cultured and then fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) for 10 minutes at room temperature. Cells were incubated overnight at 4°C with SST2 polyclonal antibody (ss-870, Gramsch Lab Germany) diluted 1:1000 in PBS (4°C overnight) and detected using Alexa Fluor 488–conjugated anti–goat and anti–rabbit IgG (1:800 in PBS containing 10% nonimmuno-goat serum; ThermoFisher Scientific Inc.). The cells were treated with Prolong Gold Antifade reagent and visualized on a Zeiss LSH 780 laser scanning microscope equipped with a 100× oil immersion lens.

Methodology for Meta-Analytic Review

A PubMed literature search was performed for all English-language publications reporting on the expression of SST2 in meningiomas and the use of octreotide therapy for the treatment of meningiomas. The meta-analysis aimed to study SST2 expression in meningiomas with regard to quantification technique and patient outcomes. The key words "somatostatin" and "meningioma" were used for the SST2 expression search, and "octreotide" and "meningioma" were used for the octreotide therapy search. All identified series and case reports were included in our analysis.

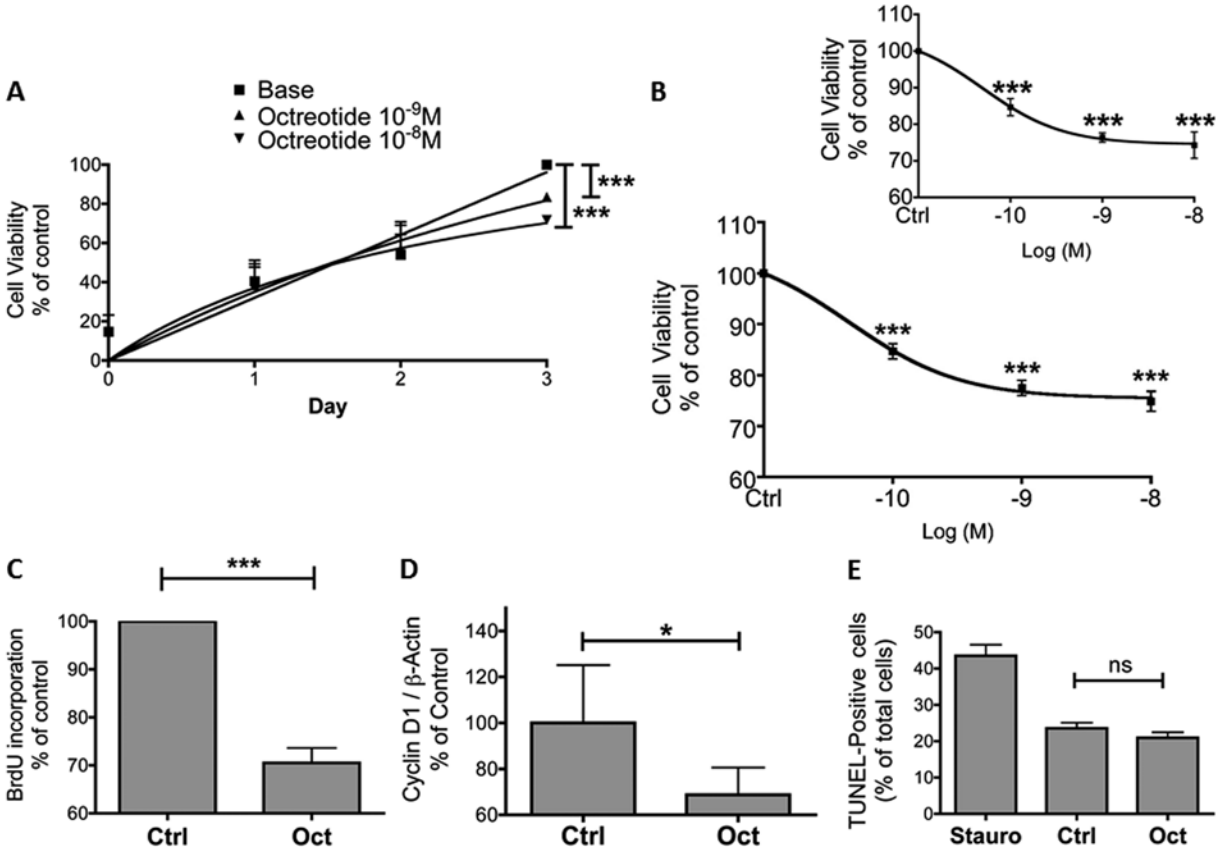


FIG. 2. Octreotide significantly decreased meningioma cell proliferation. **A:** Mean cell proliferation in 4 meningiomas (3 WHO Grade I meningiomas including 2 fibrous and 1 meningothelial subtypes and 1 WHO Grade II atypical meningioma) cultured under basal conditions (base) or treated with octreotide (10^{-9} M or 10^{-8} M for 3 days). Cell number was evaluated every day. A significant effect of octreotide was observed on the 3rd day of culture (base vs 10^{-9} M or 10^{-8} M of octreotide). **B:** Dose-effect curve of octreotide treatment (10^{-10} to 10^{-8} M for 3 days) on cell viability in 34 meningiomas (23 WHO Grade I, 10 WHO Grade II, and 1 WHO Grade III tumors) in primary culture. **Inset:** Cell viability in 10 WHO Grade II and 1 WHO Grade III tumors. Results are expressed as the mean percent cell viability versus control (untreated cells). **C:** Effect of octreotide treatment (10^{-9} M for 2 days) on BrdU incorporation in 14 meningiomas (9 WHO Grade I, 4 WHO Grade II, and 1 WHO Grade III tumors). Results are expressed as mean percent of control. **D:** Immunoblot analysis of cyclin D1 and β-actin in 10 meningiomas treated with octreotide (10^{-9} M, overnight). The results are represented as the mean percentage of cyclin D1/β-actin signal versus control. **E:** TUNEL analysis in 5 meningiomas (2 WHO Grade I and 3 WHO Grade II tumors) treated with octreotide (10^{-9} M). The number of apoptotic events was not different between octreotide-treated and untreated cells, whereas an increase was observed under staurosporine treatment (10^{-10} M). *$p < 0.05$; ****$p > 0.0001$; Ctrl = control; ns = not significant; Oct = octreotide; Stauro = staurosporine.

Statistical Analysis

Results are presented as the mean ± standard error of the mean. The statistical significance between 2 unpaired groups was determined using the Mann-Whitney U-test and between 2 paired groups by the Wilcoxon rank-sum test. To measure the strength of association between pairs of variables without specifying dependency, Spearman rank-order correlations were performed. Differences were considered to be significant at $p < 0.05$.

Results

SST2 Expression in Meningiomas

SST2 mRNA was expressed in all tumors tested (> 0.01 SST2 copy/βGus copy; Supplementary Table); SST2 expression was high (> 1 SST2 copy/βGus copy) in 74.5% of tumors (Fig. 1A).[11] No correlation was observed between the SST2 expression level and the WHO grade of the tumor. SST2 mRNA expression was significantly lower in the transitional subtype than in the meningothelial and psammous subtypes ($p = 0.04$ and $p = 0.02$, respectively). SST2 mRNA levels for all categories of meningiomas were similar to those observed in human somatotroph pituitary adenomas or GEP-NETs. SST2 protein expression was assessed by immunocytochemistry in 3 meningioma primary cell cultures. Intense membrane and dot-shaped cytosolic labeling was observed (Fig. 1B).

Decreased Cell Proliferation Due to Octreotide

Cell proliferation assays were performed on 4 randomly selected tumors (3 WHO Grade I [2 fibrous and 1 meningothelial subtypes] and 1 WHO Grade II atypical meningioma) that had been treated for 3 days under 1 of following conditions: no treatment, 10^{-9} M octreotide treatment, or 10^{-8} M octreotide treatment (Fig. 2A). Both

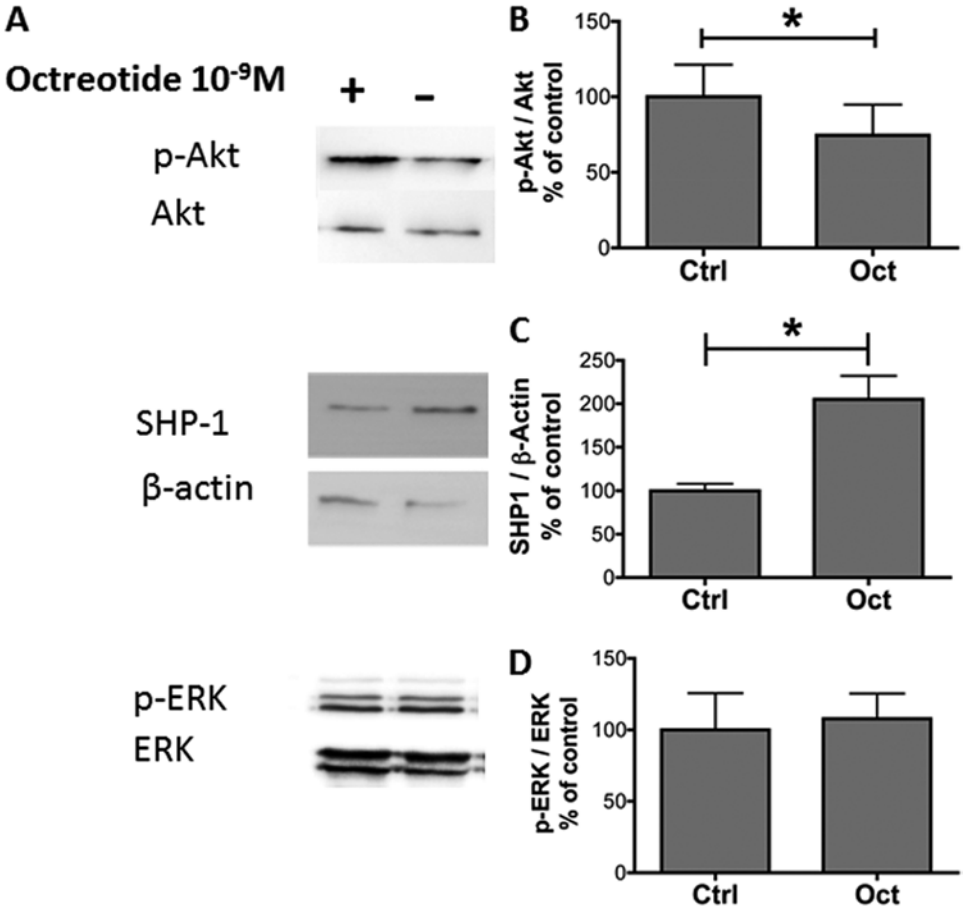


FIG. 3. Octreotide significantly decreased Akt phosphorylation (p-Akt) and increased SHP-1 expression without affecting ERK phosphorylation (p-ERK). A representative immunoblot (**A**) demonstrating levels of SHP-1, β-actin, p-Akt, total Akt, p-ERK, and total ERK1/2 after octreotide treatment (10^{-9} M, overnight). Quantification of immunoblot signals for SHP-1 versus β-actin (n = 4, **C**), p-Akt versus total Akt (n = 14, **B**), and p-ERK versus total ERK (n = 10, **D**). The results are represented as the mean percent of control. *$p < 0.05$.

concentrations of octreotide reduced cell proliferation in the 4 tested tumors.

The effect of increasing the octreotide dose (ranging from 10^{-10} to 10^{-8} M, treated for 3 days) was analyzed on 34 meningiomas (Fig. 2B): 23 WHO Grade I tumors, 10 WHO Grade II, and 1 WHO Grade III. Among the WHO Grade I tumors, there were 11 meningothelial, 8 fibrous, 5 psammous, and 3 transitional subtypes (note that some tumors have a double component and are classified as more than one subtype; Supplementary Table). A significant dose-dependent inhibition in cell viability was observed in 88% of tested tumors (Fig. 2B). Only 12% (4/34) were considered to be octreotide "nonresponders" (inhibition < 10%). Octreotide decreased cell viability by 26%, but the decrease in cell viability was not significantly different between WHO Grade I and WHO Grade II or III tumors (Fig. 2B: all WHO grades; Fig. 2B inset: WHO Grades II and III). The mean reduction in cell viability was 27% in meningothelial subtypes, 23% in fibrous, 29% in transitional, and 13% in psammous, and the latter was significantly lower than the other subtypes ($p < 0.05$, Supplementary Fig. 1). There was no significant difference in viability between the 10^{-8} M and 10^{-9} M doses of octreotide (26.5% and 27.5%, respectively). Since a dose of 10^{-9} M corresponds to the octreotide plasma concentration in patients treated for acromegaly,[49] this concentration was used for the subsequent experiments.

The cell viability results were confirmed by direct cell counting on 3 meningiomas (data not shown) and by BrdU incorporation (Fig. 2C). Octreotide treatment (10^{-9} M for 3 days) decreased BrdU incorporation in 13 of the 14 meningiomas tested (inhibition > 10%), which included 9 WHO Grade I, 4 WHO Grade II, and 1 WHO Grade III tumors. The mean decrease in cell proliferation was 31%. Moreover, octreotide decreased cyclin D1 expression (Fig. 2D and Supplementary Fig. 2).

In slow-growing tumors such as somatotroph adenomas, octreotide induced cell death by a caspase-dependent mechanism.[1] To understand the impact of octreotide on cell viability, the apoptotic pathway was considered. The number of apoptotic events, determined by TUNEL assay in 5 tumors (2 WHO Grade I and 3 WHO Grade II), was not different between octreotide-treated (10^{-9} M) and untreated cells, while a clear increase in TUNEL-positive cells was observed after treatment with the apoptosis inducer staurosporine (Fig. 2E). Moreover, octreotide treatment (10^{-9} M) did not increase caspase activity in the 6

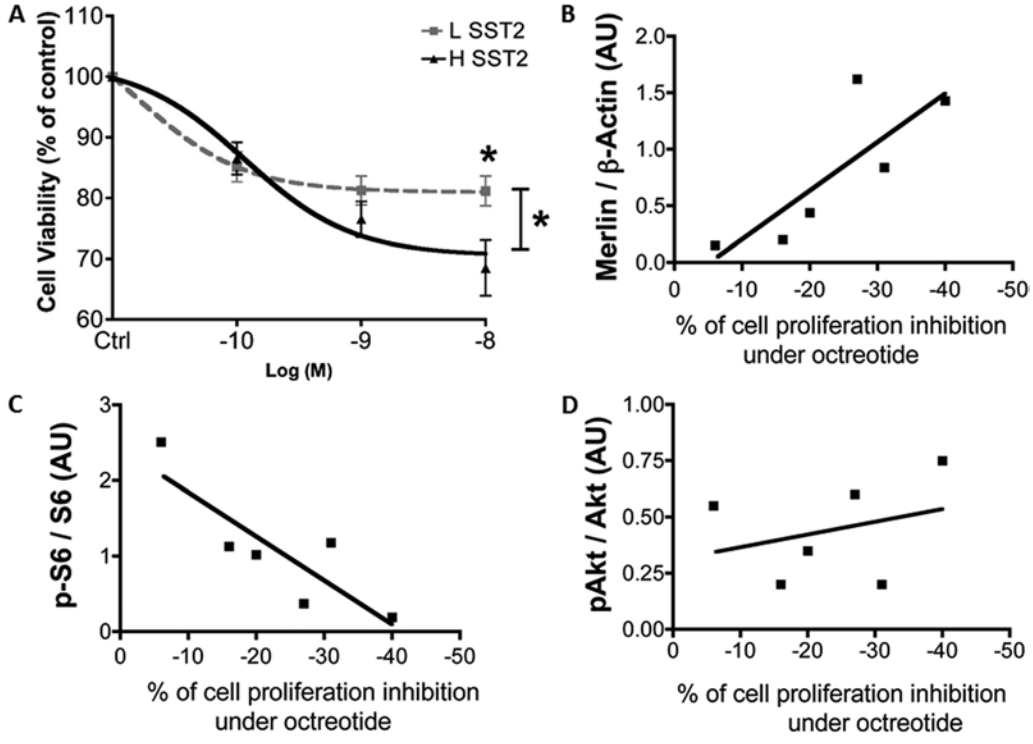


FIG. 4. Comparison of octreotide-induced inhibition of cell proliferation with SST2 mRNA expression, merlin protein expression, or S6 phosphorylation (p-S6). Dose-effect curve (**A**) of octreotide treatment on cell viability in the high-SST2 group and the low-SST2 group. Cell viability was significantly lower in the high-SST2 group than in the low-SST2 group. Correlation between cell proliferation and total merlin expression (**B**), S6 phosphorylation (**C**), or Akt phosphorylation (**D**) in 6 meningiomas treated with octreotide (3 WHO Grade I, 2 WHO Grade II, and 1 WHO Grade III). Merlin expression, S6 phosphorylation, and Akt phosphorylation were quantified by Western blot. Octreotide sensitivity was evaluated by calculating the percent inhibition of cell proliferation as assessed by BrdU incorporation with octreotide treatment (10^{-9} M). r = –0.8, p = 0.04 (B). r = 0.8, p = 0.03 (C). *p < 0.05; H SST2 = high-SST2 group; L SST2 = low-SST2 group.

meningiomas tested (3 WHO Grade I and 3 WHO Grade II; Supplementary Fig. 3).

Transduction Pathways Involved in Octreotide Effects on Meningiomas

To decipher the signal transduction pathways involved in octreotide-induced inhibition of cell proliferation, Akt, SHP-1, and ERK were analyzed by Western blotting. Octreotide treatment (10^{-9} M) significantly decreased Akt phosphorylation in 14 tested meningiomas (10 WHO Grade I and 4 WHO Grade II tumors; Fig. 3A and B) and increased total SHP-1 expression in 4 tested meningiomas (2 WHO Grade I and 2 WHO Grade II tumors; Fig. 3A and C). However, no effect was observed on ERK phosphorylation (Fig. 3A and D).

Octreotide Antiproliferative Effect

Even though psammous meningiomas, which exhibited the lowest octreotide response, and transitional meningiomas, which expressed the lowest SST2 level, were excluded, we did not observe any correlation between levels of SST2 mRNA and inhibition of cell viability by octreotide. However, when meningiomas were classified into 2 groups according to SST2 mRNA expression (low-SST2 group: < 2 SST2 copies/βGus copy [n = 16] and high-SST2 group: ≥ 2 SST2 copies/βGus copy [n = 9]), octreotide had a significantly higher inhibitory effect on cell viability in the high-SST2 group than in the low-SST2 group (Fig. 4A).

To identify other molecular markers of octreotide sensitivity, we analyzed 6 meningiomas (3 WHO Grade I, 2 WHO Grade II, and 1 WHO Grade III) for expression and phosphorylation status of 3 intracellular proteins crucial in meningiomas tumorigenesis:[20] total merlin, phospho-Akt, and phospho-S6. Merlin is encoded by the *NF2* gene and is mutated in a majority of meningiomas, resulting in a loss of protein expression. Merlin is a negative regulator of mTORC1, a downstream regulator of the PI3k/Akt/mammalian target of rapamycin (mTOR) pathway.[22] We then compared these molecular markers to the percent of cell proliferation inhibition after treatment with octreotide (10^{-9} M). Inhibition of cell proliferation was strongly positively correlated with merlin expression (p = 0.04, r = –0.8; Fig. 4B) and inversely correlated with levels of S6 phosphorylation (p = 0.03, r = 0.8), a marker of mTORC1 activity (Fig. 4C). However, we did not observe any correlation between inhibition of cell proliferation and Akt phosphorylation (Fig. 4D).

Discussion

The antitumoral effects of somatostatin analogs on

TABLE 1. Literature survey of SST2 expression in meningiomas: 1998–2016

Study	Present Study	Silva et al., 2015	Agaimy et al., 2014	Barresi et al., 2008	Durand et al., 2008		Arena et al., 2004	Schulz et al., 2000	Dutour et al., 1998
No. of tumors	50	60	68	35	22	26	42	40	20
Method	PCR	IHC	IHC	IHC	IHC	PCR	PCR	IHC	PCR
SST2 subtype A expression (%)*	100	100	87	74	64	100	79	70	100

IHC = immunohistochemistry.

* Results are expressed as the percentage of SST2-expressing tumors.

meningiomas in vivo has been previously suggested[27] but poorly documented. In fact, conducting informative clinical studies is challenging for this type of slow-growing tumor. Therefore, the relevance of many clinical studies remains limited. Somatostatin analogs are well tolerated even at high doses, highlighting the interest in this long-term treatment for slow-growing tumors such as GEP-NETs, somatotroph adenomas, and potentially meningiomas. In agreement with US Food and Drug Administration considerations, octreotide is currently one of the rare drugs recommended for the treatment of patients with meningiomas.

SST2 mRNA was detected in all 50 of the randomly selected meningiomas in our series. The level of SST2 mRNA was high in 74.5% of tested meningiomas, but there was no correlation between SST2 mRNA levels and WHO tumor grades. Our results were in agreement with immunohistochemical data from other groups, showing high SST2 expression in 69% of tumors (Table 1).[2,4,5,14,15,43,44] For the first time, we demonstrated that the range of SST2 mRNA levels in meningiomas was similar to that observed in somatotroph tumors or GEP-NETs, the 2 main tumor families targeted by somatostatin analogs in clinical practice.

Although the antitumoral effect of octreotide has been described in isolated cases of meningiomas,[18,21,38,40] only 4 clinical studies have aimed to assess this question (Table 2).[10,23,42,45] Three trials were performed on patients with aggressive and recurrent meningiomas, while 1 trial was conducted on patients with WHO Grade I skull base meningiomas who had not received prior radiotherapy or chemotherapy.[42] For all but 1 of these studies,[42] the main limitation was their short duration. Octreotide was well tolerated in all cases, regardless of the dose or the galenic form used.

According to our review of the literature (Table 2), the distribution of octreotide-treated patients into 3 groups based on outcome—stable disease, partial response, and progressive disease—revealed a statistical difference between the patients with WHO Grade I and those with WHO Grade III meningiomas (chi-square test, $p = 0.01$), with the best treatment efficacy in WHO Grade I tumors. Recently, Norden et al. conducted a study on the use of the somatostatin analog pasireotide, which binds to SST1, -2, -3, and -5 (with the highest affinity for SST5), in 18 recurrent or progressive meningiomas and reported no increase in the proportion of patients experiencing 6-month progression-free survival (PFS6).[35] The antitumoral effect of somatostatin analogs has clearly been established for patients with GEP-NETs,[8,39] while the distribution of somatostatin analog–treated patients was not statistically different from that observed for WHO Grade I meningiomas: 45% in the stable disease group, 2% in the partial remission group, and 41% in the progressive disease group.[3]

TABLE 2. Literature review of clinical studies and case reports using octreotide for meningiomas

Authors & Year	No. of Pts	Octeotide Dose	Oct Scan	IHC	WHO Grade				Op	RT	CT	PFS6	Median TTP (mos)	BRR		
					ND	I	II	III						SD	PR	PD
Clinical studies																
Simó et al., 2014	9	30-40 mg LAR	+	−	0	0	5	4	9/9	9/9	0/9	44%	4.2	3	0	6
Johnson et al., 2011	11	500 μg 3/day	−	6+/6	0	3	3	5	11/11	9/11	3	30%	4.2	8	0	3
Schulz et al., 2011	8	30 mg LAR	+	−	0	8	0	0	8/8	0/8	0/8	100%	>60	8	0	0
Chamberlain et al., 2007	16	30 mg LAR	+	−	2	6	3	5	14/16	13/16	12/16	44%	5	5	5	6
Case reports																
Rammo et al., 2016	1	30 mg LAR	+	+	0	0	0	1	1/1	1/1	1/1					
Jaffrain-Rea et al., 1998	1	100 μg 3/day	−	−	1	0	0	0	1	0	0					
García-Luna et al., 1993	3	900–1500 μg/day	−	1+/1	0	2	0	1	3/3	0/3	0/3					
Rünzi et al., 1989	1	500 μg 3/day	−	−	0	1	0	0	1	0	0					

BRR = best radiological response; CT = chemotherapy; LAR = long-acting repeatable; ND = not determined; Oct Scan = octreotide SPECT scanning; PD = progressive disease; PR = partial response; Pts = patients; RT = radiotherapy; SD = stable disease; TTP = time to progression.

TABLE 3. Literature review: assessment of PFS6 and BRR in clinical studies of octreotide for meningioma, by WHO grade*

Tumor Grade	No. of Pts	PFS6 (no. [%])	BRR (no. [%])		
			SD	PR	PD
WHO Grade I	19	13 (68.4)	13 (68.4)	3 (15.8)	3 (15.8)
Skull Base WHO Grade I	13	12 (92.3)	9 (69.2)	3 (23.1)	1 (7.7)
WHO Grade II	11	4 (36.5)	6 (54.6)	1 (9)	4 (36.4)
WHO Grade III	14	4 (28.5)	4 (28.6)	1 (7.1)	9 (64.3)

* Chamberlain et al., 2007; Johnson et al., 2011; Schulz et al., 2011; Simó et al., 2014.

According to the benchmarks of the Response Assessment in Neuro-Oncology (RANO) criteria,[24] the effects of octreotide should be considered significant in patients with WHO Grade I meningiomas when the PFS6 level averages more than 50%. A PFS6 level approaching 92% was observed in WHO Grade I skull base meningiomas (Table 3).[10,42] Overall, these data clearly demonstrate the efficacy of somatostatin analogs for WHO Grade I meningiomas. Although octreotide had a clear inhibitory effect on cell viability in vitro for WHO Grade II or III meningiomas, it did not appear to be an efficient treatment (based on PFS6 assessment) in vivo.[10,23,45] However, the PFS6 criterion does not take tumor growth rate into consideration; therefore, a putative decrease in growth rate is not assessed.

Our in vitro data clearly support the results of in vivo studies using octreotide therapy for meningiomas. Although SST2 expression was well characterized in meningiomas,[5,15,43] the cellular and molecular mechanisms triggered by somatostatin analogs remains somewhat unknown. This is essentially attributable to the difficulty in performing primary cultures for this type of tumor compared with other CNS tumors such as glioma.[4,26] In our large series of human meningiomas, octreotide clearly inhibits cell proliferation in vitro. This effect was significant (> 10%) in 88% of the tested tumors regardless of their WHO grade. Our results were in agreement with those of Arena et al., who showed a decrease in cell proliferation by thymidine incorporation in a smaller series (4/7) of meningiomas.[4] In contrast, Koper et al. reported an increase in cell proliferation with octreotide treatment using the same protocol.[26] However, experiments from the Koper study were not performed days after surgical removal, as was done in our study, but several weeks after instead. During this time lapse, many native molecular features, such as receptor membrane expression, are likely to be lost.[6,20]

Somatostatin analogs may exert their antiproliferative action directly by blocking cell division or by inducing apoptosis. In contrast to the situation for other slow-growing tumors, such as GEP-NETs[33] or somatotroph tumors,[1] the inhibition of cell viability by octreotide in meningiomas in vitro was not the result of apoptosis. Therefore, considering the direct effect of somatostatin analogs, we expected the clinical effect of octreotide to control tumor growth rather than induce tumor shrinkage.

It has been reported that SST2 mediates cell growth arrest by regulating several signal transduction pathways, including the ERK and PI3K/Akt pathways, and by activating tyrosine phosphatases.[37] In contrast to pituitary adenomas, octreotide did not modulate ERK phosphorylation levels in meningiomas; however, Akt phosphorylation was clearly inhibited. In addition, the inhibition of cell proliferation by octreotide was inversely correlated with the activation (phosphorylation) of S6 kinase, a downstream effector of mTOR. Consequently, mTOR hyperactivation may limit the antiproliferative effect of octreotide. We show that expression levels of merlin protein are strongly positively correlated to octreotide response; therefore, it may be a relevant marker for in vivo octreotide sensitivity.[22,32] The significant increase in the levels of SHP1 protein observed in our study suggests that upregulation of SHP1 could be an important step for SST2-mediated antiproliferative signaling in meningioma, as was previously demonstrated in pancreatic adenocarcinoma[31] and tumoral pituitary cells.[46]

In addition to their direct antitumoral effect, somatostatin analogs also exert crucial peritumoral action in vivo, either antiangiogenic or antiinflammatory. SSTs are expressed in growing vascular endothelial cells.[48] An antiangiogenic effect of octreotide, inhibiting VEGF signaling, has been demonstrated in neuroendocrine tumors and pituitary adenomas.[28,47] Some meningiomas lead to peritumoral brain edema,[13,19,25,34] causing increased morbidity. Peritumoral brain edema is correlated to VEGF mRNA expression levels and to meningioma vascularization.[29,34] Improvements in neurological symptoms (visual improvement and headache alleviation) have been reported under octreotide treatment without a tumor volume change.[18,21] These clinical observations emphasize the relevance of somatostatin analogs through indirect meningioma antitumoral effects.

In our study, we observed a better octreotide response on cell viability in those meningiomas expressing high levels of SST2. Nevertheless, there was no significant correlation between octreotide response and SST2 mRNA levels. For instance, in meningiomas with a transitional subtype, a significantly low level of SST2 expression was observed (lower than in psammous meningiomas), which was associated with a paradoxical significantly better octreotide effect. In agreement with our data, no correlation was observed between radiolabeled octreotide uptake during SPECT scanning and clinical octreotide antitumoral effect.[10,23] Overall, according to our data and those from the literature, the assessment of SST2 expression level seems to be an inaccurate marker in predicting octreotide response.

Clinical studies have highlighted an antitumoral effect of octreotide in WHO Grade I meningiomas and particularly in skull base meningiomas.[10,42] This could be explained by 2 molecular characteristics of these tumors, which are found in the majority of tumors from the meningothelial subtype: a high SST2 expression level combined with a low rate of *NF2* gene mutation, resulting in merlin protein expression.[14,50] Therefore, somatostatin analog treatment should be advised for extended skull base meningiomas such as petroclival meningiomas or for those that produce undesirable symptoms, are located in areas that are difficult to access via surgery, or display tumor extension incompatible with radiotherapy. Somatostatin analogs could also be an interesting and relevant alternative

for asymptomatic, slow-growing meningiomas in elderly patients or those with a degraded general status, who may have uncertain outcomes under general anesthesia and/or unreasonable surgery. Moreover, somatostatin analogs are well-tolerated drugs, allowing long-term treatment for several years, as in acromegaly or neuroendocrine tumors.

Conclusions

We demonstrated the antiproliferative activity of the somatostatin agonist octreotide in meningiomas in vitro. Clinical studies suggest an interest in octreotide for the treatment of patients with slow-growing meningiomas, particularly skull base WHO Grade I tumors. In aggressive meningiomas, the clinical effects of octreotide seem clinically insufficient. Finally, somatostatin analogs could be of interest in therapeutic strategies that combine somatostatin analogs with mTOR inhibitors, targeting one of the crucial signaling pathways involved in meningioma tumorigenesis.[20]

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Graillon, Barlier. Acquisition of data: Graillon, Defilles, Mohamed. Analysis and interpretation of data: Graillon, Romano, Defilles, Saveanu. Critically revising the article: Romano, Saveanu, Chinot, Dufour, Barlier. Approved the final version of the manuscript on behalf of all authors: Graillon. Statistical analysis: Barlier. Administrative/technical/material support: Defilles, Mohamed, Figarella-Branger, Roche, Fuentes, Chinot, Dufour. Study supervision: Barlier.

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