Resistance to octreotide LAR in acromegalic patients with high *SSTR2* expression: analysis of *AIP* expression

Resistência ao octreotide LAR em pacientes acromegálicos com alta expressão do SSTR2*: avaliação da expressão do* AIP

Leandro Kasuki[1,2], Leandro M. Colli[3], Paula C. L. Elias[3], Margaret de Castro[3], Mônica R. Gadelha[1]

SUMMARY

We present here the clinical and molecular data of two patients with acromegaly treated with octreotide LAR after non-curative surgery, and who presented different responses to therapy. Somatostatin receptor type 2 and 5 (SSTR2 and SSTR5), and aryl hydrocarbon receptor-interacting protein (AIP) expression levels were analyzed by qPCR. In both cases, high *SSTR2* and low *SSTR5* expression levels were detected; however, only one of the patients achieved disease control after octreotide LAR therapy. When we analyzed *AIP* expression levels of both cases, the patient whose disease was controlled after therapy exhibited *AIP* expression levels that were two times higher than the patient whose disease was still active. These two cases illustrate that, although the currently available somatostatin analogs bind preferentially to SSTR2, some patients are not responsive to therapy despite high expression of this receptor. This difference could be explained by differences in post-receptor signaling pathways, including the recently described involvement of AIP. Arq Bras Endocrinol Metab. 2012;56(8):501-6

[1] Endocrinology Division, Clementino Fraga Filho University Hospital, Universidade Federal do Rio de Janeiro (HUCFF-UFRJ), Rio de Janeiro, RJ, Brazil
[2] Endocrinology Unit, Bonsucesso Federal Hospital, Rio de Janeiro, RJ, Brazil
[3] Endocrinology Laboratory, Ribeirão Preto Medical School, Universidade de São Paulo (FMRP-USP), Ribeirão Preto, SP, Brazil

SUMÁRIO

Apresentamos os dados clínicos e moleculares de dois pacientes com acromegalia tratados com octreotide LAR após cirurgia não curativa, com diferentes respostas a essa terapia medicamentosa. As expressões do receptor de somatostatina tipo 2 e 5 (SSTR2 e SSTR5) e da proteína de interação com o receptor aril hidrocarbono (AIP) foram analisadas por qPCR. Em ambos os casos, foi encontrada uma expressão elevada de *SSTR2* e baixa do *SSTR5*. No entanto, o controle da doença foi obtido após tratamento com octreotide LAR em apenas um dos pacientes. Quando analisamos a expressão do *AIP* em ambos os casos, o paciente cuja doença foi controlada após a terapia medicamentosa apresentou uma expressão duas vezes maior do que a do paciente não controlado com o tratamento. Conclui-se que esses dois casos ilustram que, embora os análogos de somatostatina atualmente disponíveis se liguem preferencialmente ao *SSTR2*, alguns pacientes não respondem ao tratamento, apesar de uma elevada expressão desse receptor. Isso poderia ser explicado por alterações nas vias de sinalização pós-receptor, incluindo o envolvimento recentemente descrito da *AIP*. Arq Bras Endocrinol Metab. 2012;56(8):501-6

Correspondence to:
Mônica R. Gadelha
Centro de Pesquisa em Neuroendocrinologia, Divisão de Endocrinologia, Hospital Universitário Clementino Fraga Filho, Universidade Federal do Rio de Janeiro
Rua Professor Rodolpho Paulo Rocco, 255, sala 9F
21941-913 – Rio de Janeiro, RJ, Brazil
mgadelha@hucff.ufrj.br

Received on July/31/2012
Accepted on Sept/10/2012

INTRODUCTION

The currently available somatostatin analogs (SSAs) octreotide and lanreotide are considered cornerstones of the medical treatment for acromegaly (1,2). SSAs act mainly by binding to somatostatin receptor type 2 (SSTR2) (3). Thus, as expected, SSTR2 expression is a predictor of the response to these drugs, and patients harboring tumors that present low SSTR2 expression are resistant to SSAs (4,5). However, in some cases, acromegaly is not controlled with SSAs therapy despite a high SSTR2 expression level in the somatotro-

pinoma, indicating that although the presence of a high SSTR2 expression is essential for the action of SSAs, other factors, such as post-receptor signaling pathways, may be involved in the lack of response to SSAs in tumors presenting high expression of SSTR2 (5).

Recently, germline mutations in the *aryl hydrocarbon receptor-interacting protein (AIP)* gene have been described in the setting of familial isolated pituitary adenoma, and in seemingly sporadic young-onset pituitary adenoma patients (6,7). The patients harboring mutations in the *AIP* gene present worse response to SSAs therapy (8,9). Although somatic mutations have not been described, some sporadic tumors present low AIP expression, and patients harboring tumors with this feature have a lower probability of acromegaly control with SSAs treatment (10-12). Moreover, SSAs increase AIP expression, and, although the mechanism of action of SSAs is not completely understood, this feature appears to be important in the mechanism of action of this drug class (13).

To illustrate the importance of AIP expression in the response to SSAs therapy in acromegaly, we describe two patients whose somatotropinomas presented high *SSTR2* expression levels, but exhibited different responses to octreotide LAR (OCT-LAR) treatment.

SUBJECTS AND METHODS

This study was approved by the Ethics Committees of the Clementino Fraga Filho University Hospital/Medical School, Universidade Federal do Rio de Janeiro, and Hospital das Clínicas/Ribeirão Preto Medical School, Universidade de São Paulo. All subjects signed an informed consent before entering the study.

CASE REPORTS

Case 1

A 37 year-old female acromegalic patient was diagnosed due to a history of acral enlargement and amenorrhea. At diagnosis, she presented basal growth hormone (GH) level of 185.0 ng/mL and insulin-like growth factor type I (IGF-I) level of 1,470 ng/mL (age-adjusted normal range, 106-277). Anterior pituitary workup revealed normal basal serum cortisol (9.0 µg/dL); no hypothyroidism (TSH: 0.36 IU/mL; free T4:1.0 ng/dL), and slightly elevated prolactin of 41.0 ng/dL with LH and FSH levels in the normal range (1.0 and 2.2 mU/mL, respectively). Magnetic resonance imaging (MRI) of the sellar region revealed a 3.0 x 2.1 x 2.5 cm sellar tumor with supra-sellar extension and invasion of the cavernous sinus (Figure 1). Examination of her visual field was normal. The patient was submitted to transesphenoidal surgery, but remained with active disease after the surgical procedure. Anatomopathological study revealed a pituitary adenoma with positive immunostaining only for GH. Therefore, treatment with OCT-LAR was initiated at a dosage of 20 mg every four weeks, with control of the hormone levels after six months of medical therapy, with no further need for dose adjustment. A residual sellar lesion of 1.5 cm was present at MRI after transesphenoidal surgery and remained unchanged during follow-up. GH and IGF-I levels at diagnosis, post-surgery, and after OCT-LAR therapy are shown in table 2.

Case 2

A 46-year-old male patient presented history of asthenia, hyperhidrosis, hand edema, and decreased libido that had started three years before. At initial laboratory evaluation, he presented hyperprolactinemia (prolactin, 91.4 ng/mL; normal range, 4.1-17.7), central hypothyroidism (free T4, 0.59 ng/dL; normal range, 0.8-1.9; TSH, 4.1 mcU/mL), GH level of 3.41 ng/mL, and IGF-I level of 224 ng/mL (age-adjusted normal range, 101-267). After treatment for central hypothyroidism, GH level was 15.6 ng/mL, and IGF-I level was 555 ng/mL. MRI of the sellar region showed a 2.6 x 2.5 x 2.6 cm intra-sellar lesion with infra-sellar and right cavernous sinus extension involving the right internal carotid artery (Figure 2). The patient was submitted to surgery; however, total macroscopic resection was not achieved, although he exhibited an improvement in GH and IGF-I levels (4.0 ng/mL and 411 ng/mL, respectively). The anatomopathological study revealed a pituitary adenoma with positive immunostaining for GH and prolactin. After surgery, treatment with OCT-LAR was initiated at a dosage of 20 mg every four weeks, with posterior up-titration to 30 mg every four weeks, without controlling the disease after one year of treatment. A residual lesion in the right cavernous sinus of 1.0 cm was present at MRI after transesphenoidal surgery and remained unchanged during follow-up. GH and IGF-I levels at diagnosis, three months post-surgery and after six months of OCT-LAR therapy are shown in table 3.

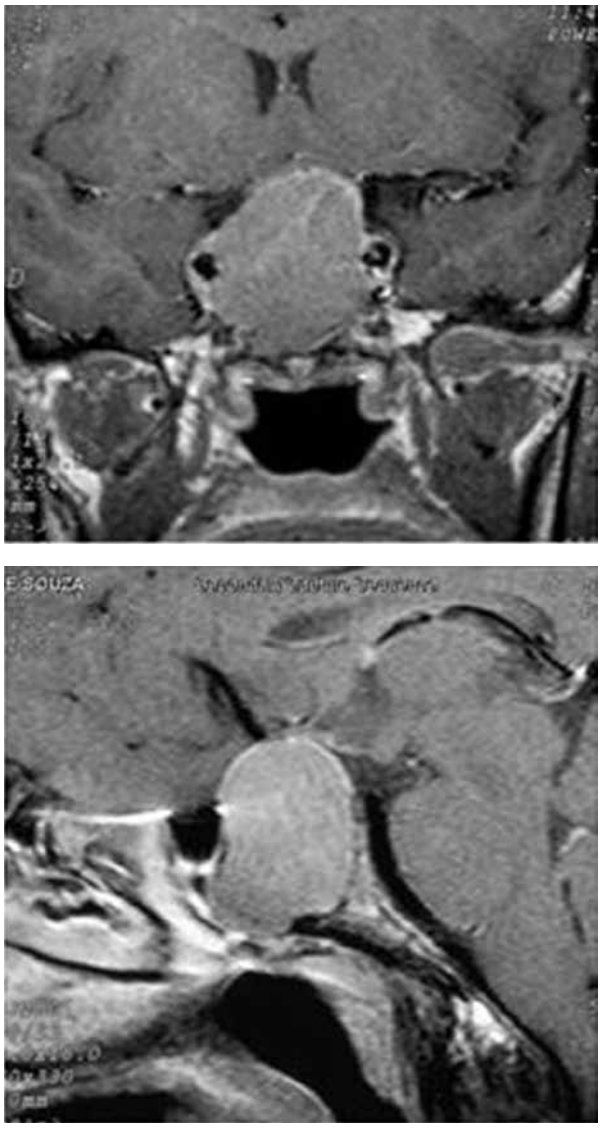

Figure 1. Magnetic resonance imaging of the sellar region (case 1).

Magnetic resonance imaging of the sellar region (**A:** coronal T1-weighted with gadolinium enhancement; **B:** Sagittal T1-weighted with gadolinium enhancement) revealing a 3.0 x 2.1 x 2.5 cm sellar tumor with infra- and supra-sellar extension with invasion of the cavernous sinus.

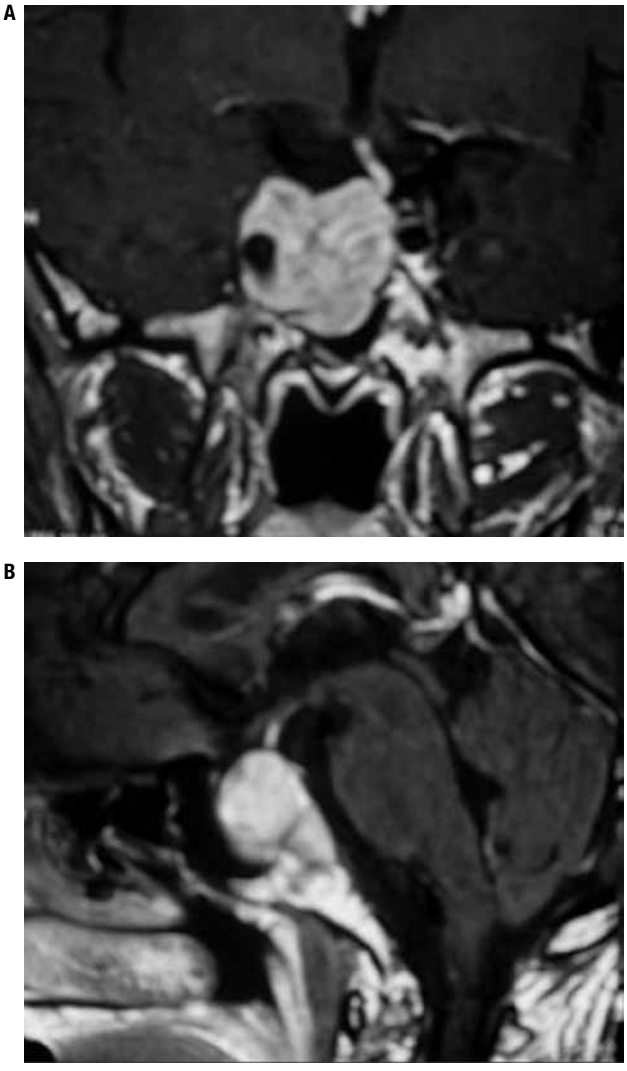

Figure 2. Magnetic resonance imaging of the sellar region (Case 2).

Magnetic resonance imaging of the sellar region (**A:** coronal T1-weighted with gadolinium enhancement; **B:** Sagital T1-weighted with gadolinium enhancement) showing a 2.6 x 2.5 x 2.6 cm intra-sellar lesion with infra-sellar and right cavernous sinus extension.

Table 1. Taqman® assays

Gene	Assays (TaqMan® Applied Biosystems)
AIP	Hs_00610222_m1*
SSTR2	Hs_00990356_m1
SSTR5	Hs00990408_s1
TBP	Hs_00427621_m1*
GUS Beta	Hs_00939627_m1*
PGK1	Hs_99999906_m1

AIP: aryl hydrocarbon receptor-interacting protein; SSTR2: somatostatin receptor type 2; TBP: TATA box-binding protein; GUSβ: β-glucuronidase; PGK1: phosphoglycerate kinase 1.

Table 2. Laboratory evaluation of patient 1

	GH (ng/mL)	IGF-I (ng/mL) NR: 106-277
Diagnosis	185.0	1470
Post-surgery (3 months)	77.0	479
Octreotide LAR 20 mg (6 months)	0.8	166
Octreotide LAR 20 mg (36 months)	0.7	104

Values of growth hormone (GH) and insulin-like growth factor type I (IGF-I) at diagnosis, after surgery and after medical therapy in patient 1; NR: normal range.

Table 3. Laboratory evaluation of patient 2

	GH (ng/mL)	IGF-I (ng/mL) NR: 101-267
Diagnosis	15.6	555
Post-surgery (3 months)	4.0	411
Octreotide LAR 20 mg (6 months)	5.39	304
Octreotide LAR 30 mg (6 months)	6.61	309

Values of growth hormone (GH) and insulin-like growth factor type I (IGF-I) at diagnosis, after surgery and after medical therapy in patient 2; NR: normal range.

Analysis of *AIP* mutations

Deoxyribonucleic acid (DNA) was extracted using the QIAamp DNA MiniKit (Qiagen, Valencia, CA, USA) from pituitary adenoma tissue according to the manufacturer's protocol. The entire coding sequence of *AIP* (NM_003977.2), the conserved splice sites (from the conserved A of the upstream branch site to +10 downstream of each exon) and 1200 base pairs of the promoter region were directly sequenced, as described elsewhere (8).

Assessment of *SSTR2*, *SSTR5* and *AIP* expression levels

SSTR2, *SSTR5* and *AIP* expression levels were analyzed by quantitative polymerase chain reaction (qPCR) using Taqman® methodology. Tumoral ribonucleic acid (RNA) was extracted using the RNeasy® Mini kit (Qiagen) according to the manufacturer's protocol. The amount and quality of the extracted RNA were measured using NanoDrop 2000® (Thermo Fischer, Wilmington, DE, USA). The MultiScribe™ and High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Carlsbad, CA, USA) were used for cDNA synthesis.

Thermocycling and fluorescence detection were performed using Applied Biosystems 7500 Real-Time PCR System®. The thermal cycling profile consisted of a preincubation step of 50°C for 2 min and denaturation at 95°C for 10 min followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 min. In addition to *SSTR2*, *SSTR5* and *AIP*, three housekeeping genes were analyzed [β-*glucuronidase* (*GUS*β), *TATA box-binding protein* (*TBP*) and *phosphoglycerate kinase 1* (*PGK1*)] as internal controls. Taqman® assays for each gene are depicted in table 1. Gene expression levels were calculated by QPCR software (14) using the efficiency of reaction (calculated for each reaction), and were compared with five normal pituitary tissues obtained from autopsies of subjects who had died of acute cardiovascular disease without evidence of previous endocrine disease.

RESULTS

Analysis of *AIP* mutations

No somatic mutations in the *AIP* gene were identified in either patient.

Analysis of *SSTR2*, *SSTR5* and *AIP* expression levels

The expression level of *SSTR2* was high in both tumors. The somatotropinoma of the patient 1 presented *SSTR2* expression that was 15.51 times higher than that of normal pituitary tissue (NPT). The somatotropinoma of the patient 2 presented *SSTR2* expression that was 22.95 times higher than that of NPT. *SSTR2* expression level was 1.5 times higher in the adenoma of patient 2 compared with the adenoma of patient 1.

SSTR5 expression was very low in both tumors in comparison with NPT. The somatotropinoma of the patient 2 presented expression 0.34 times that of NPT. *SSTR5* expression level in the somatotropinoma of patient 1 was 0.11 times that of NPT.

AIP expression of the somatotropinoma of patient 2 was similar to that of NPT (1.07x), while in patient 1, *AIP* expression level was 2.19 times higher than that of NPT. Comparing the two tumors, AIP expression was 2.05 times higher in the tumor of patient 1.

DISCUSSION

The commercially available SSAs enable the control of GH and IGF-I levels in approximately 30% of the acromegalic patients (15,16). Considering their affinity to the five SSTRs, it is expected that the expression of SSTR2 is mandatory for good response to these drugs (3). Indeed, it has been demonstrated by our group and others, that disease activity is not controlled in the presence of low SSTR2 expression (4,5,17,18). However, the presence of a high SSTR2 expression is not invariably associated with good response to SSAs therapy (5). In fact, a considerable proportion of patients with high SSTR2 expression do not achieve disease control with treatment, suggesting that other factors, such as SSTR5 expression levels, the presence of truncated isoforms of this receptor, and alterations in post-receptor signaling pathways may be involved (4,19). Our group has previously demonstrated, with another qPCR method (SYBR® Green), that the presence of a low SSTR2/

SSTR5 ratio (lower than 1.3) was associated with worse response to SSAs therapy (4).

Recent data have highlighted the importance of AIP expression in the response to SSAs therapy in acromegaly (9). Since the description of *AIP* germline mutations in familial isolated pituitary adenomas, it has been observed that patients harboring these mutations have worse response to SSAs treatment (8,9,20). Indeed, a study that compared 96 acromegalic patients with germline *AIP* mutations and 232 matched controls (acromegalic patients without mutations) demonstrated that patients harboring *AIP* mutations had a poorer response to SSAs treatment (9). The *AIP*-mutated patients had smaller decreases in GH and IGF-I levels, as well as reduced tumor shrinkage, thereby indicating that AIP may play a role in the mechanism of response to SSAs (9).

In addition to the data regarding familial acromegaly, it has been demonstrated that although no somatic mutation in the *AIP* gene has been found to date, a subset of sporadic somatotropinomas present low AIP expression (mainly the more invasive cases) (10,12,21). Our group has recently demonstrated that patients harboring tumors with low AIP protein expression have low chance of disease control with OCT-LAR therapy, independent of SSTR2 expression (11). These data reinforce the importance of AIP expression for good response to SSAs treatment.

Direct evidence of AIP involvement in the mechanism(s) of action of SSAs was provided by a study of Chahal and cols. (13). These authors observed that tumors from patients treated with lanreotide before surgery exhibited higher AIP expression than tumors from patients treated primarily by surgery. Furthermore, the authors performed an *in vitro* study and observed that treatment of GH3 cell lines with octreotide increased *AIP* expression. When *AIP* was knocked-down, the effect of octreotide in reducing cell proliferation was attenuated, and this was likely mediated through modulation of the expression of the tumor suppressor zinc-finger protein ZAC1, whose expression is known to be essential for the antiproliferative effect of SSAs (13,22).

To illustrate these recent findings in the literature, we presented two cases of acromegalic patients who were not cured by surgery, were treated with OCT-LAR, and showed different responses to treatment. In both cases, the tumors presented high *SSTR2* expression levels; however, patient 2 did not achieve disease control with OCT-LAR therapy despite having higher *SSTR2* expression than patient 1. In both cases, low expression of *SSTR5* was observed but, as previously described (4), it is the high expression of this receptor that is associated with poor response to octreotide LAR. When we compared *AIP* expression levels of both tumors, we observed that *AIP* expression was two times higher in the somatotropinoma from the patient whose disease was controlled with medical treatment (case 1). As previously discussed, the presence of a high SSTR2 expression is not always associated with good response to SSAs treatment, as in patient 2. One of the possible explanations is lower *AIP* expression, as AIP appears to be important in the post-receptor signaling of SSAs drug therapy (13).

The mechanism(s) of action of SSAs is not completely understood, and involves the regulation of ion channels (primarily potassium and calcium channels), adenylyl cyclase-cAMP-protein kinase A (PKA), mitogen-activated protein kinase (MAPK), and phosphotyrosine phosphatase (PTP) pathways (3). The exact place of AIP in this complex signaling machinery is not completely understood, and additional studies are necessary to clarify this issue.

One of the therapeutic possibilities for patient 2 is to associate cabergoline to octreotide LAR, as this approach is effective in patients with mildly elevated IGF-I levels (23). There are no reports on the possible role of AIP expression as a predictor of response to cabergoline in acromegaly, or if AIP is important in the mechanism of action of dopamine agonists.

In conclusion, the cases reported here illustrate that, although the currently available SSAs bind preferentially to SSTR2, high expression of this receptor is not always associated with good response to therapy; high AIP expression may also be important for the mechanism of action of this drug class.

Grants: this study was supported by grants from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (LK).

Disclosure: MRG received grant support from Novartis Biociências S.A. and Pfizer, and speaker fees from Novartis Biociências, Pfizer and Ipsen. LK, LMC, PCLE and MC have no conflict of interest to declare.

REFERENCES

1. Vieira Neto L, Abucham J, Araujo LA, Boguszewski CL, Bronstein MD, Czepielewski M, et al. [Recommendations of Neuroendocrinology Department from Brazilian Society of Endocrinology and

Metabolism for diagnosis and treatment of acromegaly in Brazil]. Arq Bras Endocrinol Metabol. 2011;55(2):91-105.

2. Barkan A, Bronstein MD, Bruno OD, Cob A, Espinosa-de-los-Monteros AL, Gadelha MR, et al. Management of acromegaly in Latin America: expert panel recommendations. Pituitary. 2010;13(2):168-75.
3. Ben-Shlomo A, Melmed S. Pituitary somatostatin receptor signaling. Trends Endocrinol Metab. 2010;21(3):123-33.
4. Taboada GF, Luque RM, Neto LV, Machado Ede O, Sbaffi BC, Domingues RC, et al. Quantitative analysis of somatostatin receptor subtypes (1-5) gene expression levels in somatotropinomas and correlation to in vivo hormonal and tumor volume responses to treatment with octreotide LAR. Eur J Endocrinol. 2008;158(3):295-303.
5. Wildemberg LE, Vieira Neto L, Costa DF, Nasciuti LE, Takiya CM, Alves LM, et al. Low somatostatin receptor subtype 2, but not dopamine receptor subtype 2, expression predicts the lack of biochemical response of somatotropinomas to treatment with somatostatin analogs. J Endocrinol Invest. 2012 Mar 26.
6. Vierimaa O, Georgitsi M, Lehtonen R, Vahteristo P, Kokko A, Raitila A, et al. Pituitary adenoma predisposition caused by germline mutations in the AIP gene. Science. 2006;312(5777):1228-30.
7. Cazabat L, Bouligand J, Salenave S, Bernier M, Gaillard S, Parker F, et al. Germline AIP mutations in apparently sporadic pituitary adenomas: prevalence in a prospective single-center cohort of 443 patients. J Clin Endocrinol Metab. 2012;97(4):E663-70.
8. Leontiou CA, Gueorguiev M, van der Spuy J, Quinton R, Lolli F, Hassan S, et al. The role of the aryl hydrocarbon receptor-interacting protein gene in familial and sporadic pituitary adenomas. J Clin Endocrinol Metab. 2008;93(6):2390-401.
9. Daly AF, Tichomirowa MA, Petrossians P, Heliovaara E, Jaffrain-Rea ML, Barlier A, et al. Clinical characteristics and therapeutic responses in patients with germ-line AIP mutations and pituitary adenomas: an international collaborative study. J Clin Endocrinol Metab. 2010;95(11):E373-83.
10. Kasuki Jomori de Pinho L, Vieira Neto L, Armondi Wildemberg LE, Gasparetto EL, Marcondes J, de Almeida Nunes B, et al. Low aryl hydrocarbon receptor-interacting protein expression is a better marker of invasiveness in somatotropinomas than Ki-67 and p53. Neuroendocrinology. 2011;94(1):39-48.
11. Kasuki L, Vieira Neto L, Wildemberg LE, Colli LM, de Castro M, Takiya CM, et al. AIP expression in sporadic somatotropinomas is a predictor of the response to octreotide LAR therapy independent of SSTR2 expression. Endocr Relat Cancer. 2012;19(3):L25-9.
12. Jaffrain-Rea ML, Angelini M, Gargano D, Tichomirowa MA, Daly AF, Vanbellinghen JF, et al. Expression of aryl hydrocarbon receptor (AHR) and AHR-interacting protein in pituitary adenomas: pathological and clinical implications. Endocr Relat Cancer. 2009;16(3):1029-43.
13. Chahal HS, Trivellin G, Leontiou CA, Alband N, Fowkes RC, Tahir A, et al. Somatostatin analogs modulate AIP in somatotroph adenomas: The Role of the ZAC1 Pathway. J Clin Endocrinol Metab. 2012;97(8):E1411-20.
14. Pabinger S, Thallinger GG, Snajder R, Eichhorn H, Rader R, Trajanoski Z. QPCR: application for real-time PCR data management and analysis. BMC Bioinformatics. 2009;10:268.
15. Mercado M, Borges F, Bouterfa H, Chang TC, Chervin A, Farrall AJ, et al. A prospective, multicentre study to investigate the efficacy, safety and tolerability of octreotide LAR (long-acting repeatable octreotide) in the primary therapy of patients with acromegaly. Clin Endocrinol (Oxf). 2007;66(6):859-68.
16. Colao A, Cappabianca P, Caron P, De Menis E, Farrall AJ, Gadelha MR, et al. Octreotide LAR vs. surgery in newly diagnosed patients with acromegaly: a randomized, open-label, multicentre study. Clin Endocrinol (Oxf). 2009;70(5):757-68.
17. Vieira Neto L, Taboada GF, Gadelha MR. Somatostatin receptors subtypes 2 and 5, dopamine receptor type 2 expression and gsp status as predictors of octreotide LAR responsiveness in acromegaly. Arq Bras Endocrinol Metabol. 2008;52(8):1288-95.
18. Ferone D, de Herder WW, Pivonello R, Kros JM, van Koetsveld PM, de Jong T, et al. Correlation of in vitro and in vivo somatotropic adenoma responsiveness to somatostatin analogs and dopamine agonists with immunohistochemical evaluation of somatostatin and dopamine receptors and electron microscopy. J Clin Endocrinol Metab. 2008;93(4):1412-7.
19. Duran-Prado M, Gahete MD, Martinez-Fuentes AJ, Luque RM, Quintero A, Webb SM, et al. Identification and characterization of two novel truncated but functional isoforms of the somatostatin receptor subtype 5 differentially present in pituitary tumors. J Clin Endocrinol Metab. 2009;94(7):2634-43.
20. Pinho LK, Vieira Neto L, Wildemberg LE, Moraes AB, Takiya CM, Frohman LA, et al. Familial isolated pituitary adenomas experience at a single center: clinical importance of AIP mutation screening. Arq Bras Endocrinol Metabol. 2010;54(8):698-704.
21. Raitila A, Georgitsi M, Karhu A, Tuppurainen K, Makinen MJ, Birkenkamp-Demtroder K, et al. No evidence of somatic aryl hydrocarbon receptor interacting protein mutations in sporadic endocrine neoplasia. Endocr Relat Cancer. 2007;14(3):901-6.
22. Theodoropoulou M, Zhang J, Laupheimer S, Paez-Pereda M, Erneux C, Florio T, et al. Octreotide, a somatostatin analogue, mediates its antiproliferative action in pituitary tumor cells by altering phosphatidylinositol 3-kinase signaling and inducing Zac1 expression. Cancer Res. 2006;66(3):1576-82.
23. Vilar L, Azevedo MF, Naves LA, Casulari LA, Albuquerque JL, Montenegro RM, et al. Role of the addition of cabergoline to the management of acromegalic patients resistant to longterm treatment with octreotide LAR. Pituitary. 2011;14(2):148-56.