

AIP expression in sporadic somatotropinomas is a predictor of the response to octreotide LAR therapy independent of SSTR2 expression

Dear Editor

Somatostatin receptor ligands (SRL) are currently the cornerstone of the medical treatment of acromegalic patients (Melmed *et al.* 2009). Disease control may be achieved by currently available SRL therapy in ~30–40% of patients in prospective clinical trials (Mercado *et al.* 2007). Considering the present accessibility of the diverse classes of drugs and their elevated costs, it would be valuable to identify predictive markers for the patients who are more likely to respond to SRL therapy.

Somatostatin receptor subtype 2 (SSTR2) expression seems to represent the best predictor of the response to SRL (Colao *et al.* 2011). Indeed, some studies have shown a positive correlation between the expression of SSTR2 mRNA and protein and the clinical response to SRL (Taboada *et al.* 2008, Wildenberg *et al.* 2012).

Daly *et al.* (2010) have previously observed that acromegalic patients with germline mutations in the aryl hydrocarbon receptor-interacting protein (AIP) gene exhibited a worse response to SRL therapy as compared to patients without these mutations. In addition, it has been previously demonstrated that sporadic somatotropinomas can present low AIP protein expression even in the absence of mutations (Jaffrain-Rea *et al.* 2009). Based on these data, in the current study, we sought to evaluate whether AIP expression is a predictor of octreotide LAR (OCT-LAR) response independent of SSTR2 expression in patients with sporadic somatotropinomas without AIP mutations.

Acromegalic patients consecutively operated on between 2006 and 2010 and not cured by surgical resection were included in the study. All patients signed informed consent forms before entering the study. Local ethics committee approved the study.

Exclusion criteria included previous radiotherapy for pituitary adenoma treatment, presence of AIP

mutation and/or a history of medical treatment with SRL before surgery. Biochemical assessment was performed 3 months after surgery by oral glucose tolerance test (OGTT) and evaluations of IGF1 levels. Patients were considered non-cured based on non-suppressible GH levels on OGTT and plasma IGF1 levels higher than those of age-matched normal subjects. Medical therapy with OCT-LAR was started at a dose of 20 mg every 4 weeks, and the dose was increased to 30 mg in patients with non-controlled disease after 3 months of therapy. The efficacy of medical therapy was evaluated during the last patient visit. Patients were considered to have non-controlled disease if they had plasma IGF1 levels greater than those of age-matched normal subjects or basal GH levels >1.0 ng/ml after at least 6 months of treatment with OCT-LAR at a dosage of 30 mg.

The AIP and SSTR2 expressions were analyzed through immunohistochemistry in paraffin-embedded tissue sections as previously described (Kasuki Jomori de Pinho *et al.* 2011, Wildenberg *et al.* 2011). For SSTR2, both membrane-bound and intracytoplasmic immunopositivity were considered for staging. Tumors were staged according to the percentage of stained cells: 0 (<25% stained cells – low expression) or 1 (≥25% – high expression), as previously validated by our group (Wildenberg *et al.* 2011). For the estimate of cytoplasmic AIP immunostaining, slides were scored for pattern (diffuse (score 2) or patchy (score 1)) and for intensity (strong (score 3), moderate (score 2) and weak (score 1)), and the final score was calculated by multiplying the two scores (pattern and intensity). Scores of 0 (no expression), 1 and 2 were considered low AIP expression.

The statistical analysis was performed using SPSS version 16.0 for Windows (SPSS, Inc., Chicago, IL, USA). The results were reported as median values (minimum–maximum). The Mann–Whitney non-parametric test, the Fisher exact test and χ^2 test were used as appropriate. For multivariate analysis, binary

logistic regression was performed. *P* values <0.05 were considered statistically significant.

Thirty-five samples from acromegalic patients (21 women) were included. The median age at diagnosis was 43 years (range 23–60 years). Thirty-two out of 35 tumors (91.4%) were macroadenomas. Thirteen tumors (37.1%) were mixed adenomas (positive staining for GH and prolactin), and the others were pure GH somatotropinomas. The median GH and IGF1 levels before OCT-LAR treatment were 14 ng/ml and 260% of the upper limit of normal range (ULNR), respectively.

All somatotropinomas expressed SSTR2A, with high expression observed in 29 tumors (83%). Both membrane and cytoplasmic staining of SSTR2A were observed (Fig. 1). AIP immunostaining was positive in all tumors, with 18 samples (51%) exhibiting low expression (Fig. 1). There was no difference in age, gender, hormonal staining, treatment duration, baseline GH or IGF1 levels between patients harboring somatotropinomas with low or high SSTR2A expression and between those with low or high AIP expression (Table 1). Additionally, no difference was observed in the SSTR2A expression levels between adenomas with low or high AIP expression.

In fifteen patients (43%), OCT-LAR therapy resulted in controlled disease. There was no difference between basal GH, IGF1, gender, age or hormonal staining between patients with controlled and non-controlled disease states. Treatment duration ranged from 9 to 57 months (median 28 months).

None of the patients (*n*=6) with SSTR2A expression lower than 25% (score 0) were controlled with OCT-LAR, whereas 15 out of 29 (52%) patients classified as score 1 reached a biochemically controlled disease state (*P*=0.024). Four out of 18 tumors (22%) with low AIP expression were controlled with OCT-LAR treatment, whereas 11 out of 17 (65%) patients with high AIP expression reached a biochemically controlled disease state (*P*=0.013). The control rate in the patients who presented with high AIP and SSTR2 expression was 79% (11 out of 14). The use of both tests to predict control rates yields a sensitivity of 73%, a specificity of 85%, a positive predictive value of 79%, a negative predictive value of 81% and an accuracy of 80%. After multivariate analysis, SSTR2A (*P*=0.02) and AIP (*P*=0.02) remained statistically significant predictors of disease control.

Germline mutations in the *AIP* gene are found in familial and early-onset pituitary adenoma setting, with a predomination of somatotropinomas in the mutated gene cases (Igreja et al. 2010, Tichomirowa et al. 2011). The *AIP*-mutated patients have smaller decreases in GH and IGF1 levels as well as less tumor shrinkage with SRL therapy (Leontiou et al. 2008, Daly et al. 2010, Pinho et al. 2010). These data indicate that AIP may play a role in the mechanism of response to SRL. Indeed, Chahal et al. (2009) have shown that treatment of GH3 cell lines with octreotide increased the AIP protein expression at 9 and 12 h. They also observed that patients treated with lanreotide prior to surgery exhibited tumors with higher AIP expression

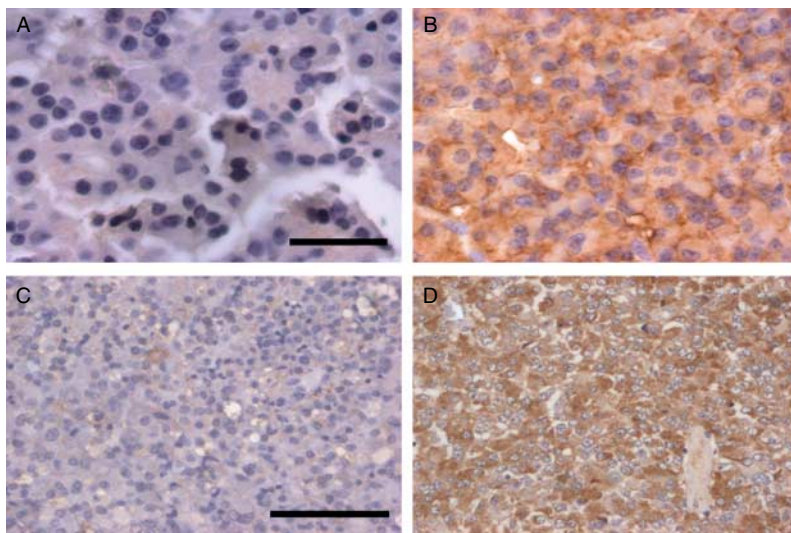


Figure 1 Examples of somatostatin receptor subtype 2 (SSTR2) and aryl hydrocarbon receptor-interacting protein (AIP) expression: (A) low SSTR2 expression (<25% of immunostained cells). (B) High SSTR2 expression (≥25% of immunostained cells). Scale bar: 500 μm. (C) Low AIP expression (score 1 – patchy and weak). (D) High AIP expression (score 6 – diffuse and strong). Scale bar: 1000 μm.

Table 1 Patient characteristics according to somatostatin receptor subtype 2 (SSTR2) and aryl hydrocarbon receptor-interacting protein (AIP) expression

	High SSTR2	Low SSTR2	P value	High AIP	Low AIP	P value
Age	46 (23–60)	36 (28–50)	0.403	43 (23–60)	45 (24–55)	0.832
Female	58.6%	66.6%	0.544	61%	58.8%	0.582
Mixed tumor (GH/PRL)	37.9%	33.3%	0.608	41.1%	33.3%	0.448
Treatment duration (months)	29 (9–57)	25 (12–35)	0.848	29 (12–57)	24 (9–48)	0.568
Baseline GH	12.6 (1.1–112)	19.4 (10–102)	0.133	29.6 (1.1–102)	19.6 (2.2–112)	0.322
Baseline IGF1 (%ULNR)	229 (128–457)	294 (203–415)	0.145	214 (128–382)	265 (137–457)	0.135

Results are presented as median (min–max). ULNR, upper limit of normal range.

than those of untreated patients. These results reinforce the potential role of AIP in the mechanism of SRL response in somatotropinomas.

In sporadic somatotropinomas, germline *AIP* mutations have been described in a small proportion of cases (mainly young patients), and no somatic mutations have been found to date (Raitila *et al.* 2007, Tichomirowa *et al.* 2011). However, a subset of sporadic tumors present with low AIP protein expression (Jaffrain-Rea *et al.* 2009, Kasuki Jomori de Pinho *et al.* 2011) even in the absence of mutations, as in our present series. In addition, our group has recently demonstrated that low AIP expression is associated with tumor invasiveness in somatotropinomas (Kasuki Jomori de Pinho *et al.* 2011).

No previous study has evaluated whether the expression of AIP may be a predictor of response to OCT-LAR therapy in sporadic somatotropinomas without *AIP* mutations. Here, we report that patients with low AIP expression are less likely to achieve disease control with OCT-LAR treatment. In addition, we demonstrate that AIP expression is a predictor of disease control independent of SSTR2 expression. Further, when both AIP and SSTR2 expression are used in combination, it is possible to predict the response to therapy with enhanced accuracy. Thus, the use of both markers may represent a useful tool in the clinical treatment decision.

In our study, we excluded those patients who were treated with SRL before surgery because SRL can increase the AIP protein expression (Chahal *et al.* 2009) and thus introduce a bias in the study.

The mechanism of resistance to OCT-LAR in patients with low AIP expression has not been described. In the present study, the expression of SSTR2 is not responsible for this decreased response to therapy, as SSTR2 expression does not differ between

adenomas with high or low AIP expression. It is probable that AIP has a direct role in the response to OCT-LAR, as AIP expression increases in cell lines treated with octreotide (Chahal *et al.* 2009). However, further studies are necessary to clarify the exact mechanism(s).

In conclusion, our study suggests that AIP expression in sporadic somatotropinomas without *AIP* mutations is a predictor of acromegaly control with OCT-LAR treatment independent of SSTR2 expression. In addition, when both markers are used in conjunction, it is possible to predict patient responses to OCT-LAR therapy with a higher accuracy than when each one is used individually.

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Declaration of interest

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