Expression of estrogen receptor alpha is associated with prolactin pituitary tumor prognosis and supports the sex-related difference in tumor growth

Etienne Delgrange2, Alexandre Vasiljevic1,3,6, Anne Wierinckx1,4, Patrick François5, Emmanuel Jouanneau1,3,7, Gérald Raverot1,3,8 and Jacqueline Trouillas1,3,6

1Université de Lyon 1, 69372 Lyon, France, 2Service d’Endocrinologie, CHU Dinant-Godinne UCL Namur, Université Catholique de Louvain, 5530 Mont-sur-Meuse, Namur, Belgium, 3Centre de Neurosciences de Lyon, INSERM S1028/CNRS UMR 5292, 69372 Lyon, France, 4Centre de Recherche en Cancérologie de Lyon, INSERM U1052/CNRS UMR 5286, 69008 Lyon, France, 5Service de Neurochirurgie, CHU de Tours, et Université François Rabelais, Tours, France, 6Centre de Pathologie Est, 7Service de Neurochirurgie and 8Fédération d’Endocrinologie, Groupement Hospitalier Est, Hospices Civils de Lyon, Lyon, France

Abstract

Context: A sex difference in the progression of prolactin (PRL) tumors has been disputed for years.

Objective: To compare tumor characteristics and postoperative clinical course between men and women, and correlate data with estrogen receptor alpha (ERα (ESR1)) expression status.

Design, patients, and methods: Eighty-nine patients (59 women and 30 men) operated on for a prolactinoma and followed for at least 5 years were selected. Tumors were classified into five grades according to their size, invasion, and proliferation characteristics. The ERα expression was detected by immunohistochemistry and a score (0–12) calculated as the product of the percentage of positive nuclei and the staining intensity.

Results: We found a significant preponderance of high-grade tumors among men and a lower surgical cure rate in men (23%) than in women (71%). Patients resistant to medical treatment were mainly men (7/8), six of whom showed tumor progression despite postoperative medical treatment, which led to multiple therapies and eventually death in three. The median score for ERα expression was 1 in men (range, 0–8) and 8 in women (range, 0–12) (P<0.0001). The expression of ERα was inversely correlated with tumor size (r = −0.59; P<0.0001) and proliferative activity. All dopamine agonist-resistant tumors and all grade 2b (invasive and proliferative) tumors (from ten men and four women) were characterized by low ERα expression.

Conclusions: PRL tumors in men are characterized by lower ERα expression, which is related to higher tumor grades, resistance to treatment, and an overall worse prognosis.

Introduction

Phenotypically, prolactin (PRL)-secreting pituitary tumors vary greatly, ranging from small indolent tumors to large invasive ones. Sex is a widely accepted factor influencing tumor size (1). The larger size of PRL tumors (prolactinoma) in men is often attributed to differences in detection, with diagnostic delays being the cause of more advanced PRL tumors in men (2). Nevertheless, young men usually present with large PRL tumors and most women of childbearing age with intrasellar tumors, even after a long duration of symptoms (3). When considering giant PRL tumors, the median age at diagnosis is almost 10 years lower in men than in women (4). PRL tumors in men are also less sensitive to dopamine agonists (DAs) (3) and often highly vascularized, whereas in women,
they frequently present as hemorrhagic tumors (5). Finally, in view of the bimodal age distribution of large PRL tumors in women, with a very low occurrence in childbearing-aged women (4, 6, 7), the presence of a functioning reproductive axis seems to restrain tumor expansion. Indeed, the proliferative activity of PRL tumors has been reported to be higher in men and older women (beyond 40 years of age) than in young women (8). These clinical data suggest that estrogens could halt PRL tumor progression.

Estrogen receptor alpha (ERα (ESR1)) expression has been detected, using different techniques, in both normal and tumoral cells secreting PRL and gonadotropins (9). Immunohistochemical (IHC) detection of ERα, used in breast cancer management, has progressively improved over the years with new generation ER-antibodies, the establishment of a semi-quantitative immunoreactive (IR) score (10), and the use of automated analysis (11). The IHC detection of ERα in pituitary PRL tumors has been studied by several authors, all of whom failed to demonstrate sex-related differences (8, 12, 13, 14, 15). However, these studies used manual immunostaining techniques, did not establish an IR score, and more recent ones (14, 15) included only five and six tumors respectively.

We therefore conducted a study on a large surgical series of patients with PRL tumors with a long-term follow-up, first to confirm the more aggressive clinical course of the disease in men (3) and its relationship with higher proliferative indexes, and secondly to correlate clinical and histological data with the IHC detection of ERα using a semi-quantitative scoring system established for breast cancer (10) and adapted to pituitary tumors. It appeared that low ERα expression was more frequent in men and associated in both sexes with higher tumor grade and DA resistance.

**Subjects and methods**

**Patients**

From our register of surgically removed pituitary tumors, we selected 89 PRL tumors resected from patients operated in two centers (Lyon and Tours), between 1988 and 2005. In case of multiple operations, samples obtained at the time of the first surgery were studied. Clinical data including patient age, gonadal status, indication for surgery, serum PRL levels, tumor size, and invasiveness were recorded. Long-term follow-up (at least 5 years) was obtained in order to compare surgical cure rates, response to adjuvant medical treatment, and outcome between women and men. Informed consent was obtained from each patient according to French law.

**Invasiveness**

Tumors were recorded as invasive if they showed invasion of the sphenoid and/or cavernous sinus on magnetic resonance imaging (MRI). In some cases, sphenoid sinus invasion was also confirmed by histology.

**Disease control**

Patients were considered as cured if they displayed postoperative normalization of PRL that was maintained until the end of follow-up, without dopaminergic therapy. Recurrence was defined as the detection of elevated PRL levels during follow-up after an initial normalization. Patients with a persistent disease (not surgically cured) and those with recurrence of hyperprolactinemia were treated with DAs. Patients in whom serum PRL levels failed to normalize despite a prolonged treatment (at least 3 months) at high doses (≥15 mg of bromocriptine daily or ≥2.0 mg of cabergoline weekly) were considered as resistant to treatment. Tumor progression was defined as evidence on MRI of regrowth despite postoperative medical treatment. In case of poor compliance, intolerance, or resistance to medical therapy, patients were re-operated and/or treated with radiotherapy.

**Histopathological diagnosis**

For each tumor, fragments were fixed in Bouin–Hollande solution and embedded in paraffin for pathological diagnosis, including IHC. Utilizing the indirect immunoperoxidase method, IHC stains were performed with antibodies directed against PRL, growth hormone, adrenocorticotropin, the alpha subunit of glycoprotein hormones (αSU), and the beta subunits of thyrotropin, luteinizing hormone, and follicle-stimulating hormone. Only tumors with predominant PRL immunostaining were considered. The following antibodies were used: anti-PRL (1/400, Immunotech, Marseille, France), MKI67 (Mib1, 1/50, Dako, Glostrup, Denmark), and anti-p53 (clone DO-7, 1/200, Novocastra Laboratories, Newcastle upon Tyne, UK). For the last two antibodies, we performed immunostaining both manually with microwave pre-treatment and automatically with Benchmark XT, Ventana Medical System (Tucson, AZ, USA).
Markers of proliferation

The proliferation rate was evaluated in each surgical specimen by the detection of mitoses and the determination of the MKI67 labeling index. The expression of p53 (TP53) was also studied, as described previously (16). For each marker, cells from ten representative high-power fields (HPF; 400× magnification) were counted with an average of 5000 nuclei/tumor. Mitoses were recorded by their absolute number. The MKI67 and p53 labeling indices were established as the percentage of tumoral cells with positive nuclei. For the grading, a mitotic count > 2 was considered positive and the threshold for MKI67 index positivity set at > 1% rather than > 3%, taking into account the use of Bouin–Holland fixative solution that results in a lower sensitivity for the MKI67 antibodies than formalin (16, 17). For p53, detection was considered as positive if there were more than ten strongly positive nuclei per 10 HPF (HPF of 0.30 mm²; 400× magnification).

Prognostic clinicopathological classification

Tumors were classified into five grades using a recently published classification (16) based on the MRI-detected invasion of cavernous and/or sphenoid sinus and their proliferation characteristics. A tumor was recorded as proliferative when at least two of the three following criteria were present: MKI67 > 1%, mitoses n > 2/10 HPF, and positive p53. The grades were as follows: grade 1a, non-invasive tumors; grade 1b, non-invasive but proliferative tumors; grade 2a, invasive tumors; grade 2b, invasive and proliferative tumors; and grade 3, metastatic tumors (with cerebrospinal or systemic metastases).

ER immunohistochemistry

The automated IHC detection of ERα protein was performed on fresh slides with a rat pre-diluted ERα or ESR1 MAB (clone SP1, ref 790-4324/25, Ventana; Roche Diagnostics) for 81 out of 89 tumors. For eight patients with a microadenoma, not enough tumors remained on the new sections to perform the analyses. In 21 cases (from four men and 17 women), the tumor was surrounded by non-tumoral pituitary. The microwave antigen retrieval technique was used to enhance IHC detection of the ER. Only nuclear staining was considered. An IR scoring system adapted from breast cancer (10) was used. This score, ranging from 0 to 12, was calculated as the product of the percentage of positive nuclei (0: 0%; 1: ≤ 10%; 2: 11–50%; 3: 51–79%; and 4: ≥ 80%) and the intensity of the staining (0: no staining; 1: mild; 2: moderate; and 3: strong). The strongest intensity observed in the non-tumoral pituitary was taken as reference. In three tumors with heterogeneous results, the most prevalent score was considered. The scoring was established independently by two pathologists (A Vasiljevic and J Trouillas), both of whom were blinded to the clinical data. Similar results were obtained. The IHC detection of ERα with the semi-quantitative score is illustrated in Fig. 1.

Statistical analysis

Data are expressed as means±S.E.M. and/or as medians. Medians were compared using the non-parametric Mann–Whitney U and Kruskal–Wallis tests. Frequencies were compared using the χ² test. Correlation coefficients were calculated by the Spearman rank order (r). Multiple linear regression analysis was performed with the ER IR score as the dependent variable, and the following parameters (that had a P value <0.10 in the univariate analysis) as explanatory variables: age, sex, PRL level, tumor size, invasion, mitotic count, MKI67, p53, tumor grade, surgical cure, and tumor progression. Predictors such as sex, invasion, tumor grade, surgical cure, and tumor progression were coded as categorical data. As some variables such as tumor size and PRL level or mitotic count and MKI67 were highly correlated (r=0.74 and 0.62 respectively), stepwise selection of predictors was performed to build a parsimonious model. Adjusted R² was used to evaluate the goodness of fit of the model. R² is the fraction of the total variance of Y that is ‘explained’ by variation in X, which has been adjusted to take into consideration the number of parameters in the model. Statistical calculations were performed using the Minitab Software (version 17.0). The level used to determine statistical significance was P<0.05.

Results

Preoperative clinical features

Of the total 89 included patients, 59 were women and 30 men. Their main clinical and pathological characteristics are summarized in Table 1. Mean age at surgery was 33±1 years among the women and 48±2 years for the men (P<0.0001). On the basis of MRI findings, 39 patients (44%) had a microadenoma and were almost exclusively women (95%). All giant tumors (n=8) were found in men. We confirmed that the tumors were larger and more often invasive in men than in women (P<0.0001).
The preponderance of large tumors in males was independent of age: among patients ≤40 years, the median tumor size was 22 mm in men and 7 mm in women (P=0.0017). Macroadenomas were more frequently invasive in men (21 of 28) than in women (seven of 22; P=0.0023).

The majority of patients (55/89; 62%) had never been treated before, although 26 women (44%) and eight men (27%) (P=0.1657) had received dopaminergic drugs before surgery (mainly bromocriptine). The indications for primary surgical treatment were patients’ preference for surgery rather than chronic medical treatment in 37 cases with intrasellar tumor, rapidly progressive loss of vision (n=9), tumor apoplexy (n=2), spontaneous rhinorrhea (n=1), and uncertainty about the correct diagnosis (typically in the presence of a large tumor and serum PRL levels below 150 μg/l) (n=6). In patients previously treated with DAs, the main indication for surgery was drug intolerance (n=13), followed by partial tumoral response despite PRL normalization (n=6) and resistance to treatment (n=5). The remaining patients were either not

Figure 1
Immunohistochemical analysis of ERα expression in PRL tumors using a semi-quantitative score calculated as the product of the percentage of positive nuclei (0: 0%; 1: ≤10%; 2: 11–50%; 3: 51–79%; and 4: ≥80%) and the intensity of the staining (0: no staining; 1: mild; 2: moderate; and 3: strong). (A) Non-tumoral pituitary (original magnification, 400×): 20% of nuclei show a strong nuclear positivity. This staining corresponds to the highest intensity in the score scale and is used as a reference for intensity ‘3’. (B) Immunonegative PRL tumor (original magnification, 200×). No nuclear staining is observed in the tumor (AD) (score 0). Non-tumoral pituitary (NTP) close to the tumor is used as a positive internal control. (C) PRL tumor with a score of 2 (original magnification, 400×); 15% of nuclei are weakly immunopositive (score: 2×1=2). (D) PRL tumor with a score of 8 (original magnification, 400×); 85% of nuclei show a moderately intense immunostaining (score: 4×2=8).
compliant to treatment \((n=2)\) or chose the surgical option after unsuccessful withdrawal of prolonged medical treatment \((n=8)\).

**Response to treatment**

The minimum postoperative follow-up was 5 years and, in 82% of cases \((73/89)\), it exceeded 10 years. Fifty-five patients displayed postoperative normalization of serum PRL levels, which was maintained until the end of follow-up in 49 patients whom we considered as surgically cured. This surgical cure was significantly more frequent among women \((n=42; 71\%)\) than men \((n=7; 23\%)\) \((P<0.0001)\). In six patients (all women), hyperprolactinemia resumed 1–9 years after initial surgical normalization. In these recurrent cases, as in patients with persistent disease \((n=34)\), we prescribed DA therapy. This postoperative medical treatment controlled the hyperprolactinemia in 25 patients but not in the remaining patients \((n=15)\). This latter group of patients showed resistance \((n=8)\) or intolerance to DAs and/or poor compliance to treatment and thus either received radiotherapy \((n=1)\) or no treatment in the few years after surgery \((n=6)\). In the patients temporarily lost to follow-up, we secondarily reinitiated medical therapy \((n=3)\), re-operated \((n=2)\), or treated with radiotherapy \((n=1)\). Patients resistant to medical treatment were mainly men \((7/8)\) and six of them (five men) showed tumor progression despite postoperative medical treatment, which required multiple treatments including radiotherapy \((n=6)\) and temozolomide \((n=2)\). Three of these patients (all men) eventually died from their tumor, two from metastatic spread and the third from invasive cerebral extensions.

**Pathological characteristics**

By IHC, the percentage of IR cells with PRL antibodies ranged from 80 to 100 in most cases \((86/89; 97\%)\). Seventeen of these tumors were plurihormonal including nine cases showing co-secretion of PRL and αSU. All PRL–αSU adenomas had been resected from women. The three tumors (all from women) with relatively low

<table>
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<th>Table 1 Sex-related comparison of clinical, biological, and pathological characteristics of PRL tumors surgically treated in 89 patients. For continuous variables, results are expressed as mean ± S.E.M. (median).</th>
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<tr>
<td><strong>Age (years)</strong></td>
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<td>PRL level (μg/l)</td>
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<td>MRI data</td>
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<td>Tumor height (mm)</td>
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<td>&lt;10 mm, n (%)</td>
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<td>10–40 mm, n (%)</td>
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<td>&gt;40 mm, n (%)</td>
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<td>Invasive tumors, n (%)</td>
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<td><strong>Tumor characteristics</strong></td>
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<td>Immunocytochemistry</td>
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<td>Monohormonal PRL, n (%)</td>
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<td>Plurihormonal PRL, n (%)</td>
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<td>Mitotic count (nb)</td>
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<td>Grade 2a, n (%)</td>
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<td>Grade 2b, n (%)</td>
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<td>Follow-up</td>
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<td>Duration (months)</td>
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<td>Surgical cure, n (%)</td>
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<td>DA resistance, n (%)</td>
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<td>Radiotherapy, n (%)</td>
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<td>Tumor progression, n (%)</td>
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<td>Death, n (%)</td>
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*Three tumors exhibited signs of malignancy (grade 3) during follow-up.

*A total of 61 patients were treated with dopamine agonists before and/or after surgery, of whom 19 were considered intolerant and/or not compliant; as such, resistance to treatment was assessable in 42.
percentage of PRL-IR cells (ranging from 50 to 60%) were monohormonal macroadenomas.

Tumors resected from men displayed a higher number of mitoses and MKI67 index than those from women (P=0.02 and P=0.001 respectively). While a slightly higher percentage of tumors from men expressed p53, this difference was not statistically significant.

Upon classifying the tumors into five grades according to invasion and proliferation, we noted a significant preponderance of high-grade tumors (2b) among men (33% vs 7%; P<0.0001). While median age at surgery did not differ among the tumor grades for men, high-grade tumors were encountered later on in women (median age 51 compared with 30 in the other groups, P=0.03).

**ERα expression and PRL tumor aggressiveness**

In the fragments of non-tumoral pituitary surrounding PRL tumors, the ERα IR score was 6 in men (n=4) as well as in women (n=17) (Fig. 1A). Regarding PRL tumors, and considering any demonstrable staining as ‘positive’, IHC revealed the expression of ERα in 68 out of 81 tumors (84%) with no significant difference between those resected from men (22/29; 76%) and those from women (46/52; 88%) (P=0.1386). However, semi-quantitative evaluation using the IR score revealed a significantly lower expression of ERα in men than in women, with respective median scores of 1 in men (range, 0–8) and 8 in women (range, 0–12) (P<0.0001) (Fig. 2). As shown in Table 2, we divided the tumors into two groups according to the IR score, with low and high ER expression being defined, respectively, by an IR score <6 or ≥6. Low expression of ER protein was significantly more frequent in the tumors from men than those from women (P<0.0001), and such tumors had significantly higher mitotic count, MKI67 index (Fig. 3), and p53 expression. Furthermore, all DA-resistant tumors, all grade 2b tumors (ten from men and four from women), and all tumors showing regrowth despite postoperative medical treatment displayed low ER expression.

**Factors correlated with ERα expression**

The expression of ERα as assessed by the IR score was correlated with several factors listed in Table 3. Testosterone level at the time of surgery was recorded in ten patients (median, 5.0 nM/L, range 0.2–11.8) but did not correlate with the IR score. In 17 women, data regarding menstrual status and/or history of estrogen administration at the time of surgery were not available, whereas 36 women had amenorrhea and six oligomenorrhea. Neither menstrual status nor previous DA treatment was related to the expression of ERα. Among variables significantly related to the ERα IR score, DA resistance was not included in the multiple regression analysis because it was not

Table 2  Comparison of the characteristics of 81 PRL tumors according to estrogen receptor alpha (ERα) expression statusa. For continuous variables, results are expressed as mean±s.e.m. (median).

<table>
<thead>
<tr>
<th></th>
<th>High ERα (n=49)</th>
<th>Low ERα (n=32)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Men, n (%)</td>
<td>7 (14)</td>
<td>22 (69)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Tumor height (mm)</td>
<td>9±1 (7)</td>
<td>25±2 (21)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Invasive tumors, n (%)</td>
<td>5 (10)</td>
<td>22 (69)</td>
<td>&lt;0.0001</td>
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<td>Cell cycle markers</td>
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<tr>
<td>Mitotic count (nb)</td>
<td>1±0 (0)</td>
<td>5±1 (3)</td>
<td>0.0011</td>
</tr>
<tr>
<td>MKI67 (%)</td>
<td>0.3±0.1 (0.0)</td>
<td>2.3±0.6 (1.0)</td>
<td>0.0008</td>
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<tr>
<td>TP53 (%)</td>
<td>0.1±0.1 (0.0)</td>
<td>0.7±0.2 (0.0)</td>
<td>0.0041</td>
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<td>Prognostic classification</td>
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<tr>
<td>Grade 1a, n (%)</td>
<td>40 (82)</td>
<td>7 (22)</td>
<td>&lt;0.0001</td>
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<tr>
<td>Grade 1b, n (%)</td>
<td>4 (8)</td>
<td>3 (9)</td>
<td></td>
</tr>
<tr>
<td>Grade 2a, n (%)</td>
<td>5 (10)</td>
<td>9 (28)</td>
<td></td>
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<tr>
<td>Grade 2b, n (%)</td>
<td>0 (0)</td>
<td>13 (41)b</td>
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<tr>
<td>Surgical cure, n (%)</td>
<td>36 (73)</td>
<td>7 (22)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>DA resistance, n (%)</td>
<td>0/17 (0)</td>
<td>8/24 (33)</td>
<td>0.0080</td>
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<tr>
<td>Tumor progression, n (%)</td>
<td>0 (0)</td>
<td>6 (19)</td>
<td>0.0016</td>
</tr>
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*aERα expression status: high if the immunoreactive score is ≥6 and low if <6.

bThree tumors exhibited signs of malignancy (grade 3) during follow-up.

cA total of 61 patients were treated with dopamine agonists before and/or after surgery; estrogen receptor status was determined in 56, of whom 15 were considered intolerant and/or not compliant; resistance to treatment was thus assessable in 41.
assessable in patients cured by a primary surgery or in those considered intolerant and/or not compliant to medical treatment which represents 40 of the 81 cases studied for ERα expression. The other 11 factors (age, sex, PRL level, tumor size, invasion, mitotic count, MKI67, p53, tumor grade, surgical cure, and tumor progression) collectively correlated significantly with the IR score (adjusted $R^2$ 0.416, F ratio 5.27, $P<0.0001$), but tumor size was the only independent predictor ($P=0.008$). Stepwise regression identified a model based on only two predictors, namely tumor size and mitotic count (ERα IR score $= 9.984 - 0.2208 \text{mm size} - 0.2269 \text{mitoses}$), to be more accurate in predicting the IR score (adjusted $R^2$ 0.441, F ratio 29.45, $P<0.0001$) compared with the model including all 11 variables.

Discussion

In this large surgical series, we have confirmed that PRL tumors are larger and more frequently invasive in men than in women, and that surgical cure is more likely in women. Considering non-invasive tumors, surgical cure was obtained in 48 out of 61 cases (79%), which further validates the recommendation made by the Pituitary Society that ‘in centres with experienced neurosurgeons, the possibility of cure by surgery vs long-term DA therapy should be discussed with the patient, and patient preference is also an indication for surgery’ (18). Owing to surgical recruitment, between 1988 and 2005, this series also included nine patients (all men) admitted for large tumors with rapidly progressive loss of vision (19), which is no more regarded as an indication for primary surgery, surgical cure being unlikely (0/9 in the present series) and improvement of visual impairment occurring promptly after initiation of DA therapy (20).

Mitosis and MKI67 expression were higher in men, as has been reported in smaller series (3, 21, 22, 23). Moreover, according to the new prognostic clinicopathological classification (16), men have higher grade tumors than women. This is associated with a worse prognosis: the grade 2b tumors of six patients (five men) progressed despite postoperative medical treatment, leading to death in three cases for which the tumor was latterly considered malignant based on metastasis or brain invasion. Our data are in agreement with the limited evidence in the literature, which notably suggests a more frequent requirement for temozolomide treatment, used in highly aggressive and/or metastatic PRL tumors, among men compared with women (sex ratio 5F/14M) (24, 25).

The reason for the more aggressive course of the disease in men remains poorly understood. What is, however, now clear is that diagnostic delay does not account for the observed difference in tumor behavior, which is already apparent in prepubertal boys (3, 4). While the presence of ER in normal PRL cells as well as in human PRL tumors is well established, questions remained as to the existence of a sex-related difference in ER expression. Until now, studies on ER expression have used a manual immunostaining technique to establish a result as positive or negative. None has attempted to establish an ‘IR score’ calculated from the intensity of staining and the percentage of IR cells, as recommended for breast cancer (10). The global proportion of PRL tumors recorded as ‘positive’ for ERα has been 45/72 (8), 8/8 (13), 3/5 (14), and 2/6 (15).
In the Law’s series (12), 21/29 (72%) PRL tumors demonstrated >5% ERα positivity, with no significant difference between men (6/10; 60%) and women (15/19; 79%). Using the same threshold, we obtained very similar figures in our series: ERα positivity in 61 out of 81 tumors (75%) including 15 out of 29 from men (52%) and 46 out of 52 from women (88%). If we regard any demonstrable staining as positive, the percentage of PRL tumors expressing ERα rises to 84%. With this type of evaluation, ERα expression does not significantly correlate with sex (22/29; 76% for men vs 46/52; 88% for women), tumor size, or invasiveness. However, concordantly with our data, Burdman et al. (14) noted that the two tumors negative for ERα were larger than the three positive ones and Kaptain et al. (12) observed that recurrent tumors (previously treated surgically) were less likely to express ERα. In the present series, we applied for the first time to pituitary tumors, an IR score adapted from studies in breast cancer to evaluate ER expression. In this way, we have herein demonstrated that a lower expression of ERα is associated with higher proliferation, is more frequently observed in tumors from men, and is clearly related to tumor grade, resistance to DA therapy, and progression despite multimodal therapy. In breast cancer, ER-positive tumors are also more likely to be well differentiated and to have a lower fraction of dividing cells. The loss of ERα might thus be a sign of lower differentiation and poorer prognosis. The expression status of ERα may thus be considered a prognostic factor, to be used alongside others such as high expression of some cell cycle proteins (26, 27) and loss of chromosome 11p (28).

Little is known about how the expression of ERα is regulated, even in human breast cancer. In the normal human pituitary, ERα is expressed at high levels in lactotropes and at somewhat lower level in gonadotropes (29). To our knowledge, no data have been published regarding a potential sex difference. We evaluated the ERα immunoreactivity in six normal pituitaries from autopsy (three men and three women; data not shown) and in the fragments of non-tumoral pituitary surrounding 21 PRL tumors. The ERα expression was scored at 6, was not different between sexes, and was maintained even if the tumor itself was negative for ERα expression (this is well illustrated in Fig. 1B). Our data indicate a lower ERα expression not only in men but also in women with high-grade tumors, regardless of the expression in the normal pituitary. We tried to correlate several clinical factors with the expression of ERα. In accordance with a previous study (12), we found no influence of previous DA treatment or of gonadal status on ERα expression, but it must be emphasized that testosterone levels at the time of surgery were available in only ten patients, that most women had amenorrhea, and that estradiol levels were not determined. Stepwise multivariate regression analysis identified tumor size and mitotic count as the best predictors of ERα IR score, but they explained together only ~50% of the variability (R² 0.44). Thus, tumor size and proliferation activity are certainly related to the ERα expression, but further studies will be required to identify other factors influencing the ERα expression in human pituitary PRL tumors.

An inverse relation between ER expression and proliferation in PRL tumors might seem surprising, especially considering the well-known stimulation of
PRL release by estrogens. Estrogen appears therefore to differentially effect cell proliferation and PRL secretion (30). Two case reports of male patients with macroprolactinomas treated with cabergoline demonstrated an increase in the PRL level following testosterone supplementation, which was reversed by the aromatase inhibitor anastrozole (23, 31). This was considered a demonstration of the negative impact of estrogens on PRL tumors, and yet there was no mention of any tumor growth. Indeed, no evidence exists for a correlation between exposure to estrogens and development or growth of PRL tumors in women treated with oral contraceptives (32) or in male-to-female transsexuals (33). During pregnancy, hyperplasia of normal PRL cells is obvious in humans and other animals. At the beginning of the 1980s, several reports underlined the occurrence of symptoms during pregnancy, suggestive of PRL tumor enlargement such as headaches and/or visual disturbance (34). Since then, this is also viewed as a strong argument in favor of a deleterious effect of estrogens on tumor growth. However, radiological evidence of tumor growth was often lacking, no histological proof of stimulation of the cell proliferation was given, and PRL tumor regrowth was mainly described after withdrawal of a short-term treatment with bromocriptine (35), which can also be the case in the absence of pregnancy (36). More recent data (37, 38, 39, 40) indicate the absence of symptomatic tumor growth in a total of 88 women treated with cabergoline for a macroprolactinoma before pregnancy. In one study (38), routine procedure included an MRI performed without contrast injection between 24 and 32 weeks of gestation, and cabergoline was reintroduced in three out of 15 patients because the pituitary contacted the optic chiasm. In another study (39), 12 patients had headaches and five unspecific visual disturbances without visual field defect, but none of them showed tumor enlargement in post partum MRI. In some patients with macroprolactinoma, post partum imaging investigation may reveal either tumor shrinkage or hemorrhage (41). In our experience, four patients operated on in an emergency setting for visual impairment had hemorrhagic PRL tumors proved by MRI, peroperative data, and histology, but low MKI67 index (J Trouillas, unpublished observations). Pregnancy has been repeatedly reported to ‘cure’ some PRL tumors (42, 43), especially microprolactinomas, which precisely overexpress ERα. Even in some animal models, estrogens have been shown to lead to inhibition of pituitary transplantable tumor growth (44, 45, 46). This inhibition of tumor growth by estrogens might explain the bimodal distribution of large PRL tumors in women (4, 5, 6) and the lower MKI67 indexes observed in women of ‘reproductive age’ (8). Altogether, our data and evidence in the literature suggest that, in men and postmenopausal women, a lower ERα expression combined with low estrogen production could enhance proliferation and give rise to high-grade (2b) tumors such as those we observed in men and older women.

In a previous study (12), the proportion of ER-positive tumors was similar in naïve (6/9: 67%) and in previously DA-treated patients (15/20: 75%). Our results also indicate that the preoperative DA treatment does not decrease the ERα expression but patients not responsive to DA treatment have highly proliferating tumors (47) and are certainly characterized by low ERα expression, which may be considered as a sign of cellular dedifferentiation.

The mechanism by which expression of ERα is associated with an inhibition of tumor growth is probably related to a DA-induced antiproliferative effect and/or to an antiangiogenic action. In addition, previous studies on Wistar rats indicated that DA-induced apoptosis of anterior pituitary cells required the presence of estrogens (48).

In conclusion, we confirm that PRL tumors are larger and more frequently invasive in men compared with women, and a surgical cure is more likely in women. Accordingly, men have higher grade tumors than women, associated with a worse prognosis. Low ERα expression, as assessed by a semi-quantitative IR score, is more frequently observed in men and is associated in both sexes with high-grade PRL tumors and resistance to treatment. These results should prompt a routine assessment of ERα expression status by IHC in PRL tumors, for which loss of ERα expression should be considered as a likely indicator of poor prognosis. Estrogens might restrain the growth of well-differentiated tumors, yet the growth of aggressive PRL tumors seems estrogen independent.

Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding
This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Acknowledgements
The authors thank Emily Witty from Angloscribe for reviewing the manuscript for the English language.
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Received 15 November 2014
Revised version received 15 February 2015
Accepted 19 March 2015