Estrogen Receptors α and β Immunohistochemical Expression: Clinicopathological Correlations in Pituitary Adenomas

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Abstract. Aim: We investigated the immunohistochemical expression of estrogen receptors alpha (ERα) and beta (ERβ) in pituitary adenoma subtypes combined with clinicopathological factors. Materials and Methods: Pituitary adenomas (n=75) were immunostained for ERα and ERβ using the streptavidin-biotin-peroxidase complex method with a monoclonal ERα antibody and polyclonal ERβ antibody. Results: Nuclear immunoreactivity for both receptors was highest among PRL, FSH/LH, null cell, and GH adenomas. ACTH, silent subtypes I and II corticotrophs, and subtype III adenomas were the least immunoreactive for both receptors. ACTH adenomas expressed significantly less ERα than FSH-LH, GH, and null cell adenomas. A significantly elevated ERα expression was observed in macroadenomas compared to microadenomas and non-invasive compared to invasive tumors. Conclusion: ERα and ERβ are differentially expressed in the various pituitary adenoma subtypes suggesting a cell-specific function for these receptors. To elucidate the role of ERα in tumor size and invasiveness, additional studies are required.

Estrogen receptors (ERs) are members of the steroid receptor gene superfamily, functioning as ligand-induced transcription factors. To date two distinct isoforms of the estrogen receptor, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) have been identified (1-3). ERα is encoded by an eight exon gene on chromosome 6 in humans (4), whereas the ERβ gene is located on chromosome 14 (5). Although both receptors have high binding affinity for estrogen, the difference in their trans-activation domains is suggestive of their distinct roles in gene activation.

Both, ERα and ERβ, are expressed and involved in the growth and differentiation of several tissue types including, prostate (6), spermatids and ovarian cells (5, 7). Studies have shown abundant expression of ERα and ERβ mRNAs in various fetal tissue types during development (8). The effects of estrogen on the pituitary include its role in angiogenesis (9) as well as regulation of adenohypophysial hormone synthesis and secretion (10, 11). Estrogen mediates hormonal effects through the binding of ER dimers to specific estrogen-responsive gene regions, thereby initiating transcriptional changes (12). Estrogen has also been shown to function as a potent pituitary cell mitogen in specific cell types (13). Both, in vitro and in vivo studies have suggested the proliferation of prolactin (PRL), thyroid stimulating hormone (TSH), follicle stimulating hormone (FSH), luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and growth hormone (GH) cells, to be associated with estrogen and the elevated expression of ERα and ERβ (14-17).

The proliferative effects of estrogen mediated through its nuclear receptors, ERα and ERβ, have been implicated in cell proliferation and tumorigenesis (18). Several intracranial tumors, including craniopharyngiomas (19), meningiomas, astrocytic neoplasms, schwannomas, and chordomas show the expression of ERs (20-22). The clonal expansion of a particular pituitary cell type through the mitogenic effects of estrogen is thought to stimulate pituitary tumor initiation and progression (23). This notion is supported through observations of higher prevalence of surgically removed PRL adenomas in women (3:1 female:male ratio) (18, 24, 25), higher incidence of PRL macroadenomas in pregnant women (26), the predominance of FSH-LH tumors in men (27, 28), and a 3- to 8-fold higher incidence of female ACTH adenomas (29). A case of the development of a pituitary PRL adenoma after high-dose estrogen administration in a transsexual patient has also been reported (30).

Expression of ERα and ERβ in human pituitary adenomas has also been reported (31). ERα transcript was reported in 109 pituitary adenomas in increasing amounts in ACTH, GH, and PRL adenomas (32). Using enzyme immunoassay,
another study reported ERα immunoreactivity in only 10 of 56 pituitary tumors (33). The highest levels were found in FSH/LH adenomas, PRL and combined GH/PRL adenomas, while no ERα was detected in GH or nonfunctioning tumors. Similarly, ERα immunohistochemical overexpression in PRL, GH/PRL, and FSH-LH adenomas has also been shown (29). ERβ mRNA co-expression with ERα and its splice variants was found in PRL, mixed GH-PRL and of FSH/LH tumors (34). Unlike ERα, which was only expressed in PRL, mixed GH/PRL and FSH/LH adenomas, ERβ mRNA was also found in null cell, GH and ACTH adenomas. Many of these studies using human tissue have also been supported by in vitro findings of ER expression in pituitary adenomas (11, 35, 36).

To date, no studies have examined the association between estrogen receptor expression and clinicopathological variables of patients with pituitary adenomas. The present work investigates the immunohistochemical expression of ERα and ERβ, and their correlation with clinicopathological variables in a large series of surgically removed pituitary adenomas of all subtypes.

Materials and Methods

Materials. The present study assessed 75 pituitary adenomas (45 females, 30 males), obtained from transsphenoidal surgery. Both clinically functioning and non-functioning adenomas were investigated. Each tumor was classified based on the criteria of the 2004 World Health Organization (WHO) Classification of Endocrine Tumors (37). Specimens included 6 PRL (4 females, 2 males), 12 GH, 11 ACTH, 21 FSH-LH (11 females, 10 males), as well as 7 silent corticotroph (5 type I, 2 type II), 9 silent subtype III, and 9 null cell adenomas.

Methods. All specimens were fixed in 10% buffered formalin, routinely processed, paraffin embedded cut at 5 μm, and stained with hematoxylin and eosin (H&E). The streptavidin-biotin-peroxidase complex method was used and antisera were directed against GH, PRL, ACTH, TSH, LH, FSH, as well as the alpha-SU of glycoprotein hormones. Methods of immunostaining, antibody sources and dilutions have previously been described (38). Electron microscopy had also been performed for diagnostic purposes. Immunostaining for ERα utilized a commercially available ERα mouse monoclonal antibody (1:100; Dako; Carpinteria, CA, USA). Similarly, a commercially available rabbit polyclonal ERβ antibody was used for the detection of ERβ (1:200; Millipore; Billerica, MA, USA). Routine deparaffinization, rehydration, and blockade of endogenous peroxidase activity were carried out. Sections were then microwaved in 0.1 mM sodium citrate buffer (pH 6.0), incubated with goat anti-serum, and exposed to the streptavidin-biotin-peroxidase complex. Diaminobenzidine served as the chromagen. Positive controls for ERα and ERβ immunoreactivity utilized formalin-fixed, paraffin-embedded breast carcinoma tissue. Replacement of the primary antibody with PBS served as a negative control. Immunopositivity for ERα and ERβ was evaluated at high magnification (×400). Both the intensity and percentage of positive cells of all specimens were studied blindly by two of the authors (BM, KK) to determine inter-observer variability. Intensity and percent positivity were evaluated for both ERα and ERβ. Intensity was assessed on a scale of 0-3 (0=none, 1=mild, 2=moderate, 3=strong). The percentage of immunopositive cells was assessed as the percent of cells exhibiting reactivity in 10 fields at high magnification (×400). Areas demonstrating necrosis, fibrosis, and artifacts were excluded. Average immunopositivity, intensity, and percent reactivity were recorded as mean observed in ten high power fields.

Statistical analysis. ANOVA (SPSS Statistical Program, SSPS Inc., Chicago, IL, USA) was used to find significant differences between pituitary adenoma subtypes. The kappa statistic was used to determine inter-observer variability. For kappa value calculations, the percentage positivity was categorized as: 0=0%, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%. The intensity of staining was categorized as: 0=None, 1=mild, 2=moderate, 3=strong. All means are reported as the mean± standard error of mean (S.E.M).

Results

ERα, ERβ cellular localization. ERα (Figure 1) and ERβ (Figure 2) immunoexpression demonstrated nuclear positivity. No cytoplasmic expression was observed for ERα or ERβ.

ERα, ERβ immunoexpression. Percentage of immunopositive cells. Of the 75 surgically obtained pituitary adenomas, ERα stained positive in 37 cases (49%). The mean percentage positivity score out of 4 for ERα immunopositivity across all pituitary adenoma subtypes was 0.67±0.09 (range, 0 to 3). Mean percent positivity scores were highest in null cell (1.04±0.23), FSH-LH (0.92±0.18), GH (0.67±0.19), and PRL adenomas (0.67±0.49). The lowest mean percentage positivity scores of ERβ immunoreactive cells were found in silent subtype I, II (0.57±0.28), subtype III (0.41±0.21), and ACTH adenomas (0.18±0.12). A significant difference was identified in ERα immunopositivity between GH and ACTH adenomas (p=0.044), FSH-LH and ACTH adenomas (p=0.002), null cell and ACTH adenomas (p=0.003).

ERα was immunoreactive in 48 of the 75 cases (64%). The mean percentage positivity score out of 4 for ERβ immunopositivity across all pituitary adenoma subtypes was 0.89±0.09 (range, 0 to 3). Mean percent positivity scores were highest in GH (1.08±0.23), PRL (1.06±0.44), and FSH-LH (1.00±0.18), and null cell adenomas (0.89±0.31). The lowest mean percentage positivity scores of ERβ immunoreactive cells were found in ACTH (0.82±0.23), silent subtype I, II (0.71±0.36), and subtype III adenomas (0.48±0.19). No significant differences were identified with respect to ERβ immunopositivity between tumor subtypes.

ERα, ERβ immunoexpression. Intensity of immunopositive cells. The mean intensity score out of 3 for ERα immunostaining across all adenoma subtypes was 0.71±0.09 (range, 0 to 3). The highest intensity of ERα immunostaining...
was observed in null cell (1.00±0.22), FSH-LH (0.97±0.19), and GH adenomas (0.78±0.23). The lowest ERα staining intensity was observed in PRL (0.67±0.49), silent subtype I, II (0.57±0.30), subtype III (0.33±0.22), and ACTH adenomas (0.33±0.22). A significant difference was identified in ERα staining intensity between ACTH and FSH-LH adenomas (p=0.045), subtype III and FSH-LH adenomas (p=0.047), subtype III and null cell adenomas (p=0.029). Although not significant, a difference in ERα staining intensity between ACTH and null cell adenomas was noted (p=0.052).
The mean intensity score out of 3 for ER\(\beta\) immunostaining across all adenoma subtypes was 0.96±0.10 (range, 0 to 3). The highest intensity of ER\(\beta\) immunostaining was observed in FSH-LH (1.17±0.21), PRL (1.17±0.48), and GH adenomas (1.06±0.22). The lowest ER\(\beta\) staining intensity was observed in null cell (0.89±0.31), ACTH (0.88±0.24), silent subtype I, II (0.81±0.40), and subtype III adenomas (0.52±0.22). No significant differences were identified with respect to ER\(\beta\) immunostaining intensity between tumor subtypes.

**ER\(\alpha\), ER\(\beta\) immunoexpression. Tumor size.** ER\(\alpha\) mean percent positivity score was higher in macroadenomas than microadenomas (0.74±0.10 and 0.27±0.14, respectively). A significant difference was identified in ER\(\alpha\) immunopositivity between macroadenomas and microadenomas (\(p=0.013\)). No significant differences were observed in ER\(\beta\) immunopositivity between macroadenomas and microadenomas.

Although the mean ER\(\alpha\) intensity score was higher in macroadenomas (0.77±0.10) than microadenomas (0.39±0.22), no significant difference was observed. Also, there was no significant difference in ER\(\beta\) staining intensity between macroadenomas and microadenomas.

**ER\(\alpha\), ER\(\beta\) immunoexpression. Tumor invasiveness.** ER\(\alpha\) mean percent positivity score was higher in non-invasive than invasive adenomas (0.74±0.12 and 0.41±0.10, respectively). A significant difference was identified in ER\(\alpha\) immunopositivity between non-invasive and invasive adenomas (\(p=0.038\)). No significant differences were observed in ER\(\beta\) immunopositivity between non-invasive and invasive adenomas. ER\(\alpha\) mean intensity score was higher in non-invasive than invasive adenomas (0.89±0.14 and 0.49±0.12, respectively). A significant difference was observed in ER\(\alpha\) staining intensity between non-invasive and invasive adenomas (\(p=0.031\)). There was no significant difference in ER\(\beta\) staining intensity between non-invasive and invasive adenomas.

**ER\(\alpha\), ER\(\beta\) immunoexpression. Gender, tumor recurrence, functioning vs. non-functioning adenomas.** No significant differences were observed in ER\(\alpha\) and ER\(\beta\) immunopositivity or immunostaining intensity between males and females, recurrent adenomas and non-recurrent adenomas, and endocrinologically functioning and non-functioning adenomas.

**Kappa value.** Inter-observer variability was low for all scores. Kappa value for both, ER\(\alpha\) and ER\(\beta\) percent positivity score was high (0.93 and 0.98, respectively). Similarly, the calculated Kappa value for both, ER\(\alpha\) and ER\(\beta\) intensity score was high (0.96 and 0.97, respectively). These values indicate almost optimal agreement between raters.

**Discussion**

**ER\(\alpha\), ER\(\beta\) expression in adenoma subtypes.** We investigated the relationship between ER\(\alpha\) and ER\(\beta\) immunoeexpression in pituitary tumor subtypes and tumor size, invasiveness and recurrence as well as patient age and sex (Table I). Our results showed null cell adenomas, FSH/LH, GH, and PRL adenomas to be the most reactive for ER\(\alpha\), with silent corticotroph subtype I and II, subtype III, and ACTH adenomas showing the lowest percent immunopositivity score for ER\(\alpha\) expression. Similarly, GH, PRL, FSH-LH, and null cell adenomas demonstrated the strongest immunoreactivity for ER\(\beta\), with the lowest
ERβ immunoexpression being exhibited by ACTH, silent subtype I and II, and subtype III adenomas. Pair-wise comparison of adenoma subtypes using ANOVA showed no significant differences in ERβ immunoexpression; ACTH adenomas expressed significantly lower ERα mean percent positivity scores compared to FSH-LH, GH, and null cell adenomas. Our findings of elevated ERα and ERβ immunoexpression in PRL and FSH-LH adenomas are in keeping with results from previous studies (11, 25, 29, 33-35, 39-41). Several investigators have established the direct action of estrogen, through its receptors, on the regulation of PRL gene transcription, synthesis, and secretion (18, 42-45). The PRL promoter contains a nonpalindromic estrogen response element that functions as a weak transcription activator. This is enhanced by the transcription factor, Pit-1, in the activation of PRL gene transcription (42). Clinical implications of these findings include the proliferation of lactotrophs during pregnancy (46, 47), growth of PRL adenomas during gestation (48), rapid increase in size and secretion of PRL adenomas following the administration of oral contraceptives (49), and the pathogenesis of lactotroph adenoma in a male-to-female transsexual following estrogen therapy (30). Similarly, estrogen has been shown to act through ERα and regulate gonadotroph cell differentiation, proliferation, and hormone production (25, 29, 50). Through the use of a biochemical analysis, Pinchon et al. (41) were the first to detect ERα in human pituitary adenomas, and identify an increase in the number of ERs in PRL- and FSH-LH-secreting adenomas following the administration of oral contraceptives (49), and the pathogenesis of lactotroph adenoma in a male-to-female transsexual following estrogen therapy (30). Similarly, estrogen has been shown to act through ERα and regulate gonadotroph cell differentiation, proliferation, and hormone production (25, 29, 50). Through the use of a biochemical analysis, Pinchon et al. (41) were the first to detect ERα in human pituitary adenomas, and identify an increase in the number of ERs in PRL- and FSH-LH-secreting adenomas. More recently, using RT-PCR and hybridization blotting, Shupnik et al. (24) demonstrated the expression of ERα and ERβ mRNA isoforms and splice variants to occur differently in prolactinomas and gonadotroph adenomas. ERα and ERβ mRNA were found to be more abundant in PRL and FSH-LH adenomas, respectively.

The differences in our results according to the expression patterns of both ERs in PRL and FSH-LH adenomas may be explained by the ability of ERα and ERβ to form heterodimers (51), which enables both proteins to influence estrogen action in lactotrophs and gonadotrophs. Expression of the mRNA splice variants also demonstrated cell type specificity, in that all or most prolactinomas contained exon 2 and 5 deletion variants, whereas a higher proportion of gonadotroph adenomas contained an exon 7 deletion variant. Additional investigations with prolactinomas have yielded a pattern of both ERs in PRL and FSH-LH adenomas may be attributable to ERs not being required in the regulation of PRL levels. Burdman et al. (54) described the absence of estrogen receptors in prolactinomas to be specific to poorly differentiated tumor cells and a sign of anaplasia. They noted that expression patterns of ERα and ERβ in lactotroph and gonadotroph adenomas as indicated by our study suggests estrogen to have a possible role in the stimulation and growth of these tumors.

Our findings indicated GH-secreting adenomas to be the most immunonegative for ERβ and highly immunoreactive for ERα when compared with other adenoma subtypes. During ontogeny of the pituitary gland, GH and PRL cells are derived from a common precursor. One key point of divergence in these two cell types may involve the regulation of the ERα gene, with up-regulation occurring as progenitors differentiate into PRL-secreting cells and down-regulation occurring as they differentiate into GH-secreting cells (55). It is thought that GH cells immunopositive for ERα are mamasomatotrophs existing in the pituitary, a notion supported by the observation that GH-releasing cells that do not produce PRL are devoid of ERα (25, 29). Interestingly, experiments using primary animal pituitary cell cultures have documented GH release and elevated GH mRNA in response to estrogen from ERα negative somatotroph cells (10, 56). This suggests a potential role for ERβ in mediating estrogen-stimulated hormone release and gene transcription. Our results indicate 5 out of 12 GH adenomas to be negative for ERα, 2 of which demonstrated immunoreactivity for ERβ. The remaining 3 GH adenomas, which were completely negative for both ERs may suggest the presence of regulatory mechanisms other than estrogen and its receptors. Our findings are also in keeping with those of Zafar et al. (25) in that ERα immunopositivity was more consistently found in densely granulated (80%) than sparsely granulated (43%) somatotroph adenomas. Given these findings, it appears that both ERs are involved in the growth of GH adenomas.

Interestingly, null cell adenomas in our study appeared to be the most immunonegative for ERβ and highly immunoreactive for ERβ when compared with other adenoma subtypes. Chaidarun et al. (34) found similar results as ERβ expression was present in all null cell adenomas and demonstrated the strongest immunoreactivity among adenoma subtypes. The expression pattern of “silent corticotroph adenomas”, silent subtypes I, II, and subtype III have not been previously investigated. Our results indicate these adenomas to be the least reactive for ERα and ERβ, thus suggesting the involvement of signaling pathways in the growth of these tumors that include receptors other than ERα and ERβ. Reduced reactivity for ERα and ERβ was also observed in ACTH-secreting adenomas examined in our study. Of the 11 ACTH adenomas, only 2 were immunoreactive for ERα, while 7 cases demonstrated slight ERβ immunoexpression. The immunonegative results for ERα expression in ACTH is in keeping with previous findings (25, 29). The presence of ERα in ACTH-secreting adenomas
has only been previously described once by Sano et al. (57), who identified two cases which contained the coexpression of LH. The two cases identified to be immunoreactive for ERα in our investigation did not demonstrate the coexpression of any other pituitary hormones. Previous work by Chaidarun et al. (34) also identified an increase in reactivity for ERβ, in which ERβ mRNA was identified in 60% of ACTH adenomas, delineating ERβ to be the main mediator of estrogen-stimulated gene expression in ACTH adenomas. Although ERβ demonstrated greater reactivity than ERα for ACTH adenomas in our study, the expression pattern for both receptors was among the lowest when compared with other adenoma subtypes. Further work is required to investigate the role of estrogen receptors in null cell adenomas, silent corticotroph subtypes I and II, and subtype III adenomas, and ACTH-secreting adenomas.

ERα expression in macro/microadenomas, invasive/non-invasive adenomas. In terms of clinicopathological investigations, we found a relationship between ERα expression and tumor size and invasiveness (Table II). Pairwise comparison of tumor size showed a significant difference in ERα expression; macroadenomas expressed significantly higher ERα mean percent positivity scores than microadenomas. Similarly, non-invasive adenomas expressed a significantly higher level of ERα expression when compared with invasive adenomas. ERα and ERβ expression did not reach significance with other clinicopathological parameters. Previous studies have also identified higher ERα expression in macro/microadenomas than microadenomas, supporting a role for estrogen in promoting pituitary tumor growth (33, 58, 59). Although no significant differences were observed, Kaptain et al. (59) described a relationship between ERα immunoreactivity in macroprolactinomas and non-invasive prolactinomas. In addition, despite not being specific to pituitary adenomas, reduced levels of ERα mRNA coupled with increased immunohistochemical staining for the Ki-67 proliferation index has been illustrated in breast cancer cells (60). Alternative studies have shown ERα immunopositive tumors to contain significantly reduced Ki-67 expression (61). Therefore, ERα may act as a proliferation inhibitory factor and thereby have increased expression in non-invasive adenomas. With respect to pituitary tumors, the relationship between tumor size and invasiveness are topics that are subject to controversy. Our results indicate that the presence of macroadenomas should not be considered as a sign of malignant potential. In addition, our findings suggest ERα to be a possible novel biomarker for predicting tumor size and invasiveness in pituitary adenomas.

In conclusion, we have demonstrated variable nuclear ERα and ERβ expression levels in different pituitary adenoma subtypes. ACTH adenomas expressed significantly lower levels of ERα than FSH-LH, GH, and null cell adenomas. No significant differences were identified in ERβ expression among adenoma subtypes. Additional clinicopathological investigations yielded significantly elevated ERα expression in macroadenomas than microadenomas and non-invasive tumors than invasive tumors. Although the expression of both ERα and ERβ in pituitary tumors is well established, the specific role of these receptors in the initiation and progression of tumor subtypes other than PRL adenomas has yet to be determined. Future studies are required to further characterize ERα as a novel biomarker for pituitary tumor size and invasiveness.

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References


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