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Mini-Review

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Estrogen Receptors in Nonfunctioning Pituitary Neuroendocrine Tumors: Review on Expression and Gonadotroph Functions

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Abbreviations: E2, 17β-estradiol; ER, estrogen receptor; FSH, follicle-stimulating hormone; GH, growth hormone; GnRH, gonadotropin-releasing hormone; GPER, G protein–coupled estrogen receptor; LH, luteinizing hormone; mRNA, messenger ribonucleic acid; NF-Pitnet, nonfunctioning pituitary neuroendocrine tumor; PR, progesterone receptor; PRL, prolactin; PTTG, pituitary tumor transforming gene; SERM, selective estrogen receptor modulator; TX, tamoxifen.

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Abstract

Estrogen (17β-estradiol or E2) is a crucial regulator of the synthesis and secretion of pituitary reproductive hormones luteinizing hormone, follicle-stimulating hormone, and prolactin. In this review, we summarize the role of estrogen receptors in nonfunctioning pituitary neuroendocrine tumors (NF-Pitnets), focusing on immunoexpression and gonadotroph cell proliferation and apoptosis. Gonadotroph tumors are the most common subtype of NF-Pitnets. Two major estrogen receptor (ER) isoforms expressed in the pituitary are estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ). Overall, estrogen actions are mostly exerted through the ERα isoform on the pituitary. The G protein–coupled estrogen receptor (GPER) located at the plasma membrane may contribute to nongenomic effects of estrogen. Nuclear immunoreactivity for ERα and ERβ was highest among gonadotroph and null cell tumors. Silent corticotroph tumors are the least immunoreactive for both receptors. A significantly elevated ERα expression was observed in macroadenomas compared with microadenomas. ERα and ERβ may act in opposite directions to regulate the Slug-E-cadherin pathway and to affect invasiveness of NF-Pitnets. In the cellular pathway, ERs regulate estrogen-induced proliferation and differentiation and impact several signaling pathways including the MAPK and PI3K/Akt pathway. Estrogen was the first-discovered inducer of pituitary tumor transforming gene 1 that was abundantly expressed in NF-Pitnets. ERα can be a potential biomarker for predicting tumor size and invasiveness as well as

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therapeutic target for NF-Pitnets. Selective estrogen receptor modulators or antiestrogen may represent as an alternative choice for the treatment of NF-Pitnets.

Key Words: estrogen, estrogen receptor alpha, estrogen receptor beta, nonfunctioning pituitary neuroendocrine tumors, gonadotroph tumors, SERMs

Pituitary adenomas, also referred to as pituitary neuroendocrine tumors (Pitnets) [1], are common neoplasms comprising ~10% to 20% of intracranial tumors [2]. Nonfunctioning pituitary neuroendocrine tumors (NF-Pitnets) are benign tumors characterized by the absence of clinical and biochemical evidence of hormonal overproduction [3]. However, these tumors can grow into large tumors and cause hypopituitarism and visual disturbances. NF-PitNETs represent more than one-third of PitNETs, with predominance in men and increasing frequency with older age [4]. NF-Pitnets represent a heterogeneous group of adenomas classified as gonadotroph adenomas, silent tumors that express only 1 pituitary hormone (adrenocorticotropin hormone; thyroid-stimulating hormone, prolactin [PRL], and growth hormone [GH]), multiple pituitary hormones (silent adenoma subtype III), or no hormone (null cell) based on hormone expression on immunohistochemical examination [5]. Most NF-Pitnets stem from gonadotropic cells, with immunostaining for follicle-stimulating hormone (FSH), LH, and/or alpha subunit of glycoprotein hormones. Among silent corticotroph adenomas, silent adenoma subtype I (densely granulated) and subtype II (sparsely granulated) can be found. The silent gonadotroph adenoma is the most common subtype [6].

17β-Estradiol regulates the synthesis and secretion of several pituitary hormones through the activation of estrogen receptors (ERs). 17β-Estradiol (E2) effects are typically mediated through 2 structurally related ERs, ERα and ERβ subtypes that function as ligand activated transcription factors [7]. In humans, ERα is encoded by an 8-exon gene on chromosome 6 [8], whereas the ERβ gene is located on chromosome 14 [9]. Evidence has demonstrated that ERα mediates the proliferative response to estrogen, whereas ERβ represses proliferation and induces differentiation. Overall, gonadotroph tumors have intermediate levels of protein and constitute a lower (50%) percentage of ERα-positive tumors, whereas the highest levels of ERα messenger ribonucleic acid (mRNA) protein and the highest percentages of ERα-containing tumors have been among PRL-containing tumors, with or without GH (70-100%) [10]. Null tumors with ERα were less frequent, and GH tumors were consistently ERα negative in several studies [11-13]. In contrast to the results with ERα, ERβ mRNA is expressed preferentially in gonadotroph tumors, much less frequently in prolactinomas, and in the majority of tumors expressing only GH or GH plus PRL [10]. In the rat, estrogen stimulates basal secretion of LH, FSH, and PRL, and sensitizes the pituitary to the gonadotropin-releasing hormone (GnRH) [14]. Estrogen also induces pituitary progesterone receptor (PR) expression in the gonadotrope and elicits GnRH self-priming [15]. Complex interactions between multiple signaling pathways are involved in estrogen regulation of hormone secretion and gonadotroph cell proliferation and apoptosis [16].

In the present article, the emphasis is on the ER immunoexpression in NF-Pitnets and its role in gonadotrophic cell proliferation and apoptosis. Finally, we reviewed the role of selective estrogen receptor modulators (SERMs) and antiestrogen as potential therapeutic agents in NF-Pitnets.

ER Immunoexpression in NF-PitNET

Through the use of a biochemical analysis, Pichon et al. [17] were the first to identify an increase in the number of ERs in gonadotroph adenomas. Subsequent study by Shupnik and colleagues [11] demonstrated the expression of ERα and ERβ mRNA isoforms and splice variants in gonadotroph adenomas by using reverse transcription polymerase chain reaction and hybridization blotting. Intriguingly, ERβ mRNAs were found to be more abundant in gonadotroph adenomas if compared with prolactinoma. ERα expression was found to be higher in NF-Pitnets than in functioning Pitnets in patients younger than 50 years [18]. In a study by Manoranjan et al. [19], null cell adenomas and gonadotroph adenomas were found to be among the most reactive for ERα. The expression pattern of silent corticotroph adenomas (silent subtypes I, II) and subtype III indicate that these adenomas were the least reactive for ERα and ERβ, with silent corticotroph adenomas showing the lowest percent immunopositivity score for ERα expression. Similarly, gonadotroph and null cell adenomas demonstrated among the strongest immunoreactivity for ERβ. Their findings of elevated ERα and ERβ immunoexpression in gonadotroph adenomas are in keeping with results from previous studies [11-13, 20, 21]. These findings may suggest that estrogen has a possible role in the stimulation and growth of these tumors. Moreover, ERα seems to be more abundantly expressed in large than in small pituitary tumors [19, 22]. ERα expression was also found to be significantly higher in invasive NF-Pitnets. Zhou et al. [23] found that ERα staining was significantly stronger in invasive than in noninvasive

NF-Pitnets. Interestingly, decreased expression of ERβ was observed in invasive NF-Pitnets. In contrast to findings by Manoranjan et al. [19], noninvasive adenomas expressed a significantly higher level of ERα expression when compared with invasive adenomas. This implies that the invasion of NF-Pitnet may be affected by the balance between ERα and ERβ expression although conflicting results have been reported regarding the role of ERα in invasiveness of NF-Pitnets. This hypothesis was also supported by the findings in ER knockout mice that demonstrated formation of gonadotropin-positive pituitary tumors in female mice at 2 years of age following loss of ERβ [24]. However, previous studies failed to find significant difference in the marker of cell proliferation (proliferating cell nuclear antigen [PCNA] index) between invasive NF-Pitnets and noninvasive NF-Pitnets [23, 25]. The Ki-67 expression was also detected, but without any significant difference between invasive NF-Pitnets and noninvasive NF-Pitnets [23]. Nevertheless, immunohistochemistry data derived from a very large tissue microarray study of NF-Pitnets demonstrate that the Ki-67 index is the most consistent marker of biological behavior in these tumors [29].

The aggressiveness of pituitary adenomas has been associated with loss of the cell adherence protein, E-cadherin [26]. Previous studies have demonstrated that ER signaling pathways play important roles in the regulation of E-cadherin [27, 28]. In a study with 41 cases of NF-Pitnets, the expression of ER, Slug, and E-cadherin were determined to evaluate the relationship of ER with the invasiveness of NF-Pitnets and its correlation with E-cadherin or Slug [23]. In invasive NF-Pitnets, nuclear ERα staining was found to be significantly stronger in contrast to nuclear ERβ staining, which was significantly weaker. Both E-cadherin mRNA and protein were decreased significantly in invasive NF-Pitnets compared with noninvasive ones. Moreover, Slug, a repressor of E-cadherin, was significantly increased in invasive NF-Pitnets. Thus, there were significant correlations between ER and Slug or E-cadherin in NF-Pitnets, in which Slug was positively correlated with ERα and inversely correlated with ERβ, whereas E-cadherin was positively correlated with ERβ and inversely correlated with ERα [23].

The role of ERα has emerged as a prognostic factor in male patients with NF-Pitnets. Low expression is related to an earlier and greater repeat intervention rate in male patients with NF-Pitnets [30]. Furthermore, the absence of ERα together with younger age appeared to predict the risk of reintervention in men with gonadotroph adenomas. The level of ERα (immunoreactive score and mRNA) was also found to be significantly correlated to the level of SSTR2 in men [30]. Studies on breast cancer cells have shown that ER and SSTR2 expressions were correlated, and it has been suggested that estrogens regulate the level of SSTR2 through ERα activation [31, 32].

Estrogen Functions in Gonadotroph Cell Proliferation and Antiapoptosis

The involvement of estrogens in the control of pituitary function has been extensively studied and reviewed [33]. It has been demonstrated that estrogens act on the biosynthesis and secretion of all hormones of the anterior pituitary. In the anterior pituitary, 2 classic targets for estrogen action are the lactotropes, where estrogen stimulates PRL, and the gonadotropes, where estrogen regulates FSH and LH. Estrogen has been shown to act through ERα and regulate gonadotroph cell differentiation, proliferation, and hormone production [11, 12, 34]. E2 selectively stimulates the proliferation of both normal and transformed gonadotrophs [24]. Estrogen has been shown to cause cellular responses through both rapid, nongenomic action and the "classical" genomic responses [40]. Estrogen exerts rapid nongenomic effects initiated at the cell surface through binding to membrane ERs. That membrane-initiated signaling could be mediated by the classic receptors ERα and ERβ trafficked to the cell membrane. It has been proposed that the 7-transmembrane G protein–coupled estrogen receptor (GPER) collaborates with membrane ERα signaling [35, 36]. GPER is a transmembrane receptor belonging to the G-protein–coupled receptor family. It was first identified in human breast cancer cells [39], and was later found to be expressed ubiquitously even in the rat brain and pituitary [38]. Previous reports have provided strong evidence of GPER expression in the pituitary gland and most of these studies focused on gonadotroph cells [37, 38]. Estrogen has been shown to be involved in sensitizing the pituitary gonadotroph to GnRH stimulus [41] by increasing expression of GnRH receptor (GnRH-R) in gonadotrophs [42], mobilizing secretory granules to the periphery of the cell [43], and recruiting the pool of gonadotrophs that are capable of responding to GnRH stimulation [44, 45]. It has also been shown that estrogen downstream pathways include cytoskeleton rearrangement [46], regulation of ion channels [47], and energy metabolism [48]. In the cellular pathway, ERs regulate estrogen induced proliferation and differentiation and impact several signaling pathways including the MAPK and PI3K/Akt pathway (Fig. 1). Yin and Arita [49] reported that gonadotrophs undergo a cyclic change in apoptotic cell death during the estrous cycle and suggested that inhibition of apoptosis at estrus could be a result of the proestrous surge of GnRH, helping to maintain the population of gonadotrophs needed for the next cycle.

Diverse lines of evidence indicate that ERα is the predominant mediator of estrogen action in the pituitary. ER knockout mice rat models have been used to investigate on

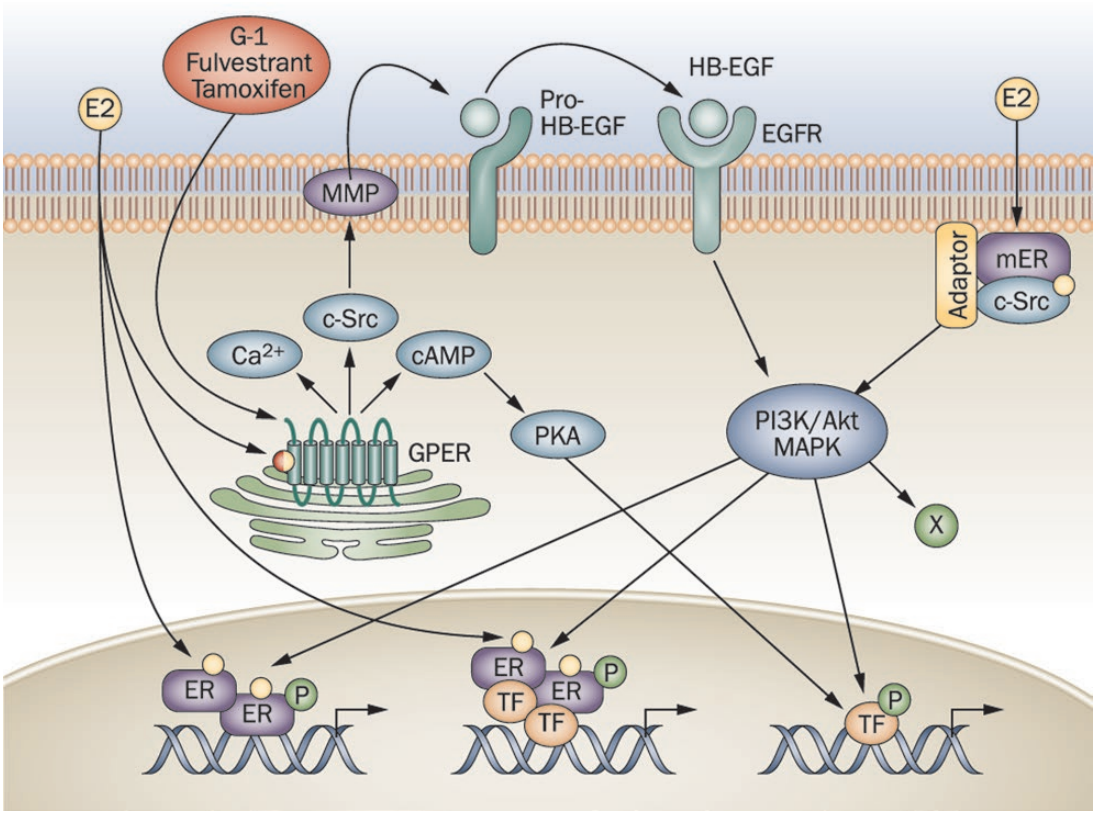

Figure 1. Nongenomic and genomic estrogen signaling pathways. Endogenous estrogens including 17β-estradiol are nonselective activators of the 3 known ERs, ERα, Erβ, and GPER. 17β-Estradiol activates nuclear ERs, inducing receptor dimerization and binding of receptor dimers to the promoters of target genes. Alternatively, activated ERs modulate the function of other classes of TFs through protein–protein interactions. Subpopulations of ERs at the plasma membrane activated by E2 interact with adaptor proteins (adaptor) and signaling molecules such as c-Src, which mediates rapid signaling via PI3K–Akt and MAPK pathways. E2, or selective agonists such as G-1, or selective estrogen receptor down-regulators, such as fulvestrant, or selective estrogen receptor modulators, such as tamoxifen, also activate GPER, which is predominantly localized intracellularly. GPER activation stimulates cAMP production, calcium mobilization and c-Src, which activates MMPs. These MMPs cleave pro-HB-EGF, releasing free HB-EGF that transactivates EGFR, which in turn activates MAPK and PI3K–Akt pathways that can induce additional rapid (nongenomic) effects (X), or genomic effects regulating gene transcription. E2-mediated transcriptional regulation may involve phosphorylation (P) of ER or other TFs that may directly interact with ER, or bind independently of ER within the promoters of target genes. Abbreviations: cAMP, cyclic adenosine 5′-monophosphate; E2, 17β-estradiol; EGFR, epidermal growth factor receptor; ER, estrogen receptor; GPER, G-protein-coupled ER; MMP, matrix metalloproteinase; pro-HB-EGF, pro-heparin-binding-epidermal growth factor; TF, transcription factor. (Figure reprinted by permission from **Springer Nature Customer Service Centre GmbH:** Nature:Nature Reviews Endocrinology. The G-protein-coupled estrogen receptor GPER in health and disease. Prossnitz ER, Barton M) [**COPYRIGHT**] (2011)

the respective roles of ERα and ERβ. Agonists for ERα were capable of inducing increased LH secretion in estrogen-primed GnRH-stimulated rat pituitaries [50]. In the study, ovariectomized rats were treated with nonsteroidal selective ligands for ER subtypes. Their effects were compared with those of estrogen actions on serum concentrations of LH, FSH, and PRL, and in vitro basal and GnRH-stimulated LH, FSH, and PRL secretion, and GnRH self-priming and pituitary PR expression. ERα was found to be the mediator of all the studied effects of estrogen in the pituitary, while ERβ activation induced PR expression and gonadectomy cell reduction only [50]. Nonetheless, absence of physiological interactions between both ER isoforms in these models can be misleading. ERα activation was shown to be primarily responsible for the reorganization of the disrupted organelle morphology seen in the gonadotroph after ovariectomy [51]. Targeted deletion of ERα in the gonadotroph was also reported to cause infertility in female mice [52].

Intriguingly, estrogen has been shown to increase pituitary tumor transforming gene (PTTG) expression. PTTG stimulates fibroblast growth factor 2 and vascular endothelial growth factor production to affect tumor growth, invasion, and angiogenesis [53]. Overexpression of PTTG leads to cellular transformation and tumor development [54, 55]. The transcription and translation levels of the PTTG both increased after estrogen administration, and under the administration of estrogen, PTTG, fibroblast growth factor 2, and vascular endothelial growth factor showed the same expression pattern, showing that estrogen is an inducer of PTTG [58]. To date, 3 studies have analyzed the PTTG expression in exclusive series of NF-Pitnets. The study by Noh et al. [56] showed PTTG expression in 100% of the

cases and a statistically significant relationship with tumor regrowth and with a higher expression in the regrowth adenomas. Ramírez et al. [29] found positivity in 99% of the samples, but no relation with invasiveness or hormonal expression. A recent study by Trott et al. [57] observed positivity in more than 50% of NF-Pitnets sample.

Selective Estrogen Receptor Modulators and Antiestrogen as Therapeutic Agents in NF PitNET

Surgery remains the first-line treatment of symptomatic NF-Pitnets. No medical therapies have been proposed so far in the guidelines of NF-PitNETs treatment although cabergoline and somatostatin analogues are able to improve visual problems and result in tumor shrinkage in 20% to 40% of tumors [59, 60]. There have been investigations into antiestrogen-based treatments, since it is already established that estrogen signaling plays a key role in the pathogenesis of pituitary adenomas [61]. The relative levels of ERα and ERβ are an important determinant of the pharmacology of antiestrogens [62]. SERMs are a group of nonsteroidal, chemically diverse substances with a common mechanism of action. SERMs show a tissue-specific behavior as they can act as estrogen agonists in some tissues but also as estrogen antagonists in others. SERMs are approved for numerous clinical applications such as treatment of breast cancer, prevention and treatment of postmenopausal osteoporosis, and acromegaly treatments [63]. The first SERM, tamoxifen (TX), was developed in the 1970s, followed by several others including raloxifene, clomifene, and bazedoxifene [64]. Among SERMs, the triphenylethylene TX was the first identified compound that exhibits mixed agonist and antagonist activities at the rat pituitary level. In cultured pituitary cells, TX was observed to be a more potent competitive antagonist of ERβ and was postulated to show a better response in ERβ-positive tumors [62, 65]. ERβ may suppress the partial agonist activity of TX on ERα. At the rat pituitary gonadotrope level, TX reduces GnRH-stimulated LH secretion, whereas, in the absence of the cognate ligand, it induces PR expression and GnRH self-priming [66, 67]. Interestingly, neither the raloxifene nor the "pure" antiestrogens (RU58668 or ICI182,780) were able to dissociate reproductive pituitary functions of ERs. Only TX was shown to do so, which may suggest involvement of selective activation of different ER isoforms in the regulation of LH, FSH, and PRL secretion [66]. Fulvestrant, is a nonselective pure ER antagonist, which has potential advantages over TX derivatives that have partial agonist actions in the pituitary. Fulvestrant appears to downregulate ER expression and block ER-mediated gene transcription [68]. Fulvestrant could inhibit pituitary adenomas growth in vivo and in vitro through inducing apoptosis [61]. Fulvestrant was shown to reduce PTTG expression in human pituitary tumors in vitro, thus supporting a role for selective antiestrogen treatment in human pituitary tumors [69].

Conclusion

In NF-Pitnets, estrogen exerts a complex regulatory pattern affecting the hormone secretion and gonadotroph cell proliferation as well as apoptosis at the same time. Future studies are required to further characterize ERα as a novel biomarker for tumor size and invasiveness. Emerging therapeutic approaches will need to be evaluated to better serve this unique group of patients as significant number of gonadotroph tumors regrow following surgical resection. SERMs may have role in the management of significant post-operative residual NF-Pitnets

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Additional Information

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