

HORMONAL CONTROL OF PITUITARY PROLACTIN-SECRETING TUMORS

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Physiology of pituitary prolactin
 - 3.1. Physiology of pituitary prolactin
 - 3.2. Prolactinomas in humans and animals
 - 3.3. Estrogen actions on prolactinomas
 - 3.4. Alcohol, estrogen and prolactinomas
 - 3.5. Xenoestrogens and prolactinomas
 - 3.6. Estrogen regulation of prolactinomas
 - 3.6.1. Estrogen receptors
 - 3.6.2. Estrogen action on protooncogenes
 - 3.6.3. Estrogen action on cell transformation
 - 3.6.4. Factors involved in estrogen-induced tumorigenesis
 - 3.6.4.1. Role of transforming growth factor- α
 - 3.6.4.2. Role of insulin-like growth factor -1
 - 3.6.4.3. Role of basic fibroblast growth factor
 - 3.6.4.4. Role of interleukins
 - 3.6.4.5. Role of nerve growth factor
 - 3.6.4.6. Role of transforming growth factor- β
4. Perspective
5. Acknowledgments
6. References

1. ABSTRACT

Prolactin secreting adenomas (prolactinomas) are the most prevalent form of pituitary tumors in humans. Prolactinomas have been linked to estrogen exposure in humans and animals. However, the mechanism by which estrogen increases mitogenesis in lactotropes, as well as other estrogen responsive cells, is not well understood. Given the complex nature of steroid hormones and their wide array of actions, it seems plausible that there are multiple ways in which estrogen can exert its cell-transforming actions. Estrogen has a wide range of actions on cells depending on the cell type, receptor levels, and other factors present in the cell. A defect at any point could play a potential role in cell transformation. The source of such defects could be the result of any of a wide range of possibilities, including genetic predisposition, prolonged exposure to sufficient levels of the steroid hormone, or other insults to the cell which lead to altered responsiveness to estrogen in some way. This review discusses the recent advances that have been made in the area of understanding estrogen action in transformation of pituitary lactotropes.

2. INTRODUCTION

Prolactinomas are tumors in prolactin-secreting lactotropes in the pituitary gland and are the most

frequently occurring neoplasm in the human pituitary (1-4). It is considered that in the general population, 1:2800 men and 1:1050 women have prolactinomas (5). In human subjects, prolactinomas occur in young as well as in older individuals (6). In addition, the existence of mixed growth hormone and prolactin-secreting adenomas are documented in a substantial number of acromegaly patients.

Prolactinomas occur both as macro and microadenomas. The purpose of this review is to briefly describe the physiological effects of prolactinomas and to summarize recent findings delineating the hormonal regulation of prolactinomas.

3. PHYSIOLOGY OF PITUITARY PROLACTIN

3.1. Physiology of pituitary prolactin

Prolactin is a polypeptide hormone found to be expressed in all mammals. Structurally it is a single linear chain of approximately 200 amino acids with a molecular weight of about 23 Kda. Prolactin is synthesized primarily by lactotropes in the anterior portion of the pituitary gland (adenohypophysis) and secreted into systemic circulation to act on various target tissues. Prolactin-like substances are also produced and act locally in the mammary gland,

Estrogen and prolactinomas

brain, lymphoid, uterine decidual cells and prostate gland. The secretion of pituitary prolactin is regulated by varieties of hypothalamic, gonadal and pituitary hormones. Several hypothalamic hormones that regulate prolactin secretion are identified; of these hormones, thyrotropin-releasing hormone (TRH), vasoactive intestinal peptide (VIP) and oxytocin stimulate and dopamine inhibits prolactin secretion (7,8). A stimulatory effect of estrogen and an inhibitory effect of progesterone on prolactin secretion are also well documented. In addition to these hormones, several pituitary-derived factors including transforming growth factor alpha (TGF- α), transforming growth factor beta (TGF- β); interleukins, VIP, and galanin effects prolactin secretion (7,8).

A major function of prolactin that is universal in female mammals is the stimulation of milk production by the mammary gland (9). Additional functions include a modulator of processes of reproduction, osmoregulation, growth and metabolism, migratory behaviors and maternal behaviors (7). Studies have revealed that at the cellular level, the effect of prolactin is mediated by two types of prolactin receptors (long and short forms of prolactin receptors) and that these receptors belong to the super family of cytokine-growth hormone-prolactin receptors (10). Molecular studies have shown that PRL action on lactogenesis is mediated by the binding of prolactin to the receptor and activation of JAK/STAT pathway (11).

3.2. Prolactinomas in humans and animals

Hyperprolactinomas is a condition in which plasma prolactin levels are elevated above normal levels. Hyperprolactinemia, with elevation of serum prolactin of more than 200 ng/ml, is characteristically associated with prolactinomas (12). Hyperprolactinemia causes reproductive dysfunction such as amenorrhea, galactorrhea and infertility in women (13). Amenorrhea and galactorrhea may occur alone or together (14). Up to 25% of patients with secondary amenorrhea have been diagnosed with hyperprolactinemia. Many of these patients showed micro-prolactin-adenomas or macro-prolactin-adenomas in the pituitary. Although treatments that alter central dopaminergic neuronal functions cause an elevated serum prolactin level, in most cases hyperprolactinemia is due to a pituitary tumor. In women prolactinomas are mainly microadenomas (less than 1 cm). These microadenomas are rarely associated with hypopituitarism or central nervous system dysfunction. Idiopathic hyperprolactinemia, without demonstrable pituitary or hypothalamic diseases, have also been identified. Men with hyperprolactinemia often exhibit gynecomastia, impotence, decreased libido and reduced reproductive hormone levels (15).

Prolactinomas are not only the most frequently occurring neoplasm in the human pituitary (1-4), but these tumors also commonly occur in laboratory animals. A 10-86% incidence of spontaneous pituitary adenomas was reported in male and old female rats of different strains, especially during aging (16-21). The incidences of pituitary adenomas in aging Wistar male and female rats, Charles River COBS male rats, and Long-Evans female

rats are 56%, 10-51% and 56% respectively. Most of these adenomas in laboratory rats are prolactinomas.

3.3. Estrogen actions on prolactinomas:

Pituitary tumors in experimental animals can be induced by estrogen. Most of these estrogen-induced pituitary tumors are prolactin- or GH- secreting tumors (22,23). In both sexes of rats, long-term elevation of serum estrogen causes hyperplasia and/or adenomas (24,25). In F-344 female rats maintenance of constant elevated systemic estradiol levels by subcutaneous implantation of 17 β -estradiol-containing silastic capsules induces prolactinomas very rapidly; within 2 weeks of estrogen implantation significant hyperplastic response of lactotropes is evident (25-28). Subcutaneous implantation of 17 β -estradiol-containing silastic capsules induces prolactinomas both in ovary intact or ovariectomized ACI rats (29). Subcutaneous implantation of DES causes pituitary hyperplasia and neoplasm in Fischer F344 rats (30). In estrogen-sensitive rats, short-term administration of the steroid stimulates lactotropes cell proliferation and in the long-term treatments results in lactotropic hyperplasia.

Estrogen also appears to promote prolactinomas in humans. There are reports of the development of a prolactinemia in a male to female transsexual receiving massive doses of estrogen (31). Some men with prolactinomas showed increased serum estradiol level due to aromatized testosterone to estradiol (32). There is evidence of growth of microprolactinoma to a macroprolactinoma during estrogen therapy (33). Women taking oral estrogen contraceptive showed higher prolactin levels (34). Women using oral contraceptive due to menstrual irregularities showed a 7-8-fold higher incidence of prolactinomas (35). Data suggest that some women are more sensitive to the lactogenic effects of exogenous estrogen and, therefore, may be at greater risk for developing prolactinomas (36). During pregnancy, estrogen levels elevate and the number of prolactin-secreting cells and serum prolactin content increases. These elevated levels of estrogen are associated with symptomatic pituitary tumor enlargement in up to 30% of women with macroadenomas (>1 cm) and may result in persistent hyperprolactinemia and postpartum amenorrhoea or galactorrhea (37). However, the risk for development of significant clinical symptoms related to tumor expansion is less than 2% in pregnant women with microadenomas (14). There appears to be a close association between the use of oral estrogenic contraceptives and the onset of amenorrhea often accompanied by galactorrhea (38-40). Hence, estrogen can be considered a risk factor for the development of prolactinomas in some laboratory animals and a certain population of humans.

3.4. Alcohol, estrogen and prolactinomas

Alcohol consumption has often been shown to be associated with increased plasma levels of estrogen in humans (15,41). The increased plasma levels of estrogen are believed to be due to an alcohol-induced increase in adrenal steroid production and conversion of these weak

Estrogen and prolactinomas

adrenal androgens to estrogen (15) or abnormal conversion of estrogen from androgen in the liver due to cirrhosis (42). Recently phytoestrogen, a biologically active substance of plant origin, has been identified in alcoholic beverages. These phytoestrogens cause reproductive disorders in animals and may cause clinically significant effects in human. Two phytoestrogens β -sitosterol and biochanin A have been found in bourbon (43,44). De-ethanolized bourbon extracts have been shown to be estrogenic both *in vivo* and *in vitro* (45) and also in normal postmenopausal women (46). Recently it has been reported that beer, the most common alcoholic beverage, contains two phytoestrogens, diadzein and genistein (47). Both diadzein and genistein are biologically active and are associated with either uterine hypertrophy or infertility in animals (48). These phytoestrogens are capable of eliciting an estrogenic response *in vitro* by competing with estradiol for uterine cytosolic estrogen receptor binding sites (49-51). Thus, alcoholics are exposed to high levels of estrogen due to increased endogenous production of estrogen or ingestion of phytoestrogen often present in beer.

There are several reports showing evidence for the existence of high levels of prolactin in chronic alcoholic men and women (41, 52-55). In a study conducted by European scientists, persistent hyperprolactinemia was observed in 16 alcoholic women during a 6-week treatment trial (52). These patients reported daily alcohol intake of 170 g for a 2-16 year period, but had no clinical evidence of alcoholic liver cirrhosis. In a study reported by Japanese scientists, 22 of 23 women admitted for alcoholism treatment had prolactin levels above normal, ranging between 27-184 ng/ml. These women reported drinking an average of 84.1 g of alcohol each day for at least 7 years. None of these patients showed liver cirrhosis but 10 had hepatitis and the rest had fatty liver (53,54). Studies conducted in a Massachusetts hospital reported hyperprolactinemia (22-87 ng/ml) in 6 of 12 alcohol-dependent women who had a history of drinking 75-247 g of alcohol per day for a minimum period of 7 years (55). Alcohol-induced hyperprolactinemia is also reported in healthy, well-nourished women during residence for 35 days in a clinical research ward (56). Sixty percent of women in the heavy drinker category (blood alcohol level, BAC, 109-199 mg/dl) and 50% of moderate drinkers (BAC, 48-87 mg/dl) showed elevated prolactin levels, and many of these drinkers had elevated prolactin levels several days after cessation of drinking. Alcohol-induced hyperprolactinemia was also evident in 66 postmenopausal women (42). The increase in prolactin levels in these patients, however, was associated with increased androgen conversion to estrogen, possibly due to liver cirrhosis.

Alcoholic men also showed elevated plasma levels of prolactin (57-59). Male alcoholic patients frequently show evidence of feminization that is manifested by gynecomastia, spider angiomas, palmar erythema and changes in body hair patterns (15). Several studies now indicate a potential role for prolactin and estrogen in the pathogenesis for the observed feminization (15). Alcoholic men show a positive association between

the presence of clinically apparent gynecomastia and elevated circulating levels of prolactin. These patients also show an elevation of plasma levels of estrogen, which is believed to be due to peripheral conversion of weak adrenal androgens to estrogen. The gynecomastia found in alcoholic patients is characterized by a proliferation of the stroma and ducts that are known to be estrogen-positive. Prolactin also may act synergistically with estrogen and adrenal steroids and may, therefore, contribute to enhanced breast hypertrophy in alcoholic men. Thus, it appears that chronic alcohol administration in humans causes increased estrogen production and prolactin elevation. Alcohol-induced hyperprolactinemia has also been demonstrated in non-human primates (60-62) and laboratory animals (63,64). Some of the macaque female monkeys showed elevated prolactin levels after chronic self-administration of high doses of alcohol (3.4g/kg/day). Interestingly, in one of these monkeys, immunocytochemical examination of the pituitary gland showed apparent pituitary hyperplasia (60). Recently we have shown that alcohol promotes estrogen-induced increases in pituitary weight and protein, and potentiates estrogen-induced lactotropic cell proliferation in ovariectomized female rats (65). Therefore, the clinical data as well as the animal data suggest that alcohol consumption is a positive risk factor for prolactinomas and hyperprolactinemia.

3.5. Xenoestrogens and prolactinomas

Xenoestrogens are environmentally occurring chemicals that mimic estrogen action by binding to estrogen receptors (66). Examples of xenoestrogens include organochlorine pollutants such as PCBs and DDT, which are known to persist in the environment and are correlated with increased incidence of breast cancer in women (67) decreased sperm count in men (68) and neurodevelopmental defects in children (69). Experimental studies have confirmed that DDT mimics estrogen in MCF-7 cells (70). Bisphenol A (BPA) is the monomeric component of polycarbonate plastics used in many consumer products and has estrogenic activity in MCF-7 cells *in vitro* (71). Recently, BPA was shown to induce hyperprolactinemia in estrogen-sensitive Fisher 344 rats by increasing prolactin gene expression in the pituitary gland. Furthermore, BPA exerts its influence at the prolactin gene transcription level by activating the estrogen response element (ERE) in pituitary cells (72). Although there is a body of evidence to support xenoestrogens as a possible public health hazard, the carcinogenic potency of these compounds is apparently much lower than that of estradiol (73,74).

3.6. Estrogen regulation of prolactinomas

The mechanisms through which estrogen induces cell transformation in a variety of tissues are not well understood (25,75-82). Given the complex nature of steroid hormones and their wide array of actions, it seems plausible that there are multiple ways in which estrogen can exert their cell transforming actions. Here we will summarize the recent advances that have been made in the area of understanding how estrogen can act on lactotropes to cause transformation.

Estrogen and prolactinomas

3.6.1. Estrogen Receptors

To understand how estrogen functions in cell proliferation and transformation, it is critical to understand the receptor mediation that occurs as a first step in the cascade that follows the introduction of estrogen into the cell. The estrogen receptor has been reported to be a single product of a single gene (83). However, there is currently much debate over this issue. Given the plethora of estrogen actions, it seems possible that there could be variants of the estrogen receptor. Recently, it has been shown that in the male rat pituitary there is a single form of estrogen receptor mRNA which is 6.2 kb, while in the female there exists an additional 5.5 kb estrogen receptor mRNA (84). The common 6.2 kb form is found at all times in male and female pituitary tissue, whereas the 5.5 kb form only occurs at proestrus and when induced by exogenous estradiol treatment. The 5.5 kb mRNA is found only in the lactotropes of the female anterior pituitary. This suggests a possible role for the 5.5 kb form in the control of lactotrope function in response to estrogenic stimuli.

The classical estrogen receptor consists of a DNA-binding region, a hormone-binding domain, and two transactivation regions. One transactivation region is in the amino terminal portion of the receptor and the other is in the region between the hormone and DNA binding domains. The importance of the two transcriptional activation regions in the receptor depends on the cell type and the target gene. In the absence of estrogen, the receptors associate with binding proteins. This most likely serves to prevent inactive receptors from binding DNA. It has been reported that there are nearly 20,000 receptors per target cell (85) and many of these may be spare receptors. The levels of estrogen receptors are generally not acutely regulated, but rather the activity of these receptors is regulated by hormone binding and the activity of kinases and phosphatases (86). The regulatory kinases and phosphatases can vary cell to cell and in any one cell depending on the presence of other factors. This is another way in which estrogen may be able to exert multiple actions

Most of the receptors are distributed throughout the nucleus (87). Histones, RNA, chromatin proteins, nuclear matrix and DNA all bind the estrogen receptor (88). The receptor complex binds several sites termed estrogen response elements (ERE). ERE refers to a DNA sequence in the regulatory regions of genes that are known to respond to treatment with estrogen. The estrogen-sensitive transcription complex including the estrogen receptor, ERE, and associated factors has not yet been defined. It is known that the EREs may vary from cell to cell (89). This suggests one way in which estrogen can exert such a wide range of effects on different cells. The introduction of estrogen into responsive cells dissociates the binding protein from the receptor; the estrogen can then bind and the receptors undergo dimerization, then bind the DNA to induce or inhibit transcription of the target gene. Estrogen can exert many actions that are not at the level of gene transcription. These include RNA processing, altering mRNA half-life, and protein synthesis,

processing, and half-life (90).

Studies over recent years suggest that members of the steroid receptor family may be recipients and transducers of signals initiated at the cell membrane (86). Estrogen is known to stimulate the protein kinase C pathway. There are reports that stimulating constituents of the signaling pathway mimics the effects of estrogen without any of the hormone present (91,92). This indicates that there is a great deal of interaction between the estrogen receptors and other signal transduction pathways. Steroids, acting through their receptors, can regulate the synthesis of growth factors and growth factor receptors (93,94). Activation of signal transduction pathways can regulate steroid receptor levels and function. Activity of the estrogen receptor may be mediated by phosphorylation as demonstrated by the ability of kinase activators and phosphates inhibitors to stimulate the transcriptional activity of the receptors even in the absence of hormone (95). Taken together, these data indicate that there is significant crosstalk between the signal transduction pathways and steroid receptors, and components of these systems may be responsible for regulating various cell functions.

3.6.2. Estrogen action on protooncogenes production

Estrogen, hormones, and growth factors stimulate characteristic patterns of *c-fos*, *c-jun*, and *c-myc* expression (96). *Fos* and *jun* form homodimers or heterodimers that function as transcription factors for genes containing AP-1 sites with which the transcription factors interact. Different dimers have similar DNA binding activity but distinct biological activities. The *c-fos* and *c-jun* can function as intracellular relay points to interconnect and coordinate signaling pathways initiated by estrogen with those initiated by other regulatory molecules. The AP-1 sites were previously thought to be specific for *fos/jun* transcription factors; however, it is now known that AP-1 sites can serve as a nonclassical estrogen responsive element (96,97).

3.6.3. Estrogen action on cell transformation

While there is a great deal known about the normal roles of estrogen and related factors, there is only a superficial understanding of the mechanism by which they function to lead to cell transformation. There is a great deal of information about the effects of estrogen on lactotropes; in regard to production or inhibition of other factors and proliferation, however, it is not yet understood which effects are critical in transformation and how the several actions are interrelated.

It is possible to directly study estrogen-induced prolactinomas using an animal model. The Fischer-344 rat strain develops tumors within 2-4 weeks of consistent estrogen exposure (25,75,82). Fischer-344 rats are more sensitive to estrogen treatment than are other strains. At present it is not known why these rats are hypersensitive to estrogen. The prolactinomas that arise in the Fischer-344 rats are structurally similar to spontaneously occurring tumors in humans and animals (17,98). Anterior pituitaries from estrogen-treated rats can be collected and

Estrogen and prolactinomas

studied after various lengths of exposure and cultured or directly assayed for any of the various responses. In this way a great deal of data has been generated toward elucidating the role of estrogen on cell transformation.

It has been demonstrated that estrogen plays a pivotal role in tumorigenesis at a very early stage before there is any visible tumor growth. It is reported that after three days of estrogen treatment Fischer-344 rats display increased enzymatic activity of pituitary RNA polymerase I, which is responsible for initiating RNA synthesis, while there is no similar change in Sprague Dawley rats that are less sensitive to estrogen mitogenic action (99). It is very likely that there exist other dissimilarities between Fischer-344 and other estrogen-insensitive rat strains; however, these have not yet been identified. It is interesting to note that there are slight structural differences in the anterior pituitaries of Fischer-344 and Sprague-Dawley rats, including a difference in the number of supporting folliculo-stellate cells (100); however, the significance of such differences is not known.

3.6.4. Factors involved in estrogen induced tumorigenesis

There are a number of factors that are both estrogen dependent and affect lactotropic proliferation, differentiation, and/or transformation. However, the relatedness of these factors in the mediation of estrogen action on cell functions is not well understood. Some such estrogen-regulated factors include epidermal growth factor (EGF), platelet derived growth factor (PDGF), insulin-like growth factor (IGF) -1, transforming growth factor alpha (TGF- α), basic fibroblast growth factor (bFGF), fibroblast growth factor-4 (FGF-4), interleukin-2 and 6 (IL-2; IL-6), nerve growth factor (NGF), and transforming growth factor-beta (TGF- β). A stimulatory action for EGF, TGF- α , PDGF, IGF-1 and IGF-2 is shown on mesenchymal cell growth. EGF, TGF- α , IGF-1 and IGF-2 also stimulates epithelial cell growth (101-102). During the developmental period, estrogen can stimulate the growth of all cell types; however, in the adult, estrogen action is restricted primarily on epithelial cells (103). It is not known why such a dichotomy exists in the adult. It could be due to expression or lack of expression of inhibitory or stimulatory factors that interact with estrogen. Estrogen's action on epithelial cells often depends on the presence of mesenchymal cells. This is yet another way in which estrogen-varied actions could occur through common receptors. The response to estrogen would depend not only on the cell displaying proliferation or differentiation, but also on neighboring cells. This is supported by the fact that estrogen actions in some cases can only be displayed *in vivo* or in specific culture conditions.

3.6.4.1. Role of transforming growth factor- α

TGF- α is one of the stimulatory growth factors that may be involved in estrogen action in the pituitary. TGF- α is produced in the lactotropes and other cells of the pituitary and the secretion of this peptide is stimulated by estrogen (106,107). TGF- α may increase estrogen-induced lactotropic cell proliferation (108). Transgenic

mice overexpressing TGF- α only in the pituitary lactotropes demonstrate lactotropic hyperplasia. By 12 months of age, all homozygous females had prolactin-positive adenomas. This indicates that TGF- α acts in the functioning of lactotropes, and, in the presence of estrogen, may play a role in lactotropic cell transformation (108).

3.6.4.2. Role of insulin like growth factor-1

In many epithelial cells IGF-1 production correlated with the increases in epithelial cell proliferation (101). It has recently been shown that IGF-1 can itself induce some cells to proliferate. IGF-1 increases *c-fos* and *c-jun* expression in some tissues and enhances the mitogenic effect of estrogen (109). IGF-1 also promotes GH3 cell growth, but only in the presence of estrogen, as the effect was diminished in the presence of an antiestrogen (110). The fact that blocking the estrogen receptor leads to a loss of IGF-1 response indicates that for GH3 cells the estrogen receptor is transcriptionally activated by intracellular peptide factor pathways. This indicates the estrogen receptor may participate in inducing mitogenesis in response to IGF-1.

3.6.4.3. Role of fibroblast growth factor

Members of the FGF family of peptides may also regulate estrogen mitogenic action on lactotropes (111). Estrogen increases the number of FGF receptors in the anterior pituitary (112). Basic FGF increases the proliferation of lactotropes but does so only in the presence of estrogen (112). FGF-4 increases prolactin secretion as well as lactotropic proliferation (113). Transfection of the *hst* gene (the gene that encodes FGF) into the GH4 cell line resulted in more aggressive tumors when injected subcutaneously than did the non-transfected cells (113). This indicates that the *hst* gene may facilitate prolactinoma development via autocrine or paracrine action of its secreted protein, FGF-4.

3.6.4.4. Role of interleukins

IL-2 and IL-6 have been shown to be expressed in the anterior pituitary and to affect cell proliferation in rat and in cell lines. IL-2 has been found to increase the production of prolactin. It has been shown that IL-2 and IL-6 regulate the expression of *c-fos* in pituitary adenoma explants (114). IL-2 has a mitogenic action on the prolactin-secreting tumor cell line GH3 cells only in the presence of estrogen (114). This may indicate that there is a functional correlation between estrogen, interleukins and protooncogenes.

3.6.4.5. Role of nerve growth factor

Recent studies have elucidated a role for NGF in lactotrope proliferation. NGF and the NGF receptor are found in the postnatal rat pituitary. Cultured pituitary cells from young rats show an increased rate of lactotropic proliferation in response to NGF (115). There are two general classes of prolactinomas, those which respond to treatment with a dopamine agonist with a cessation of lactotropic proliferation and those that do not. The non-responsive tumors lack D2 receptors on the lactotropes and express low levels of NGF (116). Recently, it has been

Estrogen and prolactinomas

demonstrated that lactotropes from a non-responsive tumor, became responsive to dopamine agonist therapy after treatment with NGF, which caused the expression of the D2 receptors (117). Hence, the NGF-mediated autocrine loop may be important for lactotropic proliferation and maintenance of the phenotype.

3.6.4.6. Role of transforming growth factor- β

The TGF- β family of peptides includes many related factors that exert numerous actions. In mammals, TGF- β is found in three highly homologous isoforms that exert their actions via heteromeric complexes of type I and type II receptors. TGF- β 1 is the most well characterized of the three TGF- β s (TGF- β 1, 2, and 3) (118). This growth factor functions in cell proliferation, differentiation, embryogenesis, tissue repair, and cell adhesion. In most epithelial cell types TGF- β 1 acts as a negative growth regulator (119-121). In mesenchymal cells TGF- β 1 has been shown to either increase or decrease cell proliferation, depending on the other factors present. Some such factors include PDGF, FGF, and EGF. TGF- β 1 can block the mitogenic actions of PDGF, FGF, EGF, and IL-1 in certain cell types (119). Given the important regulatory role of TGF- β 1 in controlling cell proliferation, it follows that a reduction in TGF- β 1 could have implications on cell transformation. TGF- β 1 actions in the transformation of cells could also be linked to the fact that it regulates the expression of certain extracellular matrix components including fibronectin, type I collagen, proteoglycans, and cell adhesion proteins (integrins) (122).

Transformation of some epithelial cell types has been associated with a loss of sensitivity to the inhibitory actions of TGF- β 1 (123). TGF- β 1 suppresses cell proliferation by increasing the expression of the pRb tumor suppressor gene, which in turn inhibits the expression of the *c-myc* protooncogene (121). Some cancer cell lines also show a decrease in TGF- β levels (119). In tumor cells, TGF- β 1 cannot suppress *c-myc* expression (121). The increased expression of *c-myc* can have effects on the cell similar to *fos/jun* and may be of significance in cell transformation as related to TGF- β 1.

TGF- β 1 is produced in and secreted by pituitary lactotropes (124,125). Our lab has shown a significant role of this growth factor in lactotropic regulation. TGF- β 1 is a potent inhibitor of lactotropic proliferation and prolactin secretion (124,126). Intrapituitary administration of TGF- β 1 resulted in inhibited prolactin release, decreased pituitary weight, and reduced DNA synthesis. Inhibitory response to TGF- β 1 is altered in anterior pituitary cell lines, including the prolactin-secreting PR1 line and the GH and prolactin-secreting GH3 cell line. Both of these cell lines show low levels of TGF- β 1 and its type II receptor (T β R-II) mRNA (127). We have shown that lactotropes exposed to estrogen express reduced levels of T β R-II and TGF- β 1 (128,129). This suggests that TGF- β 1 actions are modulated via estrogen. Hence, TGF- β 1 appears to be an important factor in estrogen-induced tumorigenesis.

4. PERSPECTIVE

Despite the many advances in recent years in the

area of estrogen-induced tumorigenesis, there is still a great deal that is not understood about this process. Here we have outlined some of the factors relevant to estrogen action on cell transformation. A great deal of information of estrogen actions on prolactinomas is derived from the studies in Fischer F344 rats. These data suggest that estrogen mitogenic action on lactotropes is a multistep process (figure 1).

The early events of estrogen action involves activation of estrogen receptors in a subset population of lactotropes, blocking the action of the inhibitory hypothalamic regulatory hormone dopamine, reducing the activity of negative growth regulator TGF- β 1 and stimulation of positive growth regulators (TGF- α and FGF). These early changes in hormonal control mechanisms lead to induction of PRL secretion and activation of cell cycle regulatory genes (e.g. *c-fos*, *c-jun*) and cell proliferation in a subset population of lactotropes. Persistent stimulation of these cellular processes is maintained due to continuous elevation of circulatory estrogen, and eventually induce spontaneous genetic errors leading to activation of oncogenes or inactivation of suppressor genes that may ultimately lead to genomic instability and transformation (26, 108, 113, 128, 129-133). The information presented represents only a portion of what is known about the actions of estrogen and there is still a larger amount that is not known. With recent technical advances including sophisticated animal models, transgenic cell lines, and new molecular techniques, the gap in our understanding of estrogen regulation of cell function should be narrowed as more questions can be addressed.

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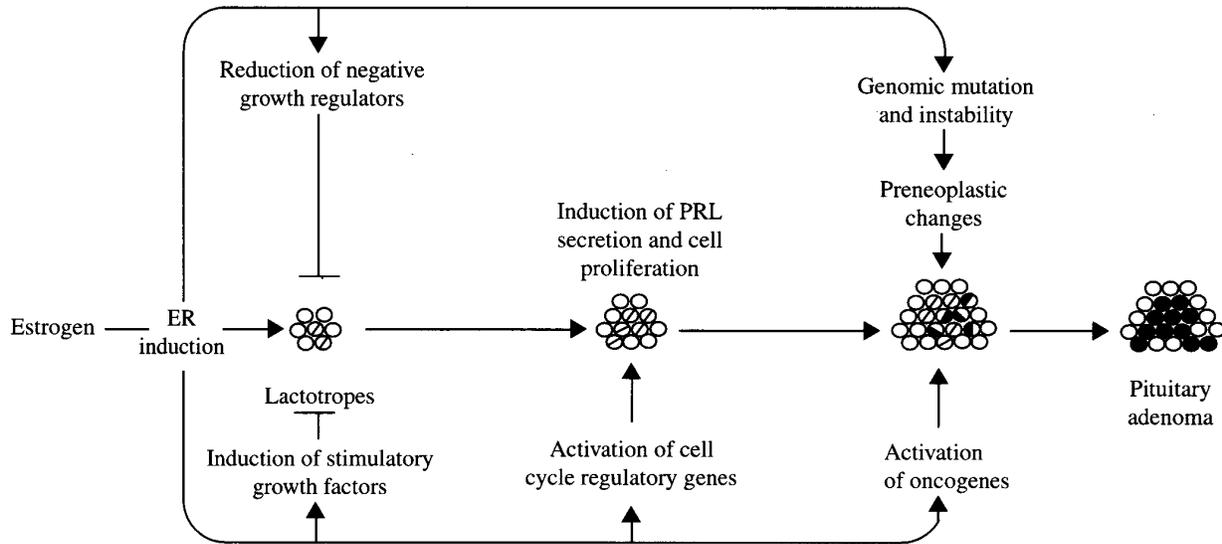


Figure 1. A schematic representation of putative mechanisms by which estrogen may induces tumors in pituitary lactotropes. Open circles are estrogen non-responsive lactotropes, middle strike circles are estrogen responsive lactotropes, half close circles are preneoplastic lactotropes and closed circles are neoplastic lactotropes.

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