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Use of letrozole as a chemopreventive agent in aromatase overexpressing transgenic mice☆

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Abstract

Our recent studies have shown that overexpression of aromatase results in increased tissue estrogenic activity and induction of hyperplastic and dysplastic lesions in mammary glands, and gynecomastia and testicular cancer in male aromatase transgenic mice. Our studies also have shown that aromatase overexpression-induced changes in mammary glands can be abrogated with very low concentrations of letrozole, an aromatase inhibitor without any effect on normal physiology. In the present study, we have examined the effect of prior low dose letrozole treatment on pregnancy and lactation. We have also investigated the effect of low dose letrozole treatment on subsequent mammary growth and biochemical changes in these animals. There was no change in the litter size, birth weight and no visible birth defects in letrozole-treated animals. Although, there was an insignificant increase in mammary growth in aged animals after 6 weeks of letrozole treatment, the levels of expression of estrogen receptor, progesterone receptor and genes involved in cell cycle and cell proliferation remained low compared to control untreated animals. These observations indicate that aromatase inhibitors such as letrozole can be used as chemopreventive agents without effecting normal physiology in aromatase transgenic mice.

Keywords: Aromatase; Transgenic mice; Aromatase inhibitors; Tamoxifen; Chemoprevention

1. Introduction

Breast cancer is one of the most prevalent types of cancer observed in women. The mitogenic and proliferative effects of estrogens have long been recognized and are known to correlate with estrogen and progesterone receptors in breast tumor tissue. Interestingly, the proportion of patients with hormone sensitive tumors is higher among postmenopausal patients than premenopausal patients [1,2]. In addition, the source of sex steroids differ between pre- and postmenopausal women. Previous studies have demonstrated the presence of aromatase, the rate limiting enzyme responsible for estrogen biosynthesis in breast tumors [3–6]. Breast tumors from postmenopausal women maintain a high estrogen content, even though the plasma levels of estradiol fall to low levels following menopause. One pathway for in situ synthesis involves the conversion of androstenedione to estrone/estradiol catalyzed by aromatase. Since aromatase was first detected in breast tumors, the problem of assessing its functional significance has attracted considerable attention and controversy [7].

An intriguing hypothesis is that local estrogen is directly involved in the initiation of either preneoplastic or neoplastic (or both) changes in mammary epithelium. To address this question directly, we have generated an aromatase transgenic mice model and showed for the first time that the transgenic virgin and postlactational females overexpressing aromatase develop various preneoplastic histopathological changes in their breast tissue [8] and males developed gynecomastia and testicular cancer [9,10]. Overexpression of aromatase results in change in the expression of a number of growth factors, oncogenes, genes involved in cell cycle, cell proliferation and cell death in mammary tissue of aromatase transgenic mice [11]. Because of the complexity of regulation in the ovary, pharmacological blockade of ovarian aromatase has been difficult in pre-menopausal patients. Interruption of estrogen biosynthesis reduces the tonic inhibitory action of estradiol on LH and FSH secretion. The reflex rise in FSH stimulates production of new aromatase and the LH increase results in increased steroidogenesis in the ovary. These two effects tend to counteract the inhibitory actions of aromatase blocking drugs on the ovary [12]. But the same endocrine regulation can be utilized as an advantage to block estrogen synthesis in other tissues such as breast where

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it is not under gonadotrophin regulation. Based on these and other observations, we hypothesized that estrogen biosynthesis in breast tissue may be regulated in pre-menopausal women by selectively blocking in situ estrogen production with aromatase inhibitors without affecting ovarian estrogen biosynthesis. This action should effectively eliminate the breast tissue estrogen-mediated initiation and progression of breast cancer. Even though a large body of literature supports the use of aromatase inhibitors as hormonal therapeutic agents to treat hormone-dependent breast cancers there is limited information pertaining to the use of aromatase inhibitors as chemopreventive agents to block the initiation and progression of this malignancy [13,14]. Results from limited preclinical experiments have shown that third -generation aromatase inhibitors posses a potent ability to prevent tumor induction and growth in carcinogen-induced rat mammary tumor models [15–17]. Based on preclinical results and their excellent activity in the metastatic setting, aromatase inhibitors are now being evaluated as adjuvants and in pilot studies for chemoprevention [18,19].

Our previous studies [20,21] have shown that aromatase overexpression-induced changes in mammary glands can be abrogated with very low concentration of letrozole. Low concentration of letrozole had no effect on normal physiology as indicated by no significant change in the circulating levels of estradiol and FSH as well as no change in estrogen responsive genes such as the PR and lactoferrin in the uterine tissue. These observations indicated that the aromatase inhibitor, letrozole can be used as a chemopreventive agent without affecting normal physiology. Current findings indicate that prior low dose letrozole treatment had no effect on subsequent pregnancy and lactation. Mammary growth and expression of ER, PR and genes involved in cell cycle and cell proliferation remained low in these animals even several months after treatment. These observations confirm that letrozole can be used to eliminate estrogen-mediated initiation of breast cancer in transgenic mice without affecting normal physiological functions.

2. Materials and methods

2.1. Aromatase overexpressing transgenic mice and animal treatments

The female aromatase transgenic mice were maintained in a standard colony. Mice were housed in a centralized animal facility accredited by the AAALAC and USDA and maintained according to the recommendations established in the *NIH Guide for the Care and Use of Laboratory Animals*. Generation of transgenic mice that overexpress aromatase (previously referred to as *int-5/aromatase* transgenic mice) in mammary glands was previously described [8]. All animals were genotyped using Southern blot analysis and age matched nontransgenic littermates were used as controls in all experiments.

2.2. Morphological and histological assessment of mammary glands and other tissues

The skin containing the mammary fat pads was fixed in 10% neutral buffered formalin for at least 24 h. The mammary glands were then dissected free from skin and processed for histological examinations [22]. Routine sections of mammary tissues, uterus and ovary were prepared after fixation by embedding in paraffin, sectioning at 5 μM, and staining with H&E.

2.3. Treatment with aromatase inhibitor or tamoxifen

Aged (16–20 weeks old) virgin aromatase transgenic females were used to investigate the effect of the aromatase inhibitor, letrozole, as a chemopreventive agent against the preneoplastic and neoplastic changes induced in mammary glands of these mice. In this study, age matched transgenic females were divided into two groups ($n = 30$ in each group), one group served as control group, the other group received daily s.c. injection of letrozole 0.5 μg letrozole/animal in 100 μl of 0.3% hydroxy propyl cellulose (in phosphate buffered saline). Control animals were given s.c. injection of vehicle. Letrozole was a gift from Drs. Ajay Bhatnagar and Dean Evans of Novartis Pharma AG (Basel, Switzerland). At the end of the 6-week treatment period, animals were divided into three groups. First group of animals was sacrificed and the mammary glands were removed and one gland each was used for histological analysis. All other glands were pooled then used for biochemical analysis as described below. The second group of animals was allowed to age (12 months) without any further treatment. The third group of animals was paired with males 2 weeks after the resting period to observe the effect of letrozole on pregnancy and lactation.

To compare the effect of tamoxifen with letrozole on abrogating aromatase-induced mammary hyperplasia, aromatase transgenic mice (16–20 weeks) were treated with 5.0 mg (60 day constant release) tamoxifen pellet ($n = 10$). At the end of experiment, the animals were sacrificed, mammary glands were collected and processed as described above. Data from these animals is compared with the first group of animals form above study to see the differences in tamoxifen and letrozole treatment.

2.4. RNA analysis

Total RNA from mice mammary tissue was isolated, following homogenization of the tissue, with the Tri Reagent (Sigma, St. Louis, MO) according to the manufacturer's protocol. The specific expression of ER, PR, PCNA, cyclin D1 and other genes was then verified by RT-PCR, using the GeneAmp RNA PCR kit (Perkin-Elmer, Foster City, CA). Use of specific primers and conditions of RT-PCR was described before [11]. Depending on the abundance of the specific mRNA species, 70 ng–1.0 μg of total RNA was

used as starting template in a reverse transcription (RT) reaction mix. The RT-PCR products were visualized on a 1% agarose gel with ethidium bromide staining. To demonstrate that equal amounts of total RNA was used from each sample for estimation of the expression of various genes, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), a house keeping gene, was used as invariant control. The densitometric data (Bio-Rad GS-700 densitometer, Hercules, CA) from ethidium bromide staining of RT-PCR products on agarose gels were used for calculating the relative differences in the expression of various mRNA levels in different tissue samples.

2.5. Western blot analysis

To isolate proteins from mammary gland and uterine tissue of both control and letrozole-treated aromatase animals, the tissues were homogenized in lysis buffer. Equal amounts (60 μg) of protein from each sample was separated on a denaturing polyacrylamide gel and transferred to a nylon membrane. Non-specific binding of antibodies were blocked by incubation for at least 4 h at room temperature with Tris-buffered saline (TBS) containing 0.05% Triton X-100 (TBST) and 5% nonfat dry milk. Membranes were then incubated with respective primary antibodies (actin, ER, PR, lactoferrin, PCNA and cyclin D1) in TBST-milk overnight at 4 °C, and specific binding was visualized by using species-specific (secondary antibodies) IgG followed by enhanced chemiluminescent detection (ECL kit; Amersham Pharmacia Biotech, New Jersey) and exposure to ECL X-ray film. Densitometric data from Western blots (X-ray image of chemiluminescent antibody-bound proteins) were used for calculating the differences in the expression of individual proteins. Expression of actin, a house keeping protein, was used for normalization as an invariant control if needed.

3. Results

3.1. Effect of prior letrozole treatment on pregnancy and lactation

Our previous studies have demonstrated that breast hyperplasia and other preneoplastic/neoplastic changes that were induced by aromatase overexpression can be abrogated by using a low dose of letrozole (0.5 μg per day per animal). This dose had no effect on uterus and ovary as well as circulating levels of estradiol and FSH [21]. To test whether prior low dose letrozole treatment had any effect on subsequent pregnancy, animals were allowed to undergo pregnancy and lactation after 2 weeks of a resting period. Data shown in Fig. 1 clearly indicate that there is no difference in the average litter size and weight (10th day) of the pups, suggesting that the prior letrozole treatment had no effect on pregnancy and lactation. We have also not seen any visible morphological or anatomical defects in the pups born to letrozole-treated mothers.

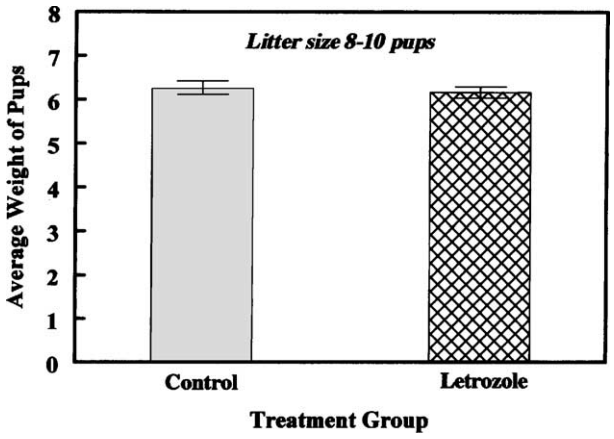

Fig. 1. Effect of letrozole treatment prior to pregnancy on birth weight and litter size of aromatase transgenic mice. Animals were treated for 6 weeks at the age of 12–16 weeks with 0.5 μg letrozole per mouse per day. After 2 weeks of resting period, the animals were paired with males. Litter size was noted immediately after the birth and weight of the pups was determined on day ten. Average data ± S.D. was used for graphical representation.

3.2. Effect of letrozole treatment on subsequent mammary growth and biochemical changes in aromatase transgenic mice

To test whether the preneoplastic/neoplastic changes abrogated by aromatase inhibitor would reappear after the treatment, we first treated the aromatase transgenic females with letrozole (0.5 μg per day per animal) for 6 weeks to completely abrogate or reduce aromatase induced hyperplastic and other changes in breast tissue. Animals were then allowed to age for 24 weeks after cessation of letrozole treatment. Mammary glands from control and letrozole-treated and aged aromatase transgenic animals were examined. Our findings indicate that the aromatase females treated with letrozole for 6 weeks and aged after ending treatment show some new or sustained mammary growth (Fig. 2A). However, compared to untreated aged controls (Fig. 2B), the growth of mammary glands in these mice is markedly reduced as indicated by the lack of tertiary branching and labulo-alveolar growth and complete lack of terminal end buds. These results suggest that mammary glands were capable of continued proliferation (growth) even after cessation letrozole treatment. However, the growth resulting from such proliferation was shown to be minimal.

To further understand the effect of letrozole treatment on subsequent mammary growth or biochemical changes, we have examined the expression of ER (α and β), PR and as well as estrogen responsive genes like TGFβ and VEGF, in mammary tissues from transgenic animals immediately after the letrozole treatment and in animals that were aged after letrozole treatment along with appropriate controls.

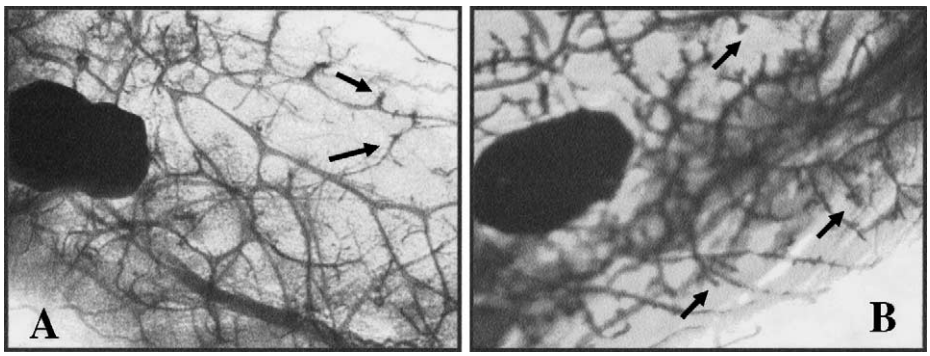

Fig. 2. Morphological features of mammary glands from aged after letrozole treatment and control aromatase transgenic mice. Mammary gland whole mounts from aromatase transgenic mice with (Panel A) and without (Panel B) letrozole treatment prior to aging. Note the lack of terminal branching, labulo-alveolar growth and terminal end buds in letrozole treated mice (Panel A) compare to control aged mice (arrow markings).

As shown in the Fig. 3, expression of ER, PR and TGFβ decreased significantly immediately after the letrozole administration and remained low even several months after the treatment. However, the levels of expression of these genes are higher in the mammary tissue of animals that were aged after being given letrozole compared to animals that were sacrificed immediately after the letrozole treatment. The only exception was that the expression of PR which remained low even in aged animals compared to the level of expression seen in animals immediately after letrozole treatment. Our data also shows a decrease in the protein levels of ER, PR, cyclin D1 and PCNA in mammary tissues of animals that were aged after letrozole treatment compared to control untreated animals (Fig. 4).

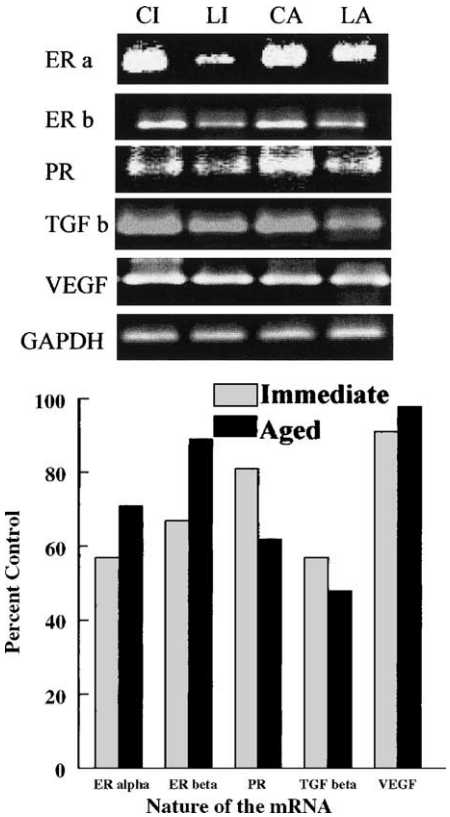

Fig. 3. Effect of letrozole on mRNA expression of ER (α and β), PR, TGFβ and VEGF immediately and aged after letrozole treatment in mammary tissue of aromatase transgenic mice. Densitometric data from RT-PCR analysis are used for graphical representation after correcting for any difference based on GAPDH levels. Animals were treated with 0.5 μg letrozole per mouse per day and aged as indicated in the methods. Data presented in top panel is a representative RT-PCR analysis of expression profile of various genes in the mammary tissue from both control (without and with aging, CI, CA), immediately (LI) and aged after letrozole treatment (LA).

3.3. *Effect of tamoxifen on aromatase-induced mammary hyperplasia and biochemical changes in the aromatase transgenic female mice*

Our previous studies [8,11,20,21] have shown that overexpression of aromatase results in the upregulation of a

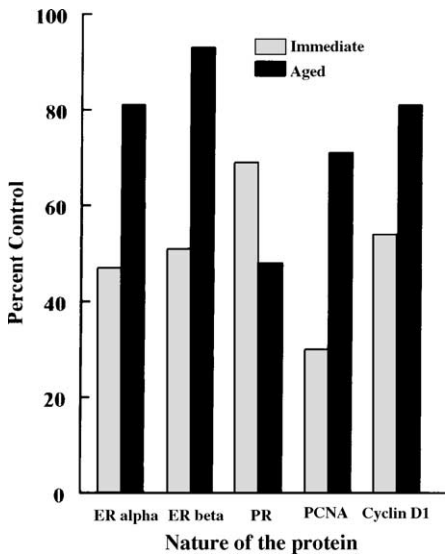

Fig. 4. Effect of letrozole on ER (α and β), PR, PCNA and cyclin D1 protein levels immediately and aged after letrozole treatment in mammary tissue of aromatase transgenic mice. Densitometric data from Western blot analysis are used for graphical representation after correcting for any difference based on actin levels. Animals were treated with 0.5 μg letrozole per mouse per day and aged as indicated in Section 2.

number of estrogen dependent genes that are under estradiol/ER regulation, and that letrozole abrogated aromatase-induced hyperplasia. To examine whether tamoxifen is equally as effective in abrogating aromatase-induced hyperplasia, we compared the mammary growth of tamoxifen-treated aromatase mice with that of letrozole-treated (0.5 μg per day per animal) animals. Our results showed that tamoxifen is also effective in abrogating aromatase-induced mammary hyperplasia; however, letrozole appears to be more effective because it even affected the growth of ductal branching (data not shown).

To further examine the differences in both treatments, we have evaluated the expression of ER, PR and various estrogen regulated genes in both letrozole and tamoxifen-treated mammary tissues. Data shown in Fig. 5 indicate that both treatments affected the expression of ER, PR and other estrogen-dependent genes. However, the letrozole was more effective in down-regulating of ER, PR, TGFβ compared to tamoxifen. No significant differences between both the treatments were seen in the effects on VEGF expression. Consistent with less proliferation and decreased mammary growth, the levels of PCNA were also low in animals that were treated with letrozole compared to tamoxifen-treated animals (Fig. 6). These studies suggest that both letrozole and tamoxifen are potent suppressors of aromatase-induced hyperplasia; however, based on both morpho-histological and biochemical analysis letrozole appears to be more effective.

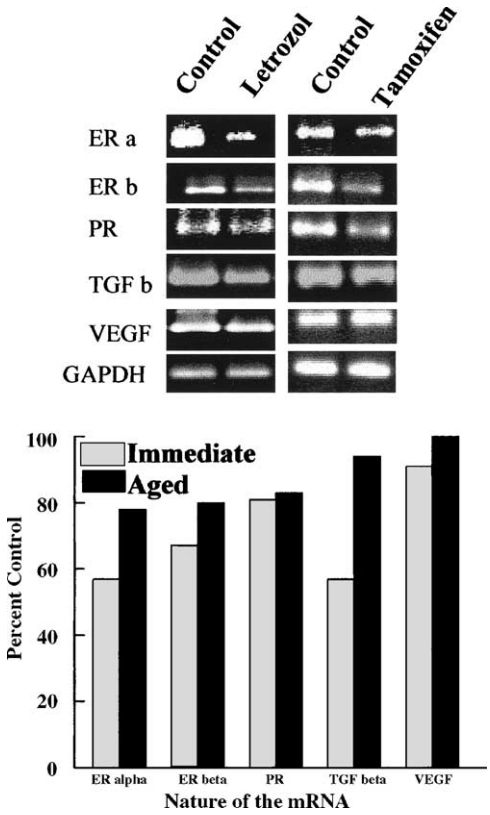

Fig. 5. Effect of letrozole or tamoxifen on the expression of ER, PR, TGFβ, VEGF in mammary tissues of aromatase transgenic mice. Densitometric data from RT-PCR analysis (top panel) is used for graphical representation after correcting for any difference based on the GAPDH levels. Tissues were harvested immediately after the treatment with letrozole or tamoxifen as indicated in Section 2.

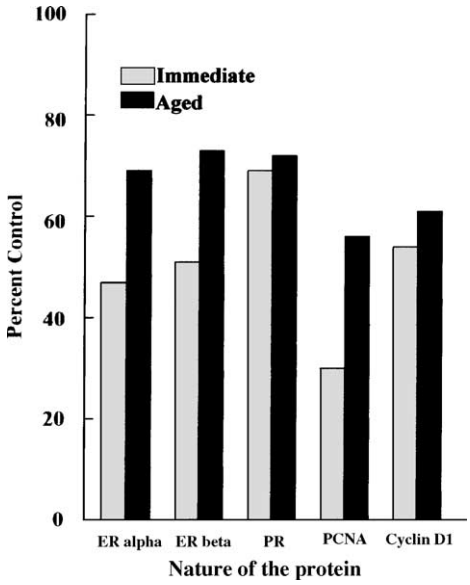

Fig. 6. Effect of letrozole or tamoxifen on ER (α and β), PR, PCNA and cyclin D1 protein levels immediately after letrozole treatment in mammary tissue of aromatase transgenic mice. Densitometric data from Western blot analysis are used for graphical representation after correcting for any difference based on actin levels. Animals were treated with either letrozole or tamoxifen aged as indicated in Section 2.

4. Discussion

Since aromatase was first detected in breast tumors [3], the problem of assessing its functional significance has attracted considerable attention and controversy. A number of recent studies provide evidence to support a biological role for tumor aromatase [23–25]. Aromatase activity and aromatase mRNA is higher in tumor bearing quadrants than in normal quadrants [26,27]. Tumor concentrations of androstenedione are known to be higher than plasma levels and contribute to significant in situ tumor estrogen synthesis [5,28,29]. Increased aromatase protein is expressed in tumors compared to normal areas of the breast as indicated by immunohistochemical localization [30]. Breast tumor-specific transcriptional regulation of aromatase gene has been described [31–33]. In addition, breast tumor aromatase activity can be correlated with markers of cell proliferation such as PCNA [11,30]. All these studies provide evidence that locally overexpressed aromatase has a direct biological role in the growth of normal breast tissue and breast tumors.

An intriguing hypothesis is that local estrogen is directly involved in the initiation of either preneoplastic or neoplastic (or both) changes in mammary epithelium. To

address this question directly, we have generated an aromatase overexpression transgenic model. Our initial studies using this model [8–10] showed that overexpression of aromatase leads to increased estrogenic activity both in female and male mammary glands and in male reproductive tissues. This activity results in the initiation of various preneoplastic/neoplastic changes in female and male mammary glands and Leydig cell tumor formation in males. We have also shown that [11] overexpression of aromatase leads to various epigenetic changes that may be involved in estrogen-induced tumorigenesis.

A large body of literature provides direct evidence that aromatase inhibitors are very effective in blocking the action of this enzyme as well as treating hormone-dependent breast tumors [19,24,34–38]. Therefore, we wanted to investigate whether aromatase inhibitors can be used as chemopreventive agents to block/reduce aromatase induced breast hyperplasia and other preneoplastic/neoplastic changes in transgenic animals. Our previous studies have clearly demonstrated that the aromatase inhibitor, letrozole, even when used in low doses is very effective in abrogating aromatase-induced breast hyperplasia [20,21].

Data presented in this paper clearly demonstrated that letrozole, even when used in low doses is very effective in abrogating aromatase-induced breast hyperplasia, moreover, animals were physiologically normal after treatment as indicated by normal pregnancy and lactation and lack of any birth defects in the pups that were born to letrozole-treated mothers.

Although, letrozole was effective in abrogating aromatase-induced mammary hyperplasia in the aromatase transgenic mice, the presence of some mammary proliferation in aged animals that were previously treated with letrozole suggests that inhibition of mammary growth is not a permanent or long-lasting one. The slight increase in the expression of ER, and other proliferation markers in aged animals were in agreement with the morphological changes. Furthermore, the decreased ductal branching in letrozole-treated mice and continued low levels of PR in these animals is consistent with the importance of ER/PR in ductal branching of mammary glands [39].

A number of previous studies [40,41] have indicated no consistent relationship between ER status and tumor aromatase levels. However, the majority of ER positive tumors showed high aromatase activity. We have recently demonstrated that overexpression of aromatase led to upregulation of ERα in mammary tissue of transgenic females and also activation of ERα in transgenic male mammary glands [11]. Our studies also have shown down regulation of ERα in mammary tissues of animals that were treated with letrozole, suggesting that lack of breast tissue estrogen leads to down-regulation of its receptors. Comparable downregulation of ER, PR and other estrogen-dependent genes in letrozole and tamoxifen-treated mammary tissue suggests that estradiol/ER-mediated mechanisms are critical for both mammary development and tumorigenesis.

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References

[1] W.L. McGuire, An update on estrogen and progesterone receptors in prognosis for primary and advanced breast cancer, in: S. Iacobelli (Ed.), Hormones and Cancer, Raven Press, New York, 1980, pp. 337–344.
[2] M.E. Lippman, R.B. Dickson, Mechanisms of growth control in normal and malignant breast epithelium, Recent. Prog. Horm. Res. 45 (1989) 383–440.
[3] W.R. Miller, A.P.M. Forrest, Oestradiol synthesis by a human breast carcinoma, Lancet II (1974) 866–868.
[4] P.K. Siiteri, 1982 Review of studies on estrogen biosynthesis in the human, Cancer Res. 42 (1982) 3269s–3275s.
[5] S.J. Santner, P.D. Feil, R.J. Santen, In situ estrogen production via the estrone sulfatase pathways in breast tumors: relative importance versus the aromatase pathway, J. Clin. Endocrinol. Metab. 48 (1984) 29–33.
[6] M.J. Reed, A.M. Owen, L.C. Lai, N.G. Colddham, M.W. Ghilchik, N.A. Shaikh, V.H.T. James, In situ oestrone synthesis in normal breast and breast tumor tissues: effect of treatment with 4-hydroxyandrostenedione, Int. J. Cancer 44 (1989) 233–237.
[7] R.R. Tekmal, R.J. Santen, Local estrogen production: is aromatase an oncogene, in: A. Manni (Ed.), Contemporary Endocrinology: Endocrinology of the breast, Humana Press, Totowa, NJ, 1999, pp. 79–82.
[8] R.R. Tekmal, N. Ramachandra, S. Gubba, V.R. Durgam, J. Mantione, K. Toda, Y. Shizuta, D.L. Dillehay, Overexpression of *int-5/aromatase* in mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities, Cancer Res. 56 (1996) 3180–3185.
[9] K. Fowler, K. Gill, N. Kirma, D.L. Dillehay, R.R. Tekmal, Overexpression of aromatase leads to development of testicular leydig cell tumors: an animal model for hormone-mediated testicular cancer, Am. J. Pathol. 156 (2000) 347–353.
[10] K. Gill, N. Kirma, K. Fowler, D.L. Dillehay, R.R. Tekmal, Aromatase overexpression in male transgenic mice results in gynecomastia and biochemical changes in mammary glands, J. Steroid. Biochem. Mol. Biol. 76 (2001) 000–000.
[11] N. Kirma, K. Gill, U. Mandava, R.R. Tekmal, Overexpression of aromatase leads to hyperplasia and changes in the expression of genes involved in apoptosis, cell cycle, growth and tumor suppressor function in the mammary glands of the transgenic mice, Cancer Res., in press.
[12] R.J. Santen, E. Samojlik, S.A. Wells, Resistance of the ovary to blockade of aromatization with aminoglutathamide, J. Clin. Endo. Metab. 51 (1980) 473–477.
[13] W.R. Miller, R.J. Santen, Aromatase Inhibition and Breast Cancer, Marcel Dekker, New York, 2001, pp. 3–309.
[14] P.E. Goss, Chemoprevention with aromatase inhibitors, in: W.R. Miller, R.J. Santen (Eds.), Aromatase Inhibition and Breast Cancer, Marcel Dekker, New York, 2001, pp. 161–181.
[15] R.A. Lubet, V.E. Steele, T.L. Casebolt, I. Eto, G.J. Kellof, C.J. Grubbs, Chemopreventive effects of the aromatase inhibitors vorozole (R-83842) and 4-hydroxyandrostenedione in the methylnitrosourea-induced mammary tumor model in Sprague-Dawley rats, Carcinogenesis 15 (1994) 2775–2780.

[16] D.E. Gunson, R.E. Steele, R.Y. Chau, Prevention of spontaneous tumors in female rats by fadrozole hydrochloride, an aromatase inhibitor, Br. J. Cancer 72 (1995) 72–75.
[17] G.J. Kellof, R.A. Lubert, R. Liberman, K. Eisenhauer, V.E. Steele, J.A. Crowell, E.T. Hawk, C.W. Boone, C.C. Sigman, Aromatase inhibitors as potential cancer chemopreventive, Cancer Epidemiol. Biomarkers Prevent. 7 (1998) 65–78.
[18] P.E. Goss, K. Strasser, Chemoprevention with aromatase inhibitors—trail strategies, J. Steroid Biochem. Mol. Biol. 79 (2001) 143–149.
[19] P.E. Goss, Anti-aromatase agents in the treatment and prevention of breast cancer, Cancer Contr. 9 (2002) 2–8.
[20] R.R. Tekmal, K. Gill, N. Kirma, K. Fowler, Aromatase overexpression and breast hyperplasia, an in vivo model: continued overexpression of aromatase is sufficient to hyperplasia maintain without circulating estrogens and use of aromatase inhibitors abrogate these preneoplastic changes in mammary glands, Endocr. Relat. Cancer 6 (1999) 307–314.
[21] U. Mandava, N. Kirma, R.R. Tekmal, Aromatase overexpression transgenic mice model: cell type specific expression and use of letrozole to abrogate mammary hyperplasia without affecting normal physiology, J. Steroid Biochem. Mol. Biol. 79 (2001) 27–34.
[22] D. Medina, Preneoplastic lesions in mouse mammary tumorigenesis, Methods Cancer Res. 7 (1973) 3–53.
[23] M.J. Reed, The role of aromatase in breast tumors, Breast Cancer Res. Treat. 30 (1994) 7–17.
[24] A.M.H. Brodie, R.J. Santen, Aromatase and its inhibitors in breast cancer—overview and perspective, Breast Cancer Res. Treat 30 (1994) 1–6.
[25] T.R.J. Evans, M.G. Rowlands, M.C. Silva, M. Law, R.C. Coombes, Prognostic significance of aromatase and estrone sulfatase enzymes in human breast cancer, J. Steroid Biochem. Mol. Biol. 44 (1993) 1583–1587.
[26] J.S. O'Neill, R.A. Elton, W.R. Miller, Aromatase activity in adipose tissue from breast quadrants: a link with tumor site, Br. Med. J. 296 (1988) 741–743.
[27] S.E. Bulun, G. Sharda, J. Rink, S. Sharma, E.R. Simpson, Distribution of aromatase P450 transcripts and adipose fibroblasts in the human breast, J. Clin. Endocrinol. Metab. 81 (1996) 1273–1277.
[28] W.R. Miller, A.P.M. Forrest, Oestradiol synthesis from C19 steroids by human breast cancers, Br. J. Cancer 33 (1976) 116–121.
[29] E. Perel, D. Wilkins, D.W. Killinger, The conversion of androstenedione to estrone and estradiol, and testosterone in breast tissue, J. Steroid Biochem. 13 (1980) 89–92.
[30] Q. Lu, J. Nakamura, A. Savinov, W. Yue, J. Weisz, D. Dabbs, J. Wolz, A. Brodie, Expression of aromatase protein and mRNA in tumor epithelial cells and evidence of functional significance of locally produced estrogen in human breast cancers, Endocrinology 137 (1996) 3061–3068.
[31] N. Harda, T. Utsumi, Y. Takagi, Tissue-specific expression of the human aromatase P450 gene by alternative use of multiple exons 1 and promoters and switching of tissue-specific exons in carcinogenesis, Proc. Natl. Acad. Sci. U.S.A. 90 (1993) 11312–11316.
[32] S. Chen, Aromatase and breast cancer, Front. Biosci. 3 (1998) d922–d933.
[33] S. Sebastian, K. Takayam, M. Shou, S.E. Bulun, Cloning and characterization of a novel endothelial promoter of the human *cyp19* (aromatase P450) gene that is upregulated in breast cancer tissue, Mol. Endocrinol. 16 (2002) 2243–2254.
[34] W.R. Miller, J.M. Dixon, Local endocrine effects of aromatase inhibitors within the breast, J. Steroid Biochem. Mol. Biol. 79 (2001) 93–102.
[35] A.U. Buzdar, A summary of second-line randomized studies of aromatase inhibitors, J. Steroid Biochem. Mol. Biol. 79 (2001) 109–114.
[36] A.M. Brodie, V.C. Njar, Aromatase inhibitors in advanced breast cancer: mechanism of action and clinical implications, J. Steroid Biochem. Mol. Biol. 66 (1998) 1–10.
[37] A.M. Brodie, Q. Lu, J. Nakamura, Aromatase in the normal breast and breast cancer, J. Steroid Biochem. Mol. Biol. 61 (1997) 281–286.
[38] A. Brodie, Q. Lu, Y. Liu, B. Long, J.P. Wang, W. Yue, Preclinical studies using the intratumoral aromatase model for postmenopausal breast cancer, Oncology 12 (1998) 36–40.
[39] G. Shyamala, Y.C. Chou, S.G. Louie, R.C. Guzman, G.H. Smith, S. Nandi, Cellular expression of estrogen and progesterone receptors in mammary glands: regulation by hormones development and aging, J. Steroid Biochem. Mol. Biol. 80 (2002) 137–148.
[40] W.R. Miller, T.J. Anderson, W.L.J. Jack, Relationship between tumor aromatase activity tumor characteristics and response to therapy, J. Steroid Biochem. Mol. Biol. 37 (1990) 1055–1059.
[41] M.C. Silva, M.G. Rowlands, M.D. Dowsett, B. Gusterson, J.A. McKinna, I. Fryatt, R.C. Coombes, Intratumoral aromatase as a prognostic factor in human breast carcinoma, Cancer Res. 49 (1980) 2588–2591.