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AROMATASE AND BREAST CANCER

Shiuan Chen

Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010

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TABLE OF CONTENTS

1. ABSTRACT

Estrogens play an important role in breast cancer development. Aromatase (CYP19), a cytochrome P450, is the enzyme that synthesizes estrogens. Aromatase is expressed at a higher level in human breast cancer tissue than in normal breast tissue using enzyme activity measurement, immunocytochemistry, and RT-PCR analysis. Cell culture, animal experiments using aromatase-transfected breast cancer cells, and transgenic mouse studies have demonstrated that in situ produced estrogen plays a more important role than circulating estrogens in breast tumor promotion. In addition, tumor aromatase has been shown to stimulate breast cancer growth in both an autocrine and a paracrine manner. RT-PCR and gene transcriptional studies have revealed that aromatase promoter switches from a glucocorticoid-stimulated promoter, I.4, in normal tissue to cAMP-stimulated promoters, I.3 and II, in cancereous tissue. Suppression of in situ estrogen biosynthesis can be achieved by the prevention of aromatase expression or by the inhibition of aromatase activity in breast tumors. While the control mechanism of aromatase expression in breast cancer tissue is not yet fully understood, aromatase-inhibitor therapy is considered for second-line treatment in patients who fail anti-estrogen therapy. Twenty to thirty percent of the patients who fail anti-estrogen treatment respond to aromatase-inhibitor treatment. Several potent and selective aromatase inhibitors have been developed and used to treat breast cancer. The binding nature of various aromatase inhibitors has been examined by computer modeling, site-directed mutagenesis of aromatase, and inhibition kinetics. The enzyme structure-function studies have led to the development of a computer model of the active site region of human aromatase. The model is used to evaluate the interaction of phytoestrogens such as flavones and isoflavones with aromatase. The study provides a molecular basis as to why isoflavones are significantly poorer inhibitors of aromatase than flavones. The phytoestrogen studies will help to determine which fruits and vegetables (those containing the appropriate phytoestrogens) should be included in the diet of postmenopausal women in order to reduce the incidence for breast cancer by inhibiting estrogen biosynthesis in breast tissue.

2. INTRODUCTION

Estrogens play an important role in breast cancer development. Approximately 60% of premenopausal and 75% of postmenopausal patients have estrogen-dependent carcinomas. The effects of estrogens on proliferation are mainly mediated by their interaction with the estrogen receptor (ER) (1). In estrogen-dependent breast tumors, estrogens are thought to induce the expression of peptide growth factors that are responsible for the proliferative responses of cancer cells (e.g., 2, 3).

Aromatase, a cytochrome P450, is the enzyme that synthesizes estrogens by catalyzing three consecutive hydroxylation reactions converting C19 androgens to aromatic C18 estrogenic steroids (see figure 1). Aromatase converts androstenedione and testosterone to estrone and estradiol, respectively.

Aromatase is expressed in a tissue-specific manner. This enzyme is mainly expressed in the ovaries of premenopausal women. A very high level of aromatase is expressed in placenta in pregnant women. In postmenopausal women and men, adipose tissue and skin cells are the major sources of estrogen production, but the aromatase activity in these tissues is significantly lower than that in ovaries and the level of circulating estrogen is much lower in postmenopausal women and men than in premenopausal and pregnant women. Since aromatase is the enzyme responsible for the synthesis of estrogens, and

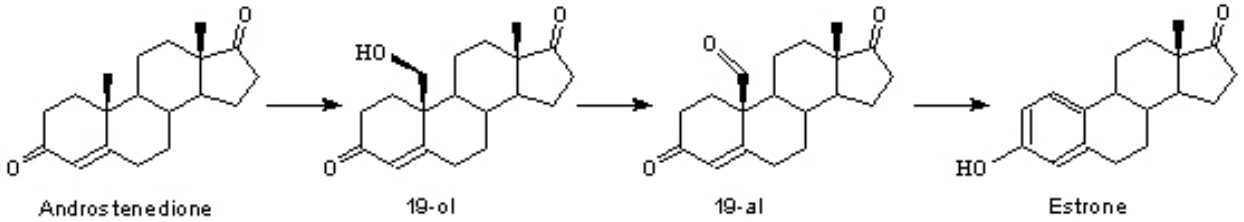

Figure 1. Aromatase converts androgens to estrogens

estrogens play a major role in the development of breast cancer, abnormal expression of aromatase in breast cancer cells and/or surrounding adipose stromal cells, especially in postmenopausal women, may have a significant influence in breast tumor maintenance and growth in breast cancer patients.

This review intends to summarize recent research from our and other laboratories in the area of aromatase expression and action in breast cancer tissue. Aromatase is expressed at a higher level in breast tumors and *in situ* estrogen biosynthesis has been shown to promote breast cancer growth in both an autocrine and a paracrine fashion. Studies related to these topics will be discussed in brief in this review (Sections 3 and 4). A major effort of our laboratory is to determine how aromatase expression in breast tumors is regulated. Gene regulation studies from our and other laboratories will be reviewed in Section 5. In addition, aromatase inhibitors are drugs used to treat breast cancer by suppressing *in situ* estrogen biosynthesis. The molecular basis of the interaction of various inhibitors with aromatase has been evaluated by site-directed mutagenesis, computer modeling, and inhibition kinetics. The findings on aromatase structure-function studies and inhibitor binding will be briefly summarized in Section 6. Finally, the potential effects of phytoestrogens and xenoestrogens on aromatase will be commented on in Section 7.

3. AROMATASE EXPRESSION IN BREAST CANCER TISSUES

James *et al.* (4) reported that aromatase activity, when measured *in vitro*, was found to be higher in breast tumors than in the fat next to the tumor or in normal breast fat. In addition, Miller and O'Neill (5) found a highly significant correlation between aromatase activity and the presence of tumors in individual quadrants of breast tissue. Recently, using quantitative PCR analysis, adipose stromal cells surrounding the cancer cells have also been reported to contain aromatase mRNA at a higher level than adipose stromal cells in noncancerous areas (6,7). It was recently reported that the concentrations of estrogens in breast tumor tissues were found to be several-fold higher than those in plasma in postmenopausal patients (8). These results support a tumor accumulation and *in situ* synthesis of estrogens. In a very recent study, we detected aromatase mRNA in 67 out of 70 breast tumor specimens (9).

Immunocytochemical analysis from our laboratory first identified the presence of aromatase in breast cancer epithelial and stromal cells (10), while others had reported the presence of aromatase only in the stromal tissue (11,12). Our findings have been recently confirmed by independent *in situ* hybridization studies and cell proliferation assays showing that aromatase is expressed in breast cancer epithelial cells (13).

Therefore, it can be stated that aromatase is expressed in breast cancer tissue, probably at a higher level than normal breast tissue, as demonstrated by enzyme activity measurement, immunocytochemistry, and RT-PCR analysis.

4. CONSEQUENCE OF AROMATASE EXPRESSION IN BREAST TUMORS

Kitawaki *et al.* (14) reported that by converting androgen to estrogen (at physiological concentrations), the endogenous aromatase enzyme in the MCF-7 breast cancer cells (ER positive cells) stimulated DNA synthesis, and the stimulation was abolished by the administration of aromatase inhibitors. In 1993, using an aromatase expressing MCF-7 cell line [expressing aromatase at a level 10 times that of the untransfected MCF-7 cell line (15)], androgen-dependent cell growth was observed (16). In addition, tumors were grown in nude mice inoculated with the aromatase transfected MCF-7 cells together with Matrigel (17). The tumor growth was accelerated by injections of androstenedione. These cell culture and nude mouse experiments using aromatase transfected MCF-7 cells demonstrate, in a direct fashion, that aromatase expressed in breast cancer cells can play a role in stimulating the growth of tumors, in an autocrine manner. Similar cell culture and nude mouse studies were also performed by Dowsett and his colleagues (18-20). In addition, results obtained from studies using a transgenic mouse model in which aromatase is over-expressed in mammary tissues indicate that *in situ* produced estrogen plays a more important role than circulating estradiol in breast tumor promotion (21).

The above described investigations have generated critical results supporting the theory that *in situ* aromatase can play a role in promoting breast tumor growth. However, the studies could not address important issues as to interactions between aromatase-expressing cell and non-aromatase-expressing cell subpopulations that may

affect growth rates and drug (e.g., aromatase inhibitor) sensitivity. In addition, it has not yet been shown whether the tumor cell subpopulations which express aromatase manifest a growth advantage within the tumors. We have carried out co-culture experiments with two aromatase expressing breast cancer cell lines (MCF-7aro and T-47Daro) together with an untransfected MCF-7 cell line, using the spheroid culture method (22).

Stable aromatase expressing MCF-7 and T-47D cell lines (i.e., MCF-7aro and T-47Daro) have been prepared by aromatase cDNA transfection and G418 (neomycin) selection. MCF-7aro was subjected to clonal purification. Testosterone (1 nM) increased cell growth to a similar degree for MCF-7/MCF-7aro co-culture (0.75 million cells each type) as with MCF-7aro only (2 to 3 fold). In addition, the enzyme specific activities remained unchanged for MCF-7/MCF-7aro co-culture samples with and without androgen treatment, indicating that estrogen produced by transfected cells can also stimulate the growth of untransfected cells. The androgen response could be inhibited by addition of an aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA) (0.01 - 0.1 mM). For MCF-7/T-47Daro co-culture experiments, a clear induction of cell growth by androgen was observed and the level of the increase was similar to that on T-47Daro only. However, for both the T-47D only or the MCF-7/T-47Daro co-culture, the aromatase activity was found to increase significantly after testosterone treatment. Since T-47Daro cells were not subjected to clonal purification, it is thought that the androgen treatment may selectively stimulate the growth of high aromatase-expressing T-47Daro cells. These results indicate that estrogen synthesized by the tumor aromatase can stimulate breast tumor growth in both an autocrine and a paracrine manner.

It is thought that in normal breast tissue aromatase is mainly expressed in adipose stromal cells and fibroblasts. The estrogens stimulate epithelial cell growth through a paracrine fashion. However, in breast tumor tissue, aromatase is found to be expressed in both stromal and cancer cells. Therefore, aromatase stimulates breast tumor growth in both an autocrine and a paracrine manner.

5. TRANSCRIPTIONAL REGULATION OF AROMATASE EXPRESSION IN BREAST TUMORS

It is known that a complex mechanism is involved in the control of human aromatase expression. Six exon Is have been reported. As indicated in figure 2, through primer extension experiments with mRNA isolated from various aromatase-expressing tissues/cells (23-27), exons I.1 and I.2, exons I.3 and I.4, and exon PII have been identified in the aromatase mRNA in placenta, adipose stromal cells, and ovary, respectively. Exon I.4 has also been found in aromatase mRNA in skin fibroblasts (25). Another exon I, I.5, has also been suggested (28). It is thought that aromatase expression in these tissues is driven by the promoters situated upstream from these exon Is, providing tissue-specific controls of aromatase expression. Phorbol ester treatment increases the expression of aromatase in human placental choriocarcinoma (JAR) cells (29). Detailed promoter functional analysis by Toda et al. (30) identified a phorbol ester-responsive enhancer element upstream from the predicted promoter I.1 region, indicating that promoter I.1(mainly used in placenta) is regulated through a protein kinase c-mediated mechanism. Aromatase expression in the ovary (primarily in granulosa cells) is under the control of gonadotropin FSH, whose action is mediated by cAMP. Results from Simpson's laboratory (24) suggest that cAMP-stimulated transcription of human aromatase in the ovary (driven by promoter II) is due in part to the action of the Ad4BP/SF-1 responsive element situated upstream from promoter II (31). The expression of aromatase in adipose tissue was found to be stimulated by glucocorticoids (26), and aromatase mRNA in adipose tissue was found to contain mainly exon I.4. Characterization of the region upstream of exon I.4 revealed the existence of a TATA-less promoter and an upstream GRE and an Sp1 sequence (32). These elements were shown to be required for expression of reporter gene constructs in the presence of serum and glucocorticoids. In addition, a GAS (interferon-r activating sequence) element was also identified near promoter I.4. Promoter I.3 was characterized functionally in our laboratory (33). This promoter is down-regulated by a silencer element (34) which overlaps with the Ad4BP/SF-1 response element and is up-regulated by a cAMP responsive element (unpublished results).

In order to understand the regulatory mechanism of aromatase expression in breast tumors, we decided to first determine which promoters are used to drive aromatase expression in breast tumors. RT-PCR using exon 1-specific primers was performed to determine the exon I usage in aromatase mRNA in 70 breast tumor specimens (9). Exon PII was been found to be present in aromatase mRNA in 73% of the specimens (49 samples) and to be the major exon I in 78% (38 of the 49 samples) of the specimens containing RNA messages with exon PII. Exon I.3 has been found to be present in aromatase mRNA in 78% of specimens (52 samples) and to be the major exon I in 60% (31 of the 52 samples) of the specimens containing messages with exon I.3. Exon I.4 was detected in a lesser number of breast tumor specimens (49%; 33 samples) and to be the major exon I in 33% (11 of the 33 samples) of the specimens containing RNA messages with exon I.4. Exon I.1 was only detected in a minimal number of breast tumor specimens. These results have shown that exons PII and I.3 are the two major exons I present in aromatase mRNA isolated from breast tumors, suggesting that promoters II and I.3 are the major promoters driving aromatase expression in breast cancer and surrounding adipose stromal cells. Further support for this finding was obtained from the fact that exons PII and 1.3 were again found to be the major exons 1 in aromatase transcripts in an adipose stromal cell line and four breast cancer cell lines (MCF-7, T-47D, SK-BR-3, and MDA-MB-231) (9). Therefore, the major promoter usage in breast tumors (both cancer cells and surrounding adipose stromal cells) (i.e., cAMP-stimulated promoters I.3 and II) is different from that in normal breast adipose tissue (i.e., glucocorticoid-

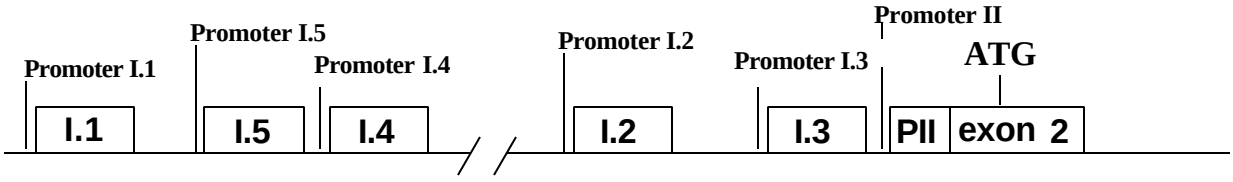

Figure 2. Scheme of alternative utilization of tissue-specific exon Is and promoters of the human aromatase gene. The region encoding the $P450_{arom}$ protein contains 9 exons (II-X), of which only exon II is shown. The translation initiation site is indicated by ATG which is located in exon II. A number of untranslated first exons are expressed in a tissue-specific fashion and encode the 5'- untranslated termini of $P450_{arom}$ mRNAs in placenta (exon 1.1 and 1.2), adipose tissue (exon 1.3 and 1.4), ovary (exon PII), skin fibroblasts (exon 1.4), and fetal liver (exon 1.5). Aromatase expression in these tissues is driven by the promoters situated upstream from these exons 1. The distance between exon 1.4 and 1.2 has not been determined due to a lack of a genomic clone which bridges the two exons.

stimulated promoter I.4). Similar findings were reported by Bulun et al. (35) and Harada (7).

Our laboratory has identified a negative regulatory element that is situated between promoters I.3 and II (see figure 3) that down regulates the action of these promoters (34). This negative regulatory element is thought to be a silencer element (S1) because it acts in an orientation- and promoter-independent manner. The position of S1 (5'-CCAAGGTCAGAAATGCTGCAATTCAAGCCA-3') was mapped by DNase I footprinting and DNA deletion analyses. The region contains three pairs of inverted repeats, as indicated by an underline, probably explaining why S1 functions in both orientations. Chloramphenicol acetyltransferase (CAT) functional analyses were performed which indicated that the transcriptional activities of both promoters I.3 and II were suppressed 2-3 fold by S1. Mutations of two inverted repeat segments (i.e., TTC and CC) in S1 destroyed its silencing action and modified the protein binding patterns in DNA mobility shift analysis. UV cross-linking experiments with 32P-labeled BrdU-substituted S1 as the probe and nuclear extracts from MCF-7 breast cancer cells and skin fibroblasts revealed that four major nuclear proteins, with molecular weights of approximately 150kd, 45kd, 30kd, and 25kd, bound to this element. Interestingly, two smaller proteins could be competed by an unlabeled fragment which contains promoter I.3. In addition, mutation of the S1 at the region CC destroyed the ability to compete with the wild-type S1 for the binding of 30kd and 25kd proteins. These results led us to propose that S1 down regulates the action of promoter I.3 (also promoter II), and the 30kd and 25kd proteins present in the nuclear extract of MCF-7 cells and skin fibroblasts are involved in the silencer action. Our laboratory is currently studying the transcriptional factors that bind to S1 and examining in detail how S1 functions.

We have identified two additional regulatory elements that are situated near promoter I.3. Recent work from our laboratory revealed that a positive regulatory region upstream from promoter I.3 could overcome the negative regulatory action of S1 (unpublished results). The positive regulatory element is a cAMP responsive element (CRE, see figure 3), and promoters I.3 and II are cAMP-regulated promoters. The position of CRE has been mapped by DNase I footprinting and DNA deletion analyses. A negative regulatory element upstream from CRE has also been found. The sequence of the regulatory element resembles a TGFß responsive element. Functional analysis is being carried out to characterize this "TGFß" responsive element. Based on results generated from exon I usage detected in normal and breast cancer tissues and from the molecular characterization of the genomic region containing promoters I.3 and II, we propose that in normal breast adipose stromal cells and fibroblasts aromatase expression is up regulated by promoter I.4 (glucocorticoid dependent), and the action of promoters I.3 and II is suppressed by the silencer (see figure 3A). However, in cancer cells and surrounding adipose stromal cells an unknown factor(s) induces cAMP response, and aromatase promoters are switched to cAMP-dependent promoters, i.e., I.3 and II. The positive regulation of CRE overcomes the negative regulation of S1. Furthermore, estrogen synthesized by aromatase is thought to down regulate the production of TGFß, suppressing the negative action of the TGFß responsive element (see figure 3B). Estrogen has been reported to suppress the synthesis of TGFß (36). This hypothesis is being carefully evaluated in our laboratory by detailed functional analysis.

Harada has demonstrated that exon I/promoter usage in tumor fibroblasts switches from I.4 to I.3 when forskolin is added (7). The factor(s) in breast tumors that induces the cAMP response has not been identified. It is thought that the induction is mediated through a paracrine fashion by factor(s) produced by breast cancer cells. Zhao et al.(37) suggested that prostaglandin PGE2 synthesized in breast cancer cells induces cAMP response in breast cancer tissue. Our mechanism involving stromal cell-cancer cell interaction would explain why aromatase expression in isolated tumor fibroblasts and adipose stromal cells is, by default, mainly driven by promoter I.4 (38).

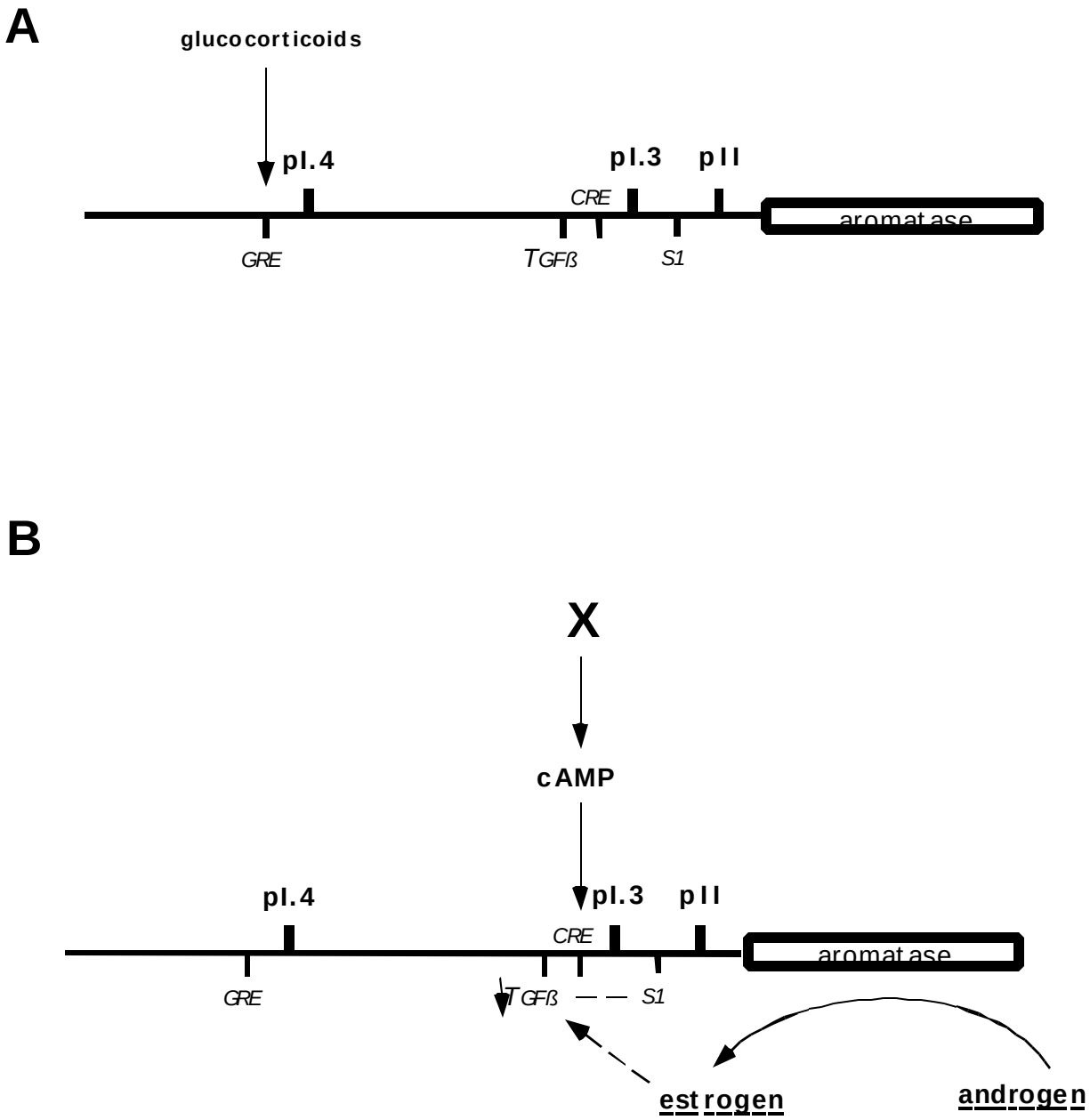

Figure 3. Proposed mechanism for different promoter usage in controlling aromatase in normal breast adipose tissue and breast tumors. pI.4, promoter I.4; pI.3, promoter I.3; and pII, promoter II.

6. AROMATASE INHIBITORS AND ENZYME STRUCTURE-FUNCTION STUDIES

Aromatase inhibitors are thought to be of value in treating estrogen-dependent breast cancer, especially in postmenopausal patients. As described above, estrogens in postmenopausal patients are mostly produced in peripheral adipose tissues and in cancer cells, and the peripheral aromatase is not under gonadotropin regulation (39). Therefore, in postmenopausal patients, complications due to a feedback regulatory mechanism which increases luteinizing hormone (LH) and follicle-stimulating hormone (FSH) after aromatase inhibitor treatment does not occur. In premenopausal women, LH and FSH stimulate the synthesis of aromatase in ovaries and may counteract the effects of some aromatase inhibitors, as has been observed for aminoglutethimide (AG, structure see figure 4) (40). In view of this, the inhibition of the enzyme has been considered as a potential therapy for breast cancer in postmenopausal women. Throughout the years, a number of very potent and highly selective aromatase inhibitors have been synthesized and tested as drugs for the treatment

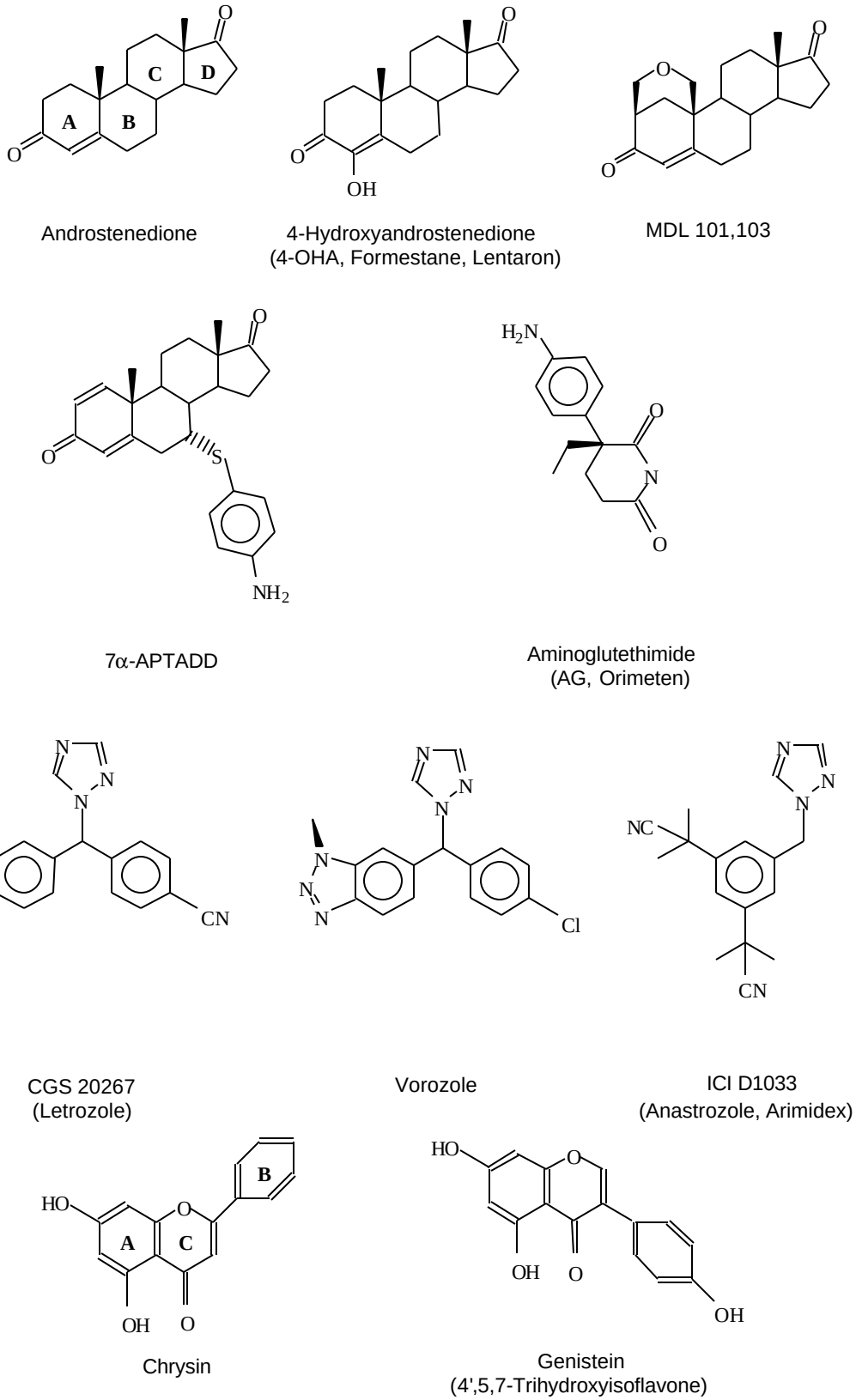

Figure 4. Structures of seven aromatase inhibitors and two phytoestrogens

of breast cancer (structures of aromatase inhibitors are shown in figure 4).

AG was initially introduced as an anticonvulsant agent and was the first aromatase inhibitor approved for use by FDA for breast cancer treatment. It was also shown to inhibit cytochrome P450 cholesterol SCC (side-chain cleavage) (41). In addition to problems caused by a lack of specificity, it has also been shown that in some patients the aromatase activity in breast tumors is significantly increased after AG treatment (5). In recent years, several potent and specific aromatase inhibitors (e.g., Lentaron, Arimidex, and Letrozole) have been developed and are being used in the treatment of breast cancer. The application of these inhibitors in treating breast cancer was recently reviewed by Brodie and Njar (42).

Aromatase inhibitor development has been based primarily on inhibitor structure-activity relationship studies. Aromatase inhibitors can be categorized into two types: steroidal and nonsteroidal inhibitors (see figure 4). In general, steroidal aromatase inhibitors are analogues of androgen substrates and nonsteroidal inhibitors perturb the catalytic properties of the heme prosthetic group of aromatase. While a number of the inhibitors have been shown to be very potent and specific inhibitors of aromatase, the exact nature of their interactions with aromatase are not known. This is especially true for nonsteroidal inhibitors since these compounds have very diverse structures. Although the structures of these compounds are different, it is thought that they bind to the active site of aromatase, as indicated by competitive inhibition of the enzyme.

We have investigated the interaction of a number of aromatase inhibitors with different aromatase mutants [using a mammalian cell expression method (15)] for evaluating the accuracy of our computer model as well as for determining the binding characteristics of different steroidal and nonsteroidal inhibitors. We have published extensively in this area (43-51). The aim of our aromatase structure-function studies has been to use results generated with steroidal inhibitors to refine our computer model and then explain results generated with nonsteroidal inhibitors using the refined model. We feel that useful information has been generated from these studies.

Our enzyme structure-function studies have revealed that two regions, the I helix (Cys-299 to Ser-312) and the "hydrophobic" pocket (Ile-474 to His-480), are important parts of the active site and make significant contributions to the binding of the substrate and conversion of androgen to estrogen. Mutations in these regions reduce the binding of the substrate and inhibitors. Characterization of two mutants, H480K and H480Q, further suggests that His-480 is hydrogen bonded to the 3-keto group of the androgen substrate (52). The molecular basis of the interaction of various inhibitors with aromatase has been discussed in details and computer models have been presented in two recent publications from our laboratory (50,51). While several important regions of aromatase have been recognized by computer modeling from several laboratories (49,50, 53-55), a few disagreements still exist among models from different groups.

Our laboratory recently succeeded in expressing aromatase using the insect cell expression method and has purified the enzyme to homogeneity (unpublished results). It is our hope that we will be able to better study the aromatase structure-function relationship using purified enzyme preparations.

7. PERSPECTIVE

Research from our and other laboratories demonstrates that aromatase is expressed at a higher level in breast cancer tissue than non-cancer tissue. RT-PCR analysis of exon I usage of breast tissue reveals that there is a switch in the aromatase promoter usage in normal tissue versus cancerous tissue, i.e., from a glucocorticoid-stimulated promoter I.4 to more efficient cAMP-stimulated promoters I.3 and II. Cell culture and animal studies indicate that *in situ* estrogen biosynthesis can promote tumor growth through both a paracrine and an autocrine manner.

Suppression of in situ estrogen biosynthesis can be achieved by the prevention of aromatase expression in breast tumors or by the inhibition of aromatase activity. It is our hope that through a understanding of the regulatory mechanism of aromatase expression in breast cancer tissue, a therapy based on suppressing aromatase expression can be developed. During the last two years, we have made significant progress in determining the promoters involved in driving aromatase expression in breast cancer tissue and in determining several important regulatory elements that may affect aromatase expression. We have identified several transcriptional factors that bind to these regulatory elements. We anticipate that we will learn a great deal about the regulatory mechanism of aromatase expression in breast cancer tissue by studying the interaction between transcriptional factors and cis-regulatory elements in the genomic region containing promoters I.3 and II.

Aromatase inhibitors is used as drugs to treat breast cancer. Complete or partial tumor regression has been reported in postmenopausal patients treated with aromatase inhibitors, such as aminoglutethimide or 4-hydroxyandrostenedione (e.g., 5,56). Aromatase-inhibitor therapy is a second-line treatment for those who fail anti-estrogen therapy. Twenty to thirty percent of the patients who fail anti-estrogen treatment respond to aromatase-inhibitor treatment. Furthermore, aromatase inhibitors may be useful as a chemopreventive agent against breast cancer by suppressing aromatase activity. Using a N-methyl-N-nitrosourea (NMU)-induced rat mammary cancer model, vorozole, an aromatase inhibitor, has been shown to be a more effective chemopreventive agent against mammary cancer than 9-cis-retinoic acid, N-(4-hydroxyphenyl)-retinamide (4-HPR), and dehydroepiandosterone (DHEA) (57,58). Vorozole at 0.08 mg/kg body weight/day (by gavage) reduced the number of mammary tumors/rat by 73%. NMU-induced tumor incidence is estrogen dependent (59), and vorozole is thought to act as a chemopreventive agent by suppressing aromatase activity in the animal.

In addition to studying the binding mechanism of known aromatase inhibitors, our laboratory has initiated an investigation on the interaction of phytoestrogens with aromatase. Phytoestrogens are plant chemicals that bind to the estrogen receptor and induce many components of estrogen action (e.g., 60-68). These compounds are thought to play a beneficial role in preventing breast cancer. They may function as antiestrogens or weak estrogens by competing with estrogens for binding to estrogen receptor (ER). However, we feel that it is also possible that some of these compounds may act in an indirect fashion by inhibiting aromatase activity, resulting in a decrease in the level of estrogen in women. This aspect has not been well studied.

Phytoestrogens such as flavones, isoflavones, and flavanones are widely present in the plant kingdom with the exception of the algae, the fungi, and the hornworts (69). These compounds are found in all parts of the higher plants: roots, stems, leaves, flowers, pollen, fruit, seeds, wood and bark. For example, chrysin (structure see figure 4) is commonly found in flowers such as the passion flower (70). The genus *Citrus* is especially rich in flavanones such as naringenin (71). Genistein (structure see figure 4) and biochanin A are known to be present in soybean extracts (72). The most significant sources of coumestrol in foods are sprouts of clover and alfalfa (73). Quercetin is present in high levels in onions (74). Adlercreutz *et al.* (75) reported that in some vegetarian women plasma genistein concentrations could be as high as 0.1 µmol/L as determined by using reverse isotope dilution gas chromatography-mass spectrometry. Adlercreutz *et al.* (76) have also estimated that the plasma level of genistein in people on a high-soy-containing diet is 1-4 µmol/L. Barnes (77) has made the following calculation. A person consuming 35 g/day of soybeans (the average amount consumed by Taiwanese) has an intake of ~ 50 µg (185 µmol) of genistein. If one assumes that genistein equilibrates with total body water (56 liters), the equilibrium plasma concentration would be 3.3 µmol/L. In a separate study, the elimination half-life of quercetin glucoside was determined to be 17 hours. A peak plasma concentration of 200 ng/ml or 0.6 µM was measured after administration of a single high dose of dietary quercetin, equivalent to 4 times the average Dutch daily intake (78). While there has not been an extensive examination of the concentrations of various phytoestrogens in our body fluids, it is estimated that the overall concentration of all flavones and isoflavones in circulation is in the micromolar range. Some flavones have been shown to inhibit aromatase in the micromolar range (79).

In a recent study (79), the inhibition profiles of four flavones [chrysin (5,7-dihydroxyflavone), 7,8-dihydroxyflavone, baicalein (5,6,7-trihydroxyflavone), galangin (3,5,7-trihydroxyflavone)], two isoflavones [genistein (4',5,7-trihydroxyisoflavone), biochanin A (5,7-dihydroxy-4'-methoxyisoflavone)], one flavanone [naringenin (4',5,7-trihydroxyflavanone)] and one naphthoflavone (? -naphthoflavone) on the wild-type and six human aromatase mutants (I133Y, P308F, D309A, T310S, I395F, and I474Y) were determined. In combination with computer modeling, the binding characteristics and the structural requirement for flavone and isoflavone phytoestrogens to inhibit human aromatase were obtained. It was found that these compounds bind to the active site of aromatase in an orientation in which their rings-A and -C mimic rings-D and -C of the androgen substrate, respectively. The study also provides a molecular basis as to why isoflavones are significantly poorer inhibitors of aromatase than flavones. Since the Women's Health Initiative is carrying out a study to test the hypothesis that a diet that is high in fruits and vegetables will lower breast cancer incidence in postmenopausal women, we anticipate that our study of phytoestrogen will generate information regarding which phytoestrogens are able to control estrogen biosynthesis. This will help to determine which fruits and vegetables, those containing proper phytoestrogens, should be included in a diet for postmenopausal women.

In addition to phytoestrogens, we have initiated a study of the interaction of xenoestrogens with aromatase. Xenoestrogens are men-made chemicals that are shown to have estrogen-like activity. Although we are at an early stage in this research, we have observed that some of these compounds can inhibit aromatase or modify aromatase expression. Therefore, it is reasonable to think that by modulating aromatase activity or expression, exposure of xenoestrogens may have an impact on breast cancer development in postmenopausal women.

The study of the involvement of aromatase in the pathogenesis and progression of breast cancer is an active field of research. Efforts are directed toward a clear understanding of the regulatory mechanism of aromatase expression in breast cancer tissue. Breast cancer prevention and treatment involving aromatase inhibitors are being carefully evaluated. The possibility of chemoprevention involving phytochemicals should not be overlooked. Estrogen is a very important hormone in women as well as in men. Aromatase research involving its expression and action in tissues other than breast has not been reviewed here. It has also been suggested that aromatase plays a role in gynecomastia in men and uterine diseases in women (35). In addition, aromatase has been studied in a number of animal species. In some species, more than one form of aromatase has been identified (80-83). Some of these isoforms of aromatase are being characterized. There is an international conference on aromatase every three years that covers various aspects of aromatase research. The IV conference was held at Lake Tahoe, California in the summer of 1996. The Proceedings of the meeting, containing forty one reports on recent developments in aromatase research, has recently been published (84).

8. ACKNOWLEDGMENTS

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Send correspondence to: Dr Shiuan Chen, Division of Immunology, Beckman Research Institute of the City of Hope, Duarte, CA 91010, Tel: (626)- 359-8111, Ext. 2601, Fax: (626)-301-8186, E-mail address, schen@ smtplink. coh.org