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MODULATION OF AROMATASE ACTIVITY AND EXPRESSION BY ENVIRONMENTAL CHEMICALS

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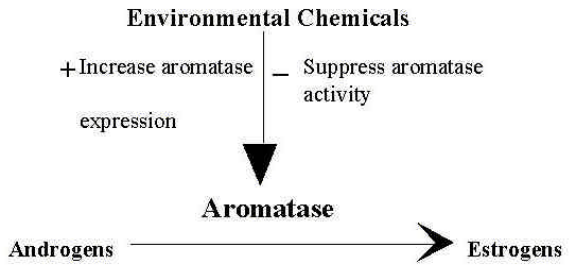
1. ABSTRACT

Aromatase is the enzyme that converts androgen to estrogen. Our laboratory has proposed and demonstrated that aromatase is an important target of some environmental chemicals. We have found that some of these compounds inhibit aromatase activity, resulting in a decrease in the level of estrogen or an increase in the level of androgen in cells. Environmental chemicals can also modify the expression of aromatase in various tissues, resulting in a change in the ratio between androgen to estrogen. The compounds that inhibit aromatase or suppress aromatase expression will behave as antiestrogens or androgen-like compounds *in vivo*. On the other hand, compounds that increase aromatase expression or enhance aromatase activity (or stability) may function as anti-androgens or estrogen-like compounds. This review will summarize and discuss findings from this and other laboratories on the effects of environmental chemicals on aromatase activity and expression.

2. INTRODUCTION

Aromatase, a cytochrome P450, catalyzes three consecutive hydroxylation reactions converting C19 androgens to aromatic C18 estrogenic steroids. Upon receiving electrons from NADPH-cytochrome P450 reductase, 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. The ovarian aromatase produces mainly estradiol which plays essential roles in many reproductive and metabolic processes. A high level of aromatase is expressed in the placenta of pregnant women. The major estrogen produced by placenta is estriol. In postmenopausal women and men, adipose tissue and skin cells are the major sources of estrogen production. However, the aromatase activity in these tissues is significantly lower than that in ovaries and the levels of circulating estrogen are much lower in men and postmenopausal women than in premenopausal and pregnant women. In men, testis, testicular germ cells, and epididymal sperm all express aromatase (1,2). In addition, during the fetal development, aromatase is expressed in the pituitary gland. The fetal expression of aromatase in the brain is believed to determine male or female metabolic patterns expressed during adult development (3). Pathologically, an abnormal over-expression of aromatase in breast tissue plays an important role in breast cancer development (4-7). Abnormal over-expression of aromatase was also found in uterine (8), testicular (9) and adrenal tumors (10,11).

Aromatase deficiency is a rare disorder and is usually caused by single base-pair changes resulting in amino acid substitution or premature stop codons (12). In most cases, the affected mother experiences virilization in



Scheme 1. Effect of environmental factors on aromatase.

he third trimester of pregnancy. Affected female newborns have pseudohemaphrodism with clitoromegaly and hypospadias. Affected male newborns are presented with tall stature secondary to failed epiphyseal fusion. Aromatase knock out (ArKO) mice have been generated by Fisher *et al.* (13), Honda *et al.* (14), and Nemoto *et al.* (15) to study the physiological importance of aromatase. Female ArKO mice are infertile as a consequence of disrupted folliculogenesis and a failure to ovulate, indicating the requirement of estrogen for normal ovarian function. Male ArKO mouse testes demonstrate arrest of spermatogenesis at the level of round spermatids and Leydig cell hyperplasia. As expected, aromatase deficiency leads to defects in bone development. The study of ArKO mice has also demonstrated the role of estrogen in supporting constitutive hepatic expression of genes involved in lipid ß-oxidation and in maintaining hepatic lipid homeostasis (15).

Both androgen and estrogen are important hormones. Not only is aromatase an essential enzyme for the synthesis of estrogen, but the expression and the activity of aromatase are tightly regulated in order to maintain the proper ratio between androgen and estrogen. As we have learned from the ArKO mouse studies, a disruption of aromatase expression and/or function has significant impacts on our health. Therefore, the potential effects of environment chemicals on the activity and the expression of aromatase should not be overlooked.

A number of environmental chemicals have been found to inhibit aromatase activity, resulting in a decrease in the level of estrogen or an increase in the level of androgen in cells (see Scheme 1). Environmental chemicals have also been found to modify the expression of aromatase in various tissues, resulting in a change in the ratio between androgen to estrogen. The compounds that inhibit aromatase or suppress aromatase expression will behave as antiestrogens or androgen-like compounds *in vivo*. On the other hand, compounds that increase aromatase expression or enhance aromatase activity (or stability) may function as anti-androgens or estrogen-like compounds. Several laboratories including ours have carried out investigations to examine the effects of environmental chemicals on aromatase activity and expression. Results from these investigations are reviewed and discussed.

3. SUPPRESSION OF AROMATASE ACTIVITY BY ENVIRONMENTAL CHEMICALS

In order to better understand the molecular basis of the inhibitory effects of environmental chemicals on aromatase activity, the structure-function relationship of human aromatase is briefly reviewed here. Aromatase was first purified to homogeneity from human placental microsomes in 1985 (16). The protein sequence information (17) was crucial for the confirmation of aromatase as a cytochrome P450 and was essential for the cloning and confirmation of human aromatase cDNA that was published between 1987 to 1989. The site-directed mutagenesis studies of aromatase 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. The molecular basis of the interaction of various inhibitors with aromatase has been discussed in detail and computer models have been reported by Kao *et al.* (18). This structural information is also useful in examining the reaction mechanism of aromatase (19). Site-directed mutagenesis experiments from our laboratory has identified a few key residues, and mutants at these positions have been found to be useful in evaluating the binding nature of anti-aromatase chemicals (20).

Phytoestrogens, such as flavones and isoflavones, are plant chemicals that bind to ER and induce many components of estrogen action. They may function as antiestrogens or weak estrogens by competing with estrogens for binding to estrogen receptor (ER). However, 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. Flavones have been demonstrated to be competitive inhibitors of aromatase with respect to the androgen substrate, with K_i values at low micromolar concentrations (21-25). The binding characteristics and the structural requirement necessary for the inhibition of human aromatase by flavone and isoflavone phytoestrogens were obtained using computer modeling and confirmed by site-directed mutagenesis (26). 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 describing why isoflavones are significantly poorer inhibitors of aromatase than flavones.

Aromatase cDNA has also been isolated from several species other than human. In some species, including pig and goldfish, more than one form of aromatase has been identified. There are three aromatase isozymes in pig: placenta, ovary, and blastocyst forms. Two isozymes, brain and ovary forms, are present in goldfish. These isozymes have different activities and different responses to known aromatase inhibitors. Since flavonoids are widespread in plants that are the major diet for goldfish, it is expected that goldfish (and other fish

populations) could be exposed to these compounds in nature. In a recent published study (27), both chrysin and 7,8-dihydroxyflavone were found to be approximately ten times more potent inhibitors of the goldfish ovary enzyme than the brain enzyme. In addition, the two compounds have similar inhibitory constants for the same isozyme. This is different from the human aromatase in that the human enzyme is four times more sensitive to chrysin than to DHF (26). These results suggest major differences, compared to the human enzyme, in the regions interacting with ring A of flavones in the active sites of these goldfish isozymes. Because the ovary isozyme has been shown to be more sensitive than the brain isozyme to environmental estrogens such as flavones, it may be possible to screen for and categorize environmental pollutants based on their selective impact on estrogen formation in goldfish ovary rather than brain. While the role of goldfish brain aromatase is not yet fully understood, these results indicate that the brain aromatase is more resistant to flavones than the ovarian aromatase. We have evaluated the effects of the same two flavones on the blastocyst and the placenta forms of pig aromatase (28). Flavones were found to be more potent inhibitors of the blastocyst enzyme than the placenta enzyme (unpublished results), implying that estrogen biosynthesis during the early phase of pregnancy is more sensitive to these environmental chemicals than the later phase of pregnancy. We feel that these results obtained from studies on goldfish and pig aromatase are very important because they indicate that environmental chemicals can have differential effects on aromatase isozymes, leading to different impacts on different organs or at different stages of the development.

The benzopyranone ring system is a molecular scaffold found in flavonoid. Brueggemeier *et al.* (29) utilize combinatorial chemistry approaches to construct small benzopyranone libraries as potential aromatase inhibitors. Several compounds in the initial libraries have demonstrated moderate aromatase inhibitory activity in screening assays.

We have developed a novel aromatase inhibitor screening method, which allows us to identify anti-aromatase activity of various environmental chemicals (30). The screen was developed by co-expressing the human aromatase and the mouse androgen receptor in yeast cells, which carry the androgen responsive ß-galactosidase reporter plasmid. Using this yeast-based assay, we have confirmed that two flavones, chrysin and α-naphtholflavone, are inhibitors of aromatase. This yeast system has allowed us to develop a high throughput screening method without using radioactive substrates to identify aromatase inhibitors as well as new ligands (non-aromatizable androgen mimics) for the androgen receptors. In addition, this screening method has also allowed us to distinguish non-androgenic aromatase inhibitors from inhibitors with androgenic activity. This yeast screening method will be very useful to screen environmental chemicals for their anti-aromatase activity and for their interaction with androgen receptor.

While it is exciting to find that isolated phytochemicals like flavones can suppress aromatase activity, it is critical to determine whether plant extracts contain sufficient amounts of phytochemicals that can inhibit aromatase activity. Our laboratory has initiated an investigation to explore whether and which fruits and vegetables contain anti-aromatase chemicals. Among the fruit group, grapes were found to contain chemicals that act as potent inhibitors of aromatase (31). The oral ingestion of grape juice was found to be able to suppress the MCF-7aro-derived tumor formation in nude mice. MCF-7aro cells are MCF-7 cells that over-express aromatase (32, 33). The nude mouse studies are very important in demonstrating the *in vivo* effects of phytochemicals. We have also demonstrated by both *in vitro* and *in vivo* experiments that red wine extract also contains potent aromatase inhibitors (34). Since white wine extract did not suppress aromatase, the active phytochemicals are thought to come from the skin and pulp of grapes. Among the vegetable group, mushrooms were found to contain chemicals that can suppress aromatase activity (35). Current efforts are devoted toward the isolation and characterization of the anti-aromatase chemicals in grapes, red wine, and mushrooms. These results indicate that some fruits and vegetables in our diet contain adequate amounts of anti-aromatase chemicals. Lee *et al.* (36) have isolated several anti-aromatase chemicals from Broussonetia papyrifera and found that the most potent chemicals (such as isolicoflavonol) suppress aromatase with IC_{50} values near 0.1 μM. Filleur *et al* (37) have isolated four lignans with anti-aromatase activity from the petrol extract of Myristica argentea mace (Myristicaceae). Weber *et al.* (38) and Lund *et al.* (39) have reported that dietary soy-phytoestrogens decrease testosterone levels, aromatase activity and prostate weight in rats.

Less is known about the anti-aromatase effects of the synthetic chemicals (i.e., xenochemicals). Drenth *et al.* (40) reported that some persistent halogenated environmental contaminants suppressed aromatase activity in the human choriocarcinoma cell line JEG-3. Hany *et al.* (41) studied the effects of developmental exposure of rats to a reconstituted PCB mixture or aroclor 1254 on aromatase activity. Saitoh *et al.* (42) reported that tributyltin and triphenyltin could inhibit aromatase activity in the human granulosa-like tumor cell line KGN at 20 ng/ml. Cooke (43) has found that these compounds are competitive inhibitors of aromatase with K_i values between 40 to 65 μM. In general, xenochemicals are weaker aromatase inhibitors than phytochemicals.

To date, there are no reports to indicate that environmental chemicals can increase aromatase activity. In our wine study (34), we have found that alcohol has a weak stimulatory effect on aromatase activity. The stimulating effect of alcohol on aromatase activity has also discussed by Purohit (44) and Dorgan *et al.* (45). The exact mechanism of the enhancement of aromatase activity by alcohol has not yet been determined. It is thought that alcohol may increase the solubility of the androgen substrate or may slightly modify the structure of aromatase.

4. MODULATION OF AROMATASE EXPRESSION BY ENVIRONMENTAL CHEMICALS

The human aromatase gene was mapped to chromosome 15, band q21.1 by *in situ* hybridization studies (46). The gene contains nine translated exons. It is known that a complex mechanism is involved in the control of human aromatase expression. At least seven untranslated exons I (I.1, I.2, I.3, I.4, I.5, I.6, and PII) have been identified. It has been found that various exon I-containing RNA messages are present at different levels in different aromatase-expressing tissues/cells. It is thought that aromatase expression in the tissues is driven by the promoters situated upstream from these exon Is, providing tissue-specific control of aromatase expression.

Our laboratory has found that promoters I.3 and II (that are mainly used in ovary and breast cancer tissue) are regulated by a responsive element, S1, which is situated between the two promoters (47). The yeast one-hybrid analysis has revealed that the proteins interacting with S1 are mainly nuclear receptors (48). One of the nuclear receptors identified is ERRα-1 (estrogen-related receptor alpha-1), which behaves as a positive regulatory element when bound to S1 (48). While estrogens are not ligands of ERRα-1, our recent results suggest that toxaphene and chlordane, two organochlorine pesticides with estrogen-like activity, behave as antagonists for this orphan nuclear receptor (49). Therefore, these compounds down-regulate aromatase expression in the ovary and breast cancer tissue by suppressing the binding of ERRα-1 to S1. Our laboratory has also found that ERα can also bind to S1 in a ligand dependent manner (50). By binding to S1, ERα suppresses the activity of promoter I.3, and this action of ERα has been interpreted as a feedback mechanism of estrogen on aromatase expression (50). Therefore, environmental chemicals that bind to ERα will modulate aromatase expression through this mechanism. The agonists of ERα suppress aromatase expression and antagonists of ERα enhance aromatase expression.

Aromatase expression in adipose tissue was found to be driven mainly by promoter I.4 that is up regulated by glucocorticoids (51). Therefore, environmental chemicals that act as the ligands of the glucocorticoid receptor would modulate aromatase expression in adipose tissue. In addition, aromatase expression in brain can be up regulated by androgen. Thus, environmental chemicals that act as the ligands of the androgen receptor would modulate aromatase expression in brain. Ligands of RAR, RXR and PPARγ have also been indicated to affect aromatase expression (52-54). Environmental chemicals that modify the activity of these receptors are expected to alter the expression level of aromatase.

The feedback mechanism *in vivo* should not be overlooked. Aromatase expression in the ovary is up regulated by peptide hormones such as FSH and LH. When the brain senses a low level of estrogen in circulation, it secretes these peptide hormones to enhance the expression of aromatase in the ovary, leading to an increase in estrogen production. On the other hand, when there is a high level of estrogen in circulation, the brain reduces the production of LH and FSH, resulting in a decrease in estrogen production in the ovary. Thus, the expression of aromatase in the ovary can be down regulated when significant levels of estrogen-like environmental chemicals are present in the circulation. In addition, steroid 5 alpha-reductase also uses testosterone as a substrate. More androgen substrates will be available for aromatase when steroid 5 alpha-reductase is suppressed. For example, female mice that lacked the type 1 5 alpha-reductase exhibited partial penetrant defects in parturition and fecundity, due to the production of an excess of estrogen due to aromatase (55). Therefore, environmental chemicals that suppress 5 alpha-reductase may lead to an increased production of estrogen because of aromatase.

Aminoglutethimide, an aromatase inhibitor, has been found to be an inducer of aromatase expression (56), and ICI 182,780, a potent antiestrogen, has been found to suppress aromatase expression (57). Saitoh *et al*. (42) have reported that tributylin, in addition to acting as an aromatase inhibitor, also suppressed aromatase expression, possibly through the promoter II region. Lovekamp and Davis (58) have found that mono-(2-ethylhexyl) phthalate can reduce aromatase transcript levels in cultured rat granulosa cells. Results from Sanderson *et al.* (59) suggest that chloro-s-triazine herbicides and metabolites can induce aromatase activity/expression in male carp hepatocytes. In addition, Shozu *et al.* (60) reported the inhibition of aromatase expression in leiomyoma of the uterus by leuprorelin acetate, a GnRH agonist that is widely used for treatment of uterine leiomyoma by down-regulation of pituitary-ovarian function. The exact modulatory mechanisms of these compounds on aromatase expression are not yet known.

Aromatase expression in some species can also be modulated by environmental temperature. This has been observed in fish (27, 61) and demonstrated in turtles (62). However, the molecular mechanism for the temperature-dependent modulation of aromatase expression has not yet been determined. It is reasonable to assume that abnormal weather changes should have an impact in the level of aromatase in some animals that lead to changes in the androgen/estrogen ratio. This may affect the composition of male vs. female population.

5. PERSPECTIVE

Environmental chemicals can modulate both aromatase activity and expression. Studies using aromatase knock out mice have demonstrated that estrogen is a very important hormone in females as well as in males. Inhibition of aromatase activity and expression by environmental chemicals will have an impact on reproductive health, bone development, and lipid metabolism in the liver. On the other hand, an over-expression of aromatase in the breast of postmenopausal women is considerated as a risk factor for breast cancer. Tekmal *et al.* (63) have produced transgenic mice that over-

express int-5/aromatase under the control of mouse mammary tumor virus enhancer/promoter. Over-expression of int-5/aromatase in mammary glands of virgin females leads to the enlargement of 40% of ducts, of which 65% had hyperplastic lesions, 20% had dysplastic lesions, and 15% had fibroadenoma lesions. Over-expression of int-5/aromatase in involuted mammary glands of transgenic females induces hyperplasia in 75-80% of ducts and glands that exhibit a range of morphological abnormalities. These results show that early exposure of mammary epithelium to *in situ* estrogen and continued exposure to *in situ* estrogen, as a result of over-expression of aromatase, appears to predispose mammary tissue to preneoplastic changes. The over production of estrogen may increase the risk of developing neoplasia and increase susceptibility to environmental carcinogens. The results further indicate that *in situ* produced estrogen plays a more important role than circulating estradiol in breast tumor promotion. The transgenic mouse studies also show that about half of the male transgenic mice overexpressing aromatase in testis were infertile and/or had larger than normal testicles (64). Gross pathology and histological analysis showed the mice to have Leydig cell tumors, unilaterally or bilaterally. These results suggest that an induction of aromatase activity and expression in men and in postmenopausal women can increase incidence of testicular cancer and breast cancer.

It is thought that unless there is an abnormally high level of exposure, the impact of environmental chemicals in premenopausal women is less significant because these women already have a high level of endogenous aromatase activity. However, environmental exposure during pregnancy could affect the fetal development. Environmental chemicals that increase aromatase activity and expression in postmenopausal women can increase their risk for breast cancer. On the other hand, chemicals that suppress aromatase activity and expression may be considered beneficial. Aromatase inhibitors have also been considered as chemopreventive agents against breast cancer by suppressing abnormal estrogen formation. 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) (65). 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, and vorozole is thought to act as a chemopreventive agent by suppressing aromatase activity in the animal. Therefore, identification of fruits and vegetables that contain anti-aromatase chemicals is considered to be very important. Diet including these types of fruits and vegetables may help protect postmenopausal women against breast cancer.

During the last several years, we and others have identified a number of environmental chemicals that can modulate aromatase activity and expression. It is critical to understand the molecular action of these chemicals as well as their potency. This information is essential to better define the effects of the chemicals. Considering the quantity of environmental chemicals, a high throughput screening method will facilitate the process to identify the chemicals that modulate aromatase activity. Our laboratory has developed a yeast screening system. However, this method still requires some refinements. New or alternative high throughput screen methods should be developed. Through our studies with fruit and vegetable extracts, we have learned that the combined effect of a mixture of chemicals cannot be overlooked. The potency of aromatase-modulating activity can be significantly increased due to the additive or synergistic effects of the mixture. The effects of the mixture should be evaluated in a systematic manner. Finally, animal experiments are essential for the *in vivo* studies of environmental chemicals. For the aromatase studies, currently there are two models: nude mouse model using aromatase-expressing cell lines and aromatase transgenic mouse model. The usage of these two animal models has been demonstrated in our studies. Additional animal models should be developed to verify the results generated with the known models.

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7. REFERENCES

1. Hess R. A, D. Bunick & J. M. Bahr: Sperm, a source of estrogen. *Environ Health Perspect* 103 (suppl. 7), 59-62 (1995)

2. Janulis L, J.M. Bahr, R. A. Hess, S. Hanssen, T. Osawa & D. Bunick: Rat testicular germ cells and epididymal sperm contain active P450 aromatase. *J. Androl* 19, 65-71 (1998)

3. MacLusky N. J, A. S. Clark, F. Naftolin & P. S. Goldman-Rakic: Estrogen formation in the mammalian brain: Possible role of aromatase in Sexual differentiation of the hippocampus and neocortex. *Steroids* 50, 459-474 (1987)

4. Esteban J, Z. Warsi, M. Haniu, P. Hall, J. E. Shively & S. Chen: Detection of intratumoral aromatase in breast carcinomas. An immunohistochemical study with clinicopathologic correlation. *Am J Pathol* 140, 337-343 (1992)

5. Bulun S. E, T. M. Price, M. S. Mahendroo, J. Aitken & E. R. Simpson: A link between breast cancer and local estrogen biosynthesis suggested by quantification of breast adipose tissue aromatase cytochrome P450 transcripts using competitive polymerase chain reaction after reverse transcription. *J Clin Endocrinol Metab* 77, 1622-1628 (1993)

6. Lu Q, J. Nakmura, A. Savinov, W. Yue, J. Weisz, D. J. Dabbs, G. Wolz & A. Brodie: Expression of aromatase protein and messenger ribonucleic acid in tumor epithelial cells and evidence of functional significance of locally produced estrogen in human breast cancers. *Endocrinology* 137, 3061-3068 (1996)
7. Harada N: Aberrant expression of aromatase in breast cancer tissues. *J Ster Biochem Mol Biol* 77, 1622-1628 (1997)
8. Sumitani H, M. Shozu, T. Segawa, K. Murakami, H. J. Yang, K. Shimada & M. Inoue: *In situ* estrogen synthesized by aromatase P450 in uterine leiomyoma cells promotes cell growth probably via an autocrine/intracrine mechanism. *Endocrinology* 141, 3852-3861 (2000)
9. Inkster S, W. Yue & A. Brodie: Human testicular aromatase: immunocytochemical and biochemical studies. *J Clin Endocrinol Metab* 80, 1941-1947 (1995)
10. Young J, S. E. Bulun, V. Agarwal, B. Couzinet, C. R. Medelson, E. R. Simpson & G. Schaison: Aromatase expression in a feminizing adrenocortical tumor. *J Clin Endocrinol Metab* 81, 3173-3176 (1996)
11. Phornphutkul C, T. Okubo, K. Wu, Z. Harel, T. F. Tracy, H. Pinar, S. Chen, P. A. Gruppuso & G. Goodwin: (2001), Aromatase P450 expression in a feminizing adrenal adenoma presenting as isosexual precocious puberty. *J Clin Endocrinol Metab* 86, 649-652 (2001)
12. Simpson E. R: Genetic mutations resulting in loss of aromatase activity in humans and mice. *J Soc Gynecol Investigation* 7, S18-S21 (2000)
13. Fisher C. R, K. H. Graves, A. F. Parlow & E. R. Simpson: Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. *Proc Natl Acad Sci USA*, 95, 6965-6970 (1998)
14. Honda S, H. Harada, S. Ito, Y. Takagi & S. Maeda: Disruption of sexual behavior in male aromatase-deficient mice lacking exons 1 and 2 of the cyp19 gene. *Biochem Biophys Res Commun* 252, 445-449 (1998)
15. Nemoto Y, K. Toda, M. Ono, K. Fujikawa-Adachi, T. Saibara, S. Onishi, H. Enzan, T. Okada & Y. Shizuta: Altered expression of fatty acid-metabolizing enzymes in aromatase-deficient mice. *J Clin Investigation* 105, 1819-1825 (2000)
16. Nakajin S, M. Shinoda & P. F. Hall: Purification to homogeneity of aromatase from human placenta. *Biochem Biophys Res Commun* 134, 704-710 (1986)
17. Chen S, J. E. Shively, S. Nakajin, M. Shinoda & P. F. Hall: Amino terminal sequence analysis of human placenta aromatase. *Biochem Biophys Res Commun* 135, 713-719 (1986)
18. Kao Y. C, L. L. Cam, C. A. Laughton, D. Zhou & S. Chen: Binding characteristics of seven aromatase inhibitors to human aromatase. A site-directed mutagenesis study. *Cancer Res* 36, 3451-3460 (1996)
19. Kao Y. C, K. R. Korzekwa, C. A. Laughton & S. Chen: Evaluation of the mechanism of aromatase cytochrome P450: A site-direct mutagenesis study. *Eur J Biochem* 268, 243-251 (2001)
20. Chen S, Y. C. Kao & C. A. Laughton: Binding characteristics of aromatase inhibitors and phytoestrogens to human aromatase. *J Steroid Biochem Mol Biol* 61,107-115 (1997)
21. Kellis J. T & L. E. Vickery: Inhibition of estrogen synthetase (aromatase) by flavones. *Science* 225, 1032-1034 (1984)
22. Ibrahim A. R & Y. J. Abul-Hajj: Aromatase inhibition by flavonoids. *J Steroid Biochem Mol Biol* 37, 257-260 (1990)
23. Adlercreutz H, C. Bannwart, K. Wahala, T. Makela, G. Brunow, T. Hase, P. J. Arosemena, J. T. Kellis & L. E. Vickery: Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J Steroid Biochem Mol Biol* 44, 147-153 (1993)
24. Campbell D. R & M. S. Kurzer: Flavonoid inhibition of aromatase enzyme activity in human preadipocytes. *J Steroid Biochem Mol Biol* 46, 381-388 (1993)
25. Wang C, T. Makela, T. Hase, H. Adlercreutz & M. S. Kurzer: Lignans and flavonoids inhibit aromatase enzyme in human preadipocytes. *J Sterold Biochem Mol Biol* 50, 205-212 (1994)
26. Kao Y. C, C. Zhou, M. Sherman, C. A. Laughton & S. Chen: Molecular basis of the inhibition of human aromatase by flavone and isoflavone phytoestrogens. A site-directed mutagenesis study. *Environmental Health Perspectives* 106, 85-92 (1998)
27. Zhao J, P. MaK, A. Tchoudakova, G. Callard & S. Chen: Different Catalytic Properties and Inhibitor Responses of the Goldfish Brain and Ovary Aromatase Isozymes. *General and Comparative Endocrinology* 123, 180-191 (2001)
28. Kao Y. C, T. Higashiyama, X. Sun, C. Yarborough, I. Choi, Y. Osawa, F. A. Simmen & S. Chen: Functional characterization of porcine blastocyst and placental aromatase isoforms. *Eur J Biochem* 267, 6134-6139 (2000)
29. Brueggemeier R. W, X. Gu, J. A. Mobley, S. Joomprabutra, A. S. Bhat & J. L. Whetstone: Effects of phytoestrogens and synthetic combinatorial libraries on aromatase, estrogen biosynthesis, and metabolism. *Ann N Y Acad Sci* 948, 51-66 (2001)
30. Mak P, F. Dela Cruz & S. Chen: A yeast screen system for aromatase inhibitors and ligands for androgen receptor: yeast cells transformed with aromatase and androgen receptor. *Environmental Health Perspectives*, 107, 855-860 (1999)
31. Chen S, X. Z. Sun, Y. C. Kao, A. Kwon, D. Zhou & E. Eng: Suppression of breast cancer cell growth with grape juice. *Pharmaceutical Biol* 36, 53-61 (1998)
32. Zhou D, D. Pompon & S. Chen: Stable expression of human aromatase cDNA in mammalian cells - A useful system for aromatase inhibitor screening. *Cancer Research*, 50, 6949-6954 (1990)
33. Sun X. Z, D. Zhou & S. Chen: Autocrine and paracrine actions of breast tumor aromatase. A three-dimensional cell culture study involving aromatase transfected MCF-7 and T-47D cells. *J Steroid Biochem Mol Biol* 63, 29-36 (1997)
34. Eng E. T, D. Williams, U. Mandava, N. Kirma, R. R. Tekmal & S. Chen: Suppression of aromatase (estrogen synthetase) by red wine phytochemicals. *Breast Cancer Research and Treatment* 67, 133-146 (2001)
35. Grube B. J, E. T. Eng, Y. C. Kao, A. Kwon & S. Chen: White button mushroom phytochemicals inhibit aromatase activity and breast cancer cell proliferation. *J Nutrition* 131, 3288-3293 (2001)
36. Lee D, K. P. Bhat, H. H. Fong, N. R. Farnsworth, J. M. Pezzuto & A. D. Kinghorn: Aromatase inhibitors from Broussonetia papyrifera. *J Nat Prod* 64, 1286-1293 (2001)

37. Filleur F, J. C. Le Bail, J. L. Durous , A. Simon & A. J. Chulia: Antiproliferative, anti-aromatase, anti-17beta-HSD and antioxidant activities of lignans isolated from Myristica argentea. *Planta Med* 67, 700-704 (2001)
38. Weber K. S, K. D. Setchell, D. M. Stocco & E. D. Lephart: Dietary soy-phytoestrogens decrease testosterone levels and prostate weight without altering LH, prostate 5 alpha-reductase or testicular steroidogenic acute regulatory peptide levels in adult male Sprague-Dawley rats. *J Endocrinol* 170, 591-599 (2001)
39. Lund T. D, R. W. Rhees, K. D. Setchell & E. D. Lephart: Altered sexually dimorphic nucleus of the preoptic area (SDN-POA) volume in adult Long-Evans rats by dietary soy phytoestrogens. *Brain Res* 914, 92-99 (2001)
40. Drenth H. J, C. A. Bouwman, W. Seinen & M. Van den Berg: Effects of some persistent halogenated environmental contaminants on aromatase (CYP 19) activity in the human choriocarcinoma cell line JEG-3. *Toxicol Appl Pharmacol* 148, 50-55 (1998)
41. Hany J, H.Lilienthal, A. Sarasin, A. Roth-Harer, A. Fastabend, L. Dunemann, W. Lichtensteiger & G. Winneke: Developmental exposure of rats to a recnstituted PCB mixture or arclor 1254: effects on organ weights, aromatase activity, sex hormone levels, and sweet preference behavior. *Toxicol Appl Pharmacol* 158, 231-243 (1999)
42. Saitoh M, T. Yanase, H. Morinaga, M. Tanabe, Y. M. Mu, Y. Nishi, M. Nomura, T. Okabe, K. Goto, R. Takayanagi & H. Nawata: Tributyltin or triphenyltin inhibits aromatase activity in the human granulosa-like tumor cell line KGN. *Biochem Biophys Res Commun* 289, 198-204 (2001)
43. Cooke G. M: Effect of organotins on human aromatase activity *in vitro*. *Toxicol Lett* 126, 121-130 (2002)
44. Purohit V: Can alcohol promote aromatization of androgens to estrogens? A review. *Alcohol* 22, 123-127 (2000)
45. Dorgan J. F, D. J. Baer, P. S. Albert, J. T. Judd, E. D. Brown, D. K. Corle, W. S. Campbell, T. J. Hartman, A. A. Tejpar, B. A. Clevidence, C. A. Giffen, D. W. Chandler, F. Z. Stanczyk & P. R. Taylor: Serum hormones and the alcohol-breast cancer association I postmenopausal women. *J Natl Cancer Inst* 93, 710-715 (2001)
46. Chen S, M. J. Besman, R. S. Sparkes, S. Zollman, I. Kilasak, T. Mohandas, P. F. Hall & J. E. Shively: Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. *DNA* 7, 27-38 (1988)
47. Zhou D & S. Chen: Characterization of a silencer element in the human aromatase gene. *Arch Biochem Biophys* 353, 213-220 (1998)
48. Yang C, D. Zhou & S. Chen: Modulation of aromatase expression in the breast tissue by ERR? -1 orphan receptor. *Cancer Res* 58, 5695–5700 (1998)
49. Yang C & S. Chen: Two organochlorine pestides, toxaphene and chlordane, are antagonists for ERR? -1 orphan receptor. *Cancer Res* 59, 4519-4524 (1999)
50. Yang C, B. Yu, D. Zhou & S. Chen: Regulation of aromatase promoter activity in human breast tissue by nuclear receptors EAR-2, COUP-TF1 (EAR-3), and RAR? . *Oncogene* 21, 2854-2863 (2002)
51. Mahendroo M. S, C. R. Mendelson & E. R. Simpson: Tissue-specific and hormonally controlled alternative promoters regulate aromatase cytochrome P450 gene expression in human adipose tissue. *J Biol Chem* 268, 19463-19470 (1993)
52. Mu Y, T. Yanase, Y. Nishi, N. Hirase, K. Goto, R. Takayanagi & H. Nawata: A nuclear receptor system constituted by RAR and RXR induces aromatase activity in MCF-7 human breast cancer cells. *Mol Cell Endocrinol* 166, 137-145 (2000)
53. Ciolino H. P, T. T. Wang & N. Sathyamoorthy: Inhibition of aromatase activity and expression in MCF-7 cells by the chemopreventive retinoid N-(4-hydroxy-phenyl)-retinamide. *Br J Cancer* 83, 333-337 (2000)
54. Rubin G. L, Y. Zhao, A. M. Kalus & E. R. Simpson: Peroxisome proliferator-activated receptor gamma ligands inhibit estrogen biosynthesis in human breast adipose tissue: possible implication for breast cancer therapy. *Cancer Res* 60, 1604-1608 (2000)
55. Mahendroo M. S, K. M. Cala, C. P. Landrum & D. W. Russell: Fetal death in mice lacking 5 alpha-reductase type 1 caused by estrogen excess. *Mol Endocrinol* 11, 917-927 (1997)
56. Kao Y. C, T. Okubo, X. Z. Sun & S. Chen: An induction of aromatase expression by aminoglutethimide, an aromatase inhibitor that is used to treat breast cancer in postmenopausal women. *Anti-Cancer Res* 19, 2049-2056 (1999)
57. Chen S, D. Zhou, C. Yang, T. Okubo, Y. Kinoshita, B. Yu, Y. C. Kao & T. Itoh: Modulation of aromatase expression in human breast tissue. *J. Steroid Biochem Mole Biol* 79, 35-40 (2001)
58. Lovekamp T. N & B. J. Davis: Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells. *Toxicol Appl Pharmacol* 172, 217-224 (2001)
59. Sanderson J. T, R. J. Letcher, M. Heneweer, J. P. Giesy & M. van Den Berg: Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. *Environ Health Perspect* 109, 1027-1031 (2001)
60. Shozu M, H. Sumitani, T. Segawa, H. J. Yang, K. Murakami & M. Inoue: Inhibition of *in situ* expression of aromatase p450 in leiomyoma of the uterus by leuprorelin acetate. *J Clin Endocrinol Metab* 86, 5405-5411 (2001)
61. Baroiller J. F & H. D'Cotta: Environment and sex determination in farmed fish. *Comp Biochem Physiol C Toxicol Pharmacol* 130, 399-409 (2001)
62. Place A. R, J. Lang, S. Gavasso & P. Jeyasuria: Expression of P450(arom) in Malaclemys terrapin and Chelydra serpentina: a tale of two sites. *J Exp Zool* 290, 673-690 (2001)
63. Tekmal R. R, N. Ramachandra, S. Gubba, V. R. Durgam, K. Toda, Y. Shizuta & D. Dillehay: Overexpression of int-5.aromatase in the mammary glands of transgenic mice results in the induction of hyperplasia and nuclear abnormalities. *Cancer Res* 56, 3180-3185 (1996)
64. Fowler K, K. Gill, N. Kirma, D. Dillehay & R. R. Tekmal: Overespression of aromatase leads to development of testicular leydig cell tumors: An animal model for hormone-mediated testicular cancer. *Am J Pathol* 156, 347-353 (2000)
65. Lubet R, V. E. Steele, R. DeCoster, C. Bowden, M. M. Juliana, I. Eto, G. J. Kelloff & C. J. Grubbs: Chemopreventive effects of the aromatase inhibitor

vorozole (R 83842) in the methylnitrosourea-induced mammary cancer model. *Carcinogenesis* 19, 1345-1351 (1998)

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