Chapter 2
Structure, Regulation and Polymorphisms of the Aromatase Gene

Nobuhiro Harada

Abstract Human aromatase is widely expressed in various tissues and shows complicated regulation by both inductive and suppressive factors. The aromatase gene has the unique characteristic of having multiple exons available for use as exon 1, which are flanked with unique promoters. Tissue-specific expression of aromatase is regulated by alternative use of these exons. The exon 1 termed exon I.4 (1b) is the one that is mainly used in breast tissues. However, during cancer development it is often switched from exon I.4 (1b) to exon I.3 (1c) or exon PII (1d), which causes enhancement of aromatase expression in cancer-associated adipocytes and fibroblasts. The aromatase gene is further regulated at both the transcriptional and post-transcriptional levels through PKA-, PKC-, and tyrosine kinase receptor-mediated signaling pathways that employ prostaglandin E_2 and class 1 cytokines. Epigenetic modifications of the aromatase gene and microRNA-mediated aromatase regulation may play a critical role in breast cancer progression. Several genetic polymorphisms in the aromatase gene may be prognostic factors of disease and may influence response to aromatase inhibitors.

Abbreviations

AP-1	Acivator protein-1
ATF-2	Activating transcription factor-2
CAA	Carcinoma-associated adipocytes
CAF	Carcinoma-associated fibroblasts
C/EBP	CCAAT/enhancer binding protein
CRE	cAMP-responsive element

N. Harada (✉)
Department of Biochemistry, School of Medicine, Fujita Health University, Toyoake, Aichi 470-1192, Japan
e-mail: nharada@fujita-hu.ac.jp

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A. Larionov (ed.), *Resistance to Aromatase Inhibitors in Breast Cancer*, Resistance to Targeted Anti-Cancer Therapeutics 8,
DOI 10.1007/978-3-319-17972-8_2

CREB	CRE binding protein
E2	17β-estradiol
ERK	Extracellular signal-regulated kinase
GAS	γ-interferon activation site
GPER	G protein-coupled estrogen receptor
GRE	Glucocorticoid responsive element
IHC	Immunohistochemical staining
LRH-1	Liver receptor homologue-1
MAPK	Mitogen-activated protein kinase
PGE_2	Prostaglandin E_2
PI3K	Phosphoinositide 3-kinase
PKA	Protein kinase A
PKC	Protein kinase C
PPAR	Peroxisome proliferator-activated receptor
RT-PCR	Reverse transcriptase-polymerase chain reaction
SF-1	Steroidogenic factor-1
SNP	Single nucleotide polymorphism
Sp1	Specificity protein 1

Introduction: Tissue-Specific Expression of Human Aromatase

Aromatase (estrogen synthase, cyp19a gene product) is a unique member of the cytochrome P450 superfamily. This enzyme is a terminal component of the electron transport system in the endoplasmic reticulum, accepts electrons from NADPH via NADPH-cytochrome P450 reductase, and catalyzes aromatization of androgens to estrogens by three successive hydroxylations and elimination of the carbon atom in position 19 of androgens, which is a rate-limiting step in estrogen biosynthesis [1]. Initially, high aromatase enzyme activity was reported to be localized in ovary and placenta, and to participate in female reproductive functions through the production of estrogens. However, later new sensitive assays using high-performance liquid chromatography with tandem mass spectrometry (LC/MS/MS), reverse transcriptase-polymerase chain reaction (RT-PCR), and immunocytochemical staining have shown that aromatase is expressed not only in female gonadal tissues but also in male gonadal tissues such as the testis and epididymis, as well as in extra-gonadal tissues such as the prostate, brain, liver, skin, adrenal gland, hair follicles, and adipose, bone, and vascular tissues [2–5]. While it is well known that aromatase expression can be found in estrogen-dependent breast cancer tissues, its expression has also been observed in endometrial carcinoma and in liver, gastric, pancreatic, colorectal, lung, ovarian, and prostatic cancers [6, 7].

There is a complicated regulation of aromatase in a tissue-specific manner by both inductive and suppressive factors. Thus, the expression of aromatase in placenta-derived cells is induced by phorbol esters [8] and suppressed by insulin [9], whereas its expression in the ovary is antithetically induced by insulin [10] and suppressed by phorbol ester [11]. Similarly, dexamethasone is an inducer of aromatase in the skin or adipose tissue [12], whereas it is a suppressor of aromatase in the ovary [13]. Aromatase is induced by the gonadotropin (e.g., FSH, LH, or hCG)-initiated cyclic AMP (cAMP)-protein kinase A (PKA) intracellular signaling system in many tissues except for the brain, in which aromatase is known to be mainly induced by androgens [14]. These observations indicate that aromatase expression is strictly regulated by tissue-specific regulatory factors, supporting the concept of intracrinology through local production of estrogens by aromatase in multiple tissues.

Structure of the Human Aromatase Gene

Analysis of aromatase transcripts in various human tissues indicated the presence of tissue-specific unique nucleotide sequences at the 5′-ends of mRNA, following which there is a common translated nucleotide sequence that encodes 503 amino acids, indicating that aromatase proteins have the same amino acid sequence in all tissues. Interestingly, aromatase transcripts are transcribed from different positions on the human aromatase gene in a tissue-dependent manner [2]. Aromatase gene clones were subsequently isolated from a human genomic DNA library. The human aromatase gene (*cyp19a1*) is present in the haploid genome as a single copy and spans about 123 kb at the 15q21.1 region of chromosome 15 [15]. Analysis of the exon-intron organization of the gene showed that all of the tissue transcripts are composed of 10 exons (Fig. 2.1). The unique sequences that are observed in the 5′-ends of the transcripts are scattered over approximately 100 kb

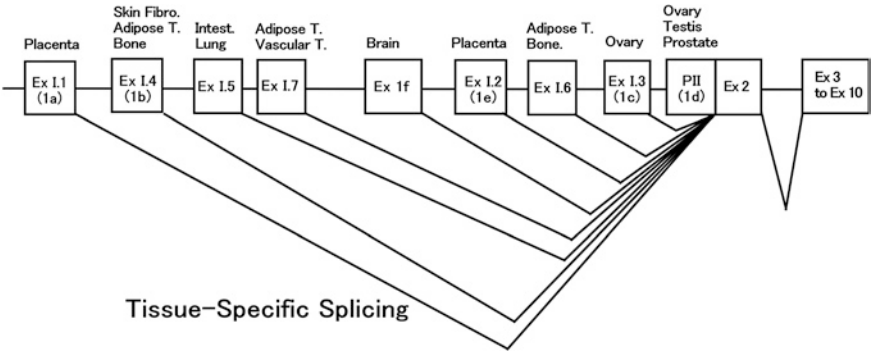

Fig. 2.1 Gene structure of the human aromatase. Multiple exons 1, encoding the only 5′-untranslational region of the human aromatase gene are tissue-specifically spliced and connected to exon (Ex) 2

upstream of exon 2, whereas the translated coding sequence was identified as exons 2-10 that lie within about 35 kb of the 3′-end of the gene [1, 2]. This gene organization suggests that the human aromatase gene includes multiple promoters and that the aromatase transcript is tissue-specifically spliced from the multiple alternative exons available for exon 1 (Fig. 2.1).

To date, nine unique alternative versions for exon 1 have been isolated (Fig. 2.1). Each exon 1 is used in a tissue-specific manner; I.1 (1a) and I.2 (1e) in the placenta, I.3 (1c) and PII (1d) in the ovary and testis, I.4 (1b) in adipose tissue, I.5 in the fetal lung and intestine, I.6 in adipose and bone tissues, I.7 in adipose and vascular endothelial tissues, and 1f in the brain [1, 16]. This selective utilization of these exons is possible due to tissue-specific promoters that flank each exon 1. The promoter structures of the exons that are predominantly used as exon 1 in the major estrogen-producing tissues are shown in Fig. 2.2 [17]. The 5′-upstream promoter regions of these exons have binding sites for diverse tissue-specific regulatory factors such as glial cell missing 1a (GCM 1a), activator protein-2γ (AP-2γ), LIM homeodomain box-2 (Lhx-2), and apolipoprotein regulatory protein-1 (ARP-1)/chicken ovalbumin upstream promoter-transcription factor II (COUP-TFII). These promoter regions also contain regulatory elements such as the glucocorticoid responsive element (GRE), γ-interferon activation site (GAS), activator protein-1 (AP-1) and cAMP-responsive element (CRE), steroidogenic factor-1 (SF-1) binding sites, as well as basic transcription elements such as a TATA box, a CAAT box, and a GC box. Exons I.1 (1a) and 1f contain

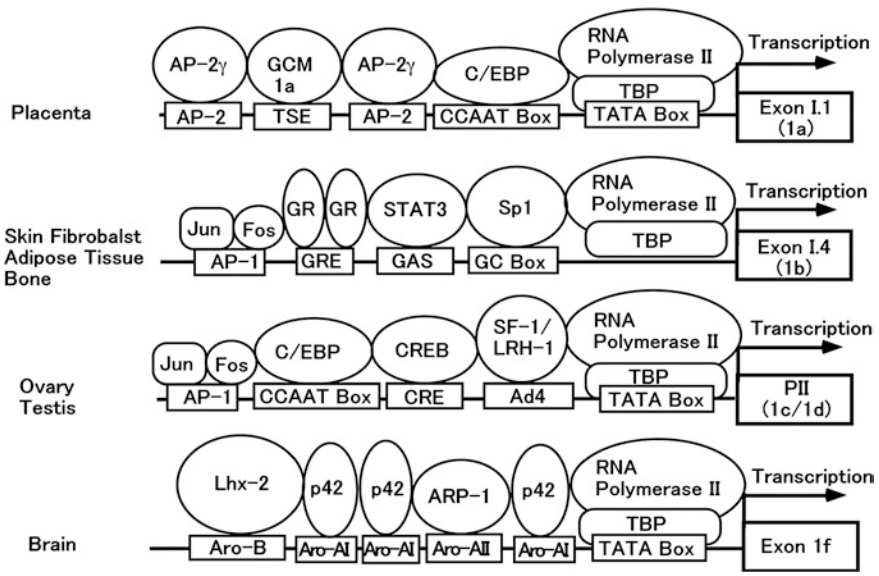

Fig. 2.2 Schematic promoter structures of exons I.1 (1a), I.4 (1b), PII (1c/1d), and 1f of the human aromatase gene. Factors are shown as *ovals* and cis-acting DNA elements as *boxes*. Details are in the text

binding sites for the trophoblast (placenta)-specific transcription factors GCM 1a and AP-2γ [18] and for the neuron-specific and/or developmental stage-specific transcription factors Lhx-2 and ARP-1/COUP-TFII [19], respectively, which illustrate remarkable differences in tissue-specific transcription regulation in the placenta and brain, respectively. Most of the exons that encode an exon 1, except for exons I.4 (1b) and I.7, have a TATA box in the proximal promoter region and form stable transcription pre-initiation complexes mediated by binding of TBP (TATA binding protein) to the TATA box. However, instead of a TATA box in the proximal promoter regions, exon I.4 (1b) and exon I.7 have a GC box, which is a specificity protein 1 (Sp1) binding site and is often found in housekeeping genes, and a GATA box, which is the binding site of a hematopoietic transcription factor, respectively [20]. Transcription from exon I.4 (1b) seems to be somewhat unstable, judging from the several transcriptional initiation sites observed in the transcripts. Switching of exon I.4 (1b) that is used in these transcripts to exon I.2 (1c)/PII (1d) is frequently observed when the tissues are exposed to cancerous or inflammatory conditions [2, 21]. This switching may be possibly explained by the relative instability of the transcription initiation complex on exon I.4 (1b). Of note, exon I.2 (1c)/PII (1d), which is predominantly used in gonadal tissues, has both CRE and AP-1 sites in the proximal promoter region for the binding of CRE binding protein (CREB)/activating transcription factor-2 (ATF-2) and c-Fos/c-Jun, respectively, suggesting that the aromatase in gonadal tissues is transcriptionally induced by cAMP/PKA and diacylglycerol (DAG)/protein kinase C (PKC) intracellular signals derived from hormones such as FSH and LH. This regulation may also explain the dynamic changes in aromatase expression in the ovary that are a response to the reproductive cycle. Transcriptional factors interacting with alternative promoters of aromatase gene in breast cancer will be discussed in more details this chapter.

Expression of Aromatase in Breast Cancer Stroma

Healthy breast tissues express low baseline levels of aromatase transcripts from exon I.4 (1b) under non-stimulated conditions. However, once the breast tissues tend towards carcinogenesis, in many cases transcription switches from using exon I.4 (1b) to using exon I.2 (1c)/PII (1d) [21], and levels of aromatase mRNA and catalytic activity are significantly increased in adjacent adipose tissue [22].

Aromatase has been reported to be localized in stromal spindle cells as well as in tumor epithelial cells of breast cancer tissues based on immunohistochemical staining (IHC) [6, 23]. High levels of aromatase mRNA expression have also been detected by a combination of RT-PCR analysis and laser capture microdissection of stromal cells, which supports previous IHC studies that stromal cells display the highest positivity for aromatase in many breast cancer cases [24]. Because cancer cells at the early stage of cancer development are usually surrounded by a large number of stromal cells, estrogen production by aromatase in

the stromal cells could play an important role in proliferation of the cancer cells. It has been suggested that the interaction between malignant epithelial cells and the stromal cells or inflammatory cells in breast cancer is important for tumor proliferation and progression [25]. In particular, invasive malignant tumor cells interact with surrounding adipocytes (carcinoma-associated adipocytes; CAA), fibroblasts (carcinoma-associated fibroblasts; CAF), and inflammatory cells, and induce a desmoplastic reaction in the surrounding stroma that is accompanied by breast cancer progression [26]. These dense fibrous or connective tissues that are formed in the desmoplastic reaction secrete various kinds of cytokines or bioactive substances, leading to induction of aromatase and consequently progression of the breast cancer by enhanced production of local estrogens [27]. Indeed, high-frequency switching from exon I.4 (1b) to exon I.2 (1c)/PII (1d) and enhanced expression of aromatase mRNA are often observed in invasive scirrhous cancers [21]. Fibroblast-like mesenchymal preadipocytes have been reported to be able to express high amounts of aromatase and this ability disappears upon differentiation into mature adipocytes [28]. In the desmoplastic reaction, cytokines such as TNFα and IL-11 that are secreted from the cancer cells promote the formation of fibrous cells thereby suppressing adipocyte differentiation in the stroma and causing elevation of aromatase expression in CAFs (Fig. 2.3) [29]. The suppressive effect of TNFα and IL-11 is believed to be mediated by selective down-regulation of the CCAAT/enhancer binding protein α (C/EBPα) and peroxisome proliferator-activated receptor γ (PPARγ), which are essential factors for adipocyte differentiation. Consequently the large quantities of estrogens produced by the elevated aromatase

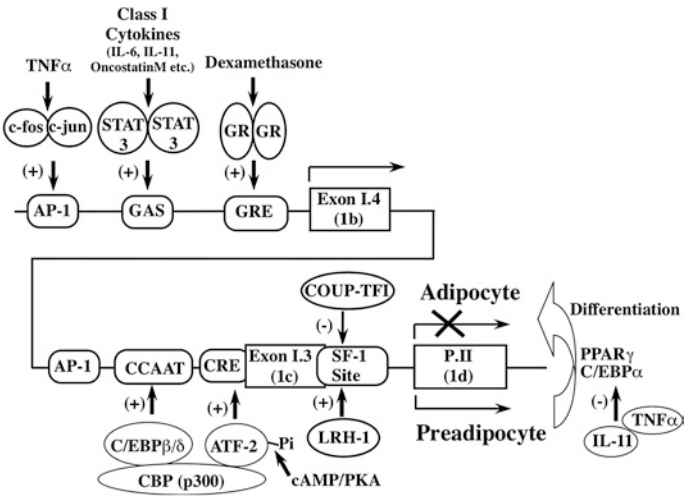

Fig. 2.3 Proposed regulation of aromatase gene expression in breast cancer tissue. Stimulatory (+) and inhibitory (−) transcription factors are differentially bound to promoter regions of exons I.4 (1b), I.3 (1c) and promoter II (1d) of the human aromatase gene in breast cancer tissue. Anti-adipogenic cytokines TNFα and IL-11 play an important role in E2 production through inhibitory effects on the differentiation of adipose tissue

in the CAFs promote the proliferation of estrogen-dependent cancer cells and, at the same time, act on the cancer cells to induce transcriptional activation of the *IL-11* gene, which ultimately forms a positive feedback and acts on CAFs and pre-adipocytes to increase TNFα receptor (TNFR1) mRNA [29].

It was previously suggested that the G protein-coupled receptor 30 (GRP30) acts as a membrane-bound mediator to interpret rapid non-genomic actions of estrogens. This receptor, also called the G protein-coupled estrogen receptor (GPER), was shown to be localized in the endoplasmic reticulum of CAFs by IHC and to increase expression of the CAF aromatase through the EGF receptor (EGFR)/extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathway that is activated by estrogens, tamoxifen and the GPER agonist G1, thereby promoting CAF proliferation and cell-cycle progression (Fig. 2.4) [30]. These findings suggest that GPER/EGFR/ERK signaling may be involved in the progression of endocrine-resistant breast cancer cells by elevation of aromatase expression and estrogen production.

Aromatase expression is locally increased in stromal cells proximal to breast cancer cells by their switching of transcription from exon I.4 (1b) to one of the alternative exons I.2 (1c)/PII (1d), whereas aromatase is expressed at normal levels in distal stroma, which cells display a low switching frequency [21]. This finding may indicate that cytokines or bioactive substances that are secreted into the region proximal to the breast cancer as a result of cancer–stroma interactions contribute to exon 1 switching. Indeed, low levels of aromatase transcripts from exon I.4 (1b) were observed in isolated cultured breast stromal cells, whereas increased levels of aromatase transcripts from exon I.2 (1c)/PII (1d) were often observed in co-culture of stromal cells with cancer cells [31]. Because this increase was reproduced by replacing the cancer cells with their culture supernatant, the participation

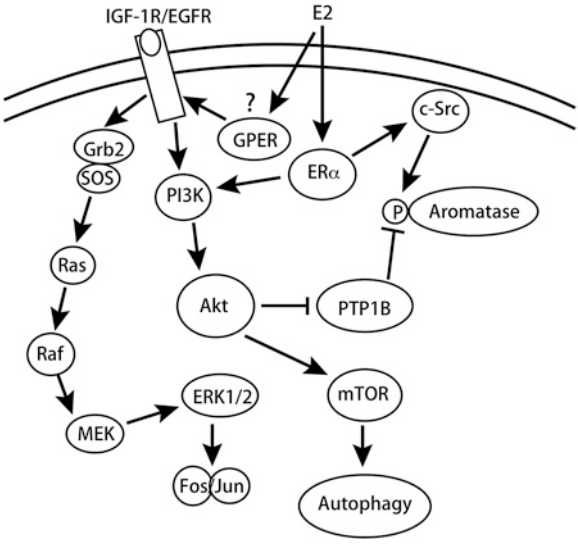

Fig. 2.4 Proposed model of potential signaling pathways from IGF-1 receptor/EGF receptor and E2 which modulate cell proliferation and aromatase activity in breast cancer cells

of humoral factor(s) secreted from the cancer cells in this exon switching and aromatase transcriptional activation was suggested. Simpson et al. reported that stromal aromatase in breast cancer tissues is induced by TNFα, ceramide, prostaglandin E_2 (PGE_2), and class 1 cytokines such as IL-6, IL-11, Leukemia inhibitory factor (LIF) and Oncostatin M (Fig. 2.3) [32, 33]. In other experiments, Yamaguchi et al. [25] evaluated the transactivation of an estrogen-estrogen receptor (ER) complex resulting from cancer-stromal cell interactions in the cancer microenvironment by using a reporter with an estrogen response element that drove expression of the green fluorescent protein gene (ERE-GFP reporter). When human breast cancer-derived MCF-7 cells that were stably transformed with this ERE-GFP reporter were co-cultured with stromal cells isolated from 67 different breast cancer patients, transactivation of the reporter gene in MCF-7 cells was observed to varying extents, depending on the case. All of these observations are consistent with the concept of a positive feedback loop of aromatase induction in stromal cells by cancer cell-derived humoral factors and of estrogen-dependent proliferation in cancer cells by cancer-stromal interaction.

Transcriptional Regulation of the Aromatase Gene in Breast Cancer Tissues

In breast cancer tissues, of the multiple exons that are available for exon 1, the aromatase gene is mainly transcribed from exons I.3 (1c), I.4 (1b), I.7, and PII (1d), and this transcription is controlled by transcription factors that interact with regulatory elements on the exon 1 flanking promoter regions (Fig. 2.2) [34]. The GRE, GAS, and AP-1 sites on the proximal promoter region of exon I.4 (1b) are predominantly used in non-malignant breast tissues. Zhao et al. [35] reported the induction of transcriptional activation from exon I.4 (1b) in breast adipose stromal cells by class 1 cytokines that were secreted by breast cancer cells or by lymphoid cells/macrophages (Fig. 2.3). These cytokines bound to gp130 cytokine receptors on the stromal cells resulting in the phosphorylation of Signal Transducer and Activator of Transcription 3 (STAT3) through activation of tyrosine kinases (JAK1/JAK2/TYK2) of the Janus activation kinase (JAK) family. STAT 3 subsequently dimerized and caused transcriptional activation by binding to the GAS site on the promoter. The aromatase in adipose stromal cells has also been reported to be induced by TNFα and its intracellular downstream factor, ceramide. Since TNFα is secreted by mature adipocytes, this finding indicates the participation of a type of adipokine in the induction of aromatase in breast tissues [33]. Aromatase induction by TNFα is considered to involve the mitogen-activated protein kinase (MAPK) signalling pathway, because it is inhibited by the p38 MAPK inhibitor, SB203580. As there is an incomplete AP-1 site in the promoter upstream of GAS, TNFα is considered to promote the binding of c-Fos and c-Jun to this AP-1 site through activation of MAPK signaling.

Frequent switching of the aromatase exon 1 that is used in breast cancer tissues from the dominant exon I.4 (1b) to exon I.3 (1c)/PII (1d) has been observed, concomitant with increased expression of aromatase [21]. Because the promoter regions of exon I.2 (1c) and PII (1d) partially overlap, there is similar transcriptional regulation of these two exons. They both share an AP-1 and a CRE that is present on the common region. For this reason, transcription from both of these exons is commonly regulated by various inducible factors in gonadal tissues. As shown in Fig. 2.3, and proposed by Simpson et al., transcription from exon I.3 (1c)/PII (1d) is suppressed in normal breast tissues by binding of the transcriptional suppressor, COUP-TFI, to an SF-1 binding site on the promoter, whereas surprisingly, transcription from this exon is activated with the progression of breast cancer [36]. This conversion from inhibition to activation is enabled by decreased expression of COUP-TFI and increased expression of liver receptor homolog-1 (LRH-1), a transcriptional activator with high affinity for SF-1 binding sites, and is accompanied by cancer progression [28]. Additionally, progressive cancer cells and inflammatory cells frequently secrete PGE_2 or cytokines, which activate the transcription factors ATF-2 and c-Fos/c-Jun in adipose stromal cells through PKA- and PKC-activating intracellular signals, respectively [37]. Consequently, transcription from exon I.3 (1c)/PII (1d) is enhanced by binding of activated ATF-2 and c-Fos/c-Jun to the CRE and AP-1 sites, respectively, on the promoter region. In addition, the adipocyte differentiation factors C/EBPα and PPARγ were reported to cause transcriptional suppression of the aromatase gene by lowering LRH-1 expression, whereas C/EBPβ, a preadipocyte differentiation factor, was suggested to participate in transcriptional activation by forming a stable transcription complex with the transcriptional coactivator, p300/CREB binding protein (CBP) [38], and AFT-2 which is activated by cAMP-PKA signaling from PGE_2 (Fig. 2.3) [39]. While the conditioned medium of cultured breast cancer cells is known to increase aromatase mRNA levels in cultured adipose stromal cells, it also induces expression of C/EBPβ and the formation of a stable transcription complex consisting of C/EBPβ, AFT-2 and p300/CBP, resulting in induction of aromatase [40]. As this induction was not completely suppressed by inhibitors of cyclooxygenase-2 (COX-2) and adenylate cyclase, the conditioned medium may contain other unknown aromatase-inducible factors in addition to PGE_2 that might synergistically induce aromatase. PGE_2 in breast cancer tissues was shown to increase the binding affinity of LRH-1 for the SF-1 binding site on the promoter of PII (1d) and to simultaneously induce aromatase in adipose stromal cells [28]. Therefore, non-steroidal anti-inflammatory drugs (NSAIDs) may be expected to suppress aromatase gene expression trough promoter PII and proliferation of breast cancer cells by inhibition of COX-2, a rate-limiting enzyme in PGE_2 synthesis. It was shown that the CREB-regulated co-activator CRTC2 binds directly to the PII (1d) promoter of the aromatase gene in preadipocytes and activates expression of the aromatase gene through mechanisms involving LKB1-AMP kinase (AMPK) that is regulated in response to PGE_2 [41, 42]. Tamoxifen-resistant breast cancer cells also displayed increased expression of aromatase

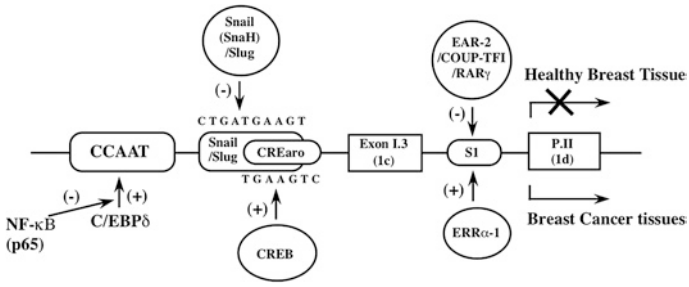

Fig. 2.5 Proposed mechanism of aromatase gene expression in healthy and malignant breast tissues. Stimulatory (+) and inhibitory (−) transcription factors are differentially bound to promoter regions of exon I.3 (1c) and promoter II (1d) of the human aromatase gene

together with phosphorylation of Akt, ERK and the p38 kinase and the resulting phosphoinositide 3-kinase (PI3K)/Akt-dependent CREB activation induced the expression of aromatase [43].

Chen et al. [44] identified an S1 site (silencer element), a CREaro site, and a Snail/Slug site on the promoter region of exon I.3 (1c)/PII (1d) using a yeast one-hybrid screening system and a promoter assay (Fig. 2.5). The S1 site, which is just upstream of PII (1d), includes the SF-1 binding site within it. Transcriptional repressors of COUP-TFI, V-erbA related protein 2 (EAR-2), and retinoic acid receptor γ (RARγ) bind to this S1 site in healthy breast tissues and suppress expression of aromatase [45]. The expression levels of these repressors decrease with cancer progression and in their stead ERRα-1, a transcriptional activator, binds to the S1 site, leading to an increase in transcription from PII (1d) [45]. That study also indicated that two types of CREB, CREB-1 and CREB-related factor, bind to CREaro, an incomplete CRE site (5′-TGAAGTCA-3′) that is just upstream of exon I.3 (1c), and induce transcriptional activation of the aromatase gene [46]. Two transcription factors with zinc-finger motifs, Snail (a human homologue of SnaH) and Slug, were implicated to regulation of this promoter by yeast one-hybrid screening [47]. Their DNA binding sequence (5′-CTGATGAAGT-3′) largely overlaps with that of CREaro. Because the expression of aromatase in breast cancer tissues showed an inverse correlation with that of SnaH, it was suggested that SnaH is a transcriptional repressor that binds to the Snail/Slug site. Indeed, the expression of aromatase is suppressed due to overexpression of SnaH in healthy breast tissues [47]. However, in breast cancer tissues, in which PGE_2 is secreted from cancer cells or inflammatory cells and activates cAMP-PKA signals in the stromal cells, CREB family factors are activated and bind to the CREaro site. Because there is a 6-base pair overlap between the DNA sequences of the CREaro and the Snail/Slug sites, binding of the CREB factors to the CREaro site hinders binding of SnaH to the Snail/Slug site, resulting in release of aromatase expression from SnaH suppression and the induction of aromatase [44].

Epigenetic Regulation of Aromatase

The above-described transcriptional regulation of aromatase has been studied since 1980s. More recently the epigenetic modification has also attracted attention as an aromatase regulatory mechanism and was shown to play an important role in the regulation of aromatase in physiological processes such as in temperature-dependent sex determination [48], placental development [49], and the ovarian sexual cycle [50]. Since epigenetic mechanisms regulate aromatase in human breast adipose fibroblasts, it was suggested that DNA methylation in the promoter region of the aromatase gene might contribute to this regulation [51]. In contrast to the well-studied role of epigenetic DNA methylation, little is known to date regarding the potential role of histone modifications as regulatory mechanisms of the human aromatase gene. Epigenetic histone modifications make it possible to regulate the expression of diverse genes through the regulation of DNA-chromatin interactions by using combinations of histone methylation, acetylation, phosphorylation, glycosylation, ubiquitination, and ADP-ribosylation. Generally, trimethylation of histone 3 lysine 4 (H3K4me3) and lysine 36 (H3K36me3) or acetylation of histone 3 lysine 9 (H3K9ac), lysine 14 (H3K14ac), and lysine 27 (H3K27ac) are observed in activated genes, whereas trimethylation of histone 3 lysine 9 (H3K9me3) and lysine 27 (H3K27me3) are observed in repressed genes. Lee et al. [52] recently showed that a decreased level of H3K4me3 and an increased level of H3K27me3 were found at the promoter region of the rat aromatase gene in ovary granulosa cells, concomitant with a decrease in aromatase gene expression. Methodical investigation will be required to evaluate the exact contribution of epigenetic regulation of the aromatase gene in breast cancer tissues in relationship to local estrogen supply and breast cancer progression.

Genetic Polymorphisms of the Human Aromatase Gene Associated with AI Response and Susceptibility to Breast Cancer

As estrogens are considered to be an important risk factor for the incidence and development of breast cancer, the transcriptional regulation of aromatase has been intensively studied as described above in order to elucidate its etiological role in this disease. In addition to transcriptional regulation, genetic variants of the aromatase gene including short tandem repeat polymorphisms and single nucleotide polymorphisms (SNPs) have also been shown to be associated with aromatase activity and are potential prognostic factors for breast cancer susceptibility. Indeed, several genetic polymorphisms of the aromatase gene have been reported to potentially affect aromatase gene expression, clinicopathological factors, prognostic factors, refractory factors, and cancer susceptibility.

Kristensen et al. [53] observed five different short tandem repeat polymorphisms of $(TTTA)_n$ in the intron 5 of the aromatase gene and showed that the $(TTTA)_{12}$ repeat allele is more frequent in Caucasian white women with breast cancer than in healthy controls. In contrast, Probst-Hensch et al. [54] reported the opposite result, showing no significant association of breast cancer susceptibility with any alleles of $(TTTA)_n$ repeats in African-American, Japanese, Latin, and non-Latin white populations. Since the allele distribution of the $(TTTA)_n$ repeat polymorphism varied depending on ethnic differences, the discrepancy between the results of the groups might reflect ethnic differences.

Ma et al. [55, 56] identified 88 SNP alleles of the human aromatase gene by exone re-sequencing in 240 subjects representing different ethnical groups with follow-up analysis on 10,592 cases and 11,720 controls. These studies firmly established functional associations between aromatase variation and function, with potential clinical implications for estrogen-dependent conditions, including breast cancer. A considerable number of other authors have also reported correlations of SNP alleles with levels of aromatase mRNA and activity, clinical factors, and susceptibility to breast cancer as well as to other cancers has been found to date (Table 2.1). A typical example is the genotype distribution of an SNP that is found in the 3′-untranslated region of exon 10 [57]. The TT genotype of this SNP was found at significantly higher frequency in breast cancer patients than in controls. Higher frequency was especially notable in patients with stage III and IV cancers and with tumors larger than 5 cm. The TT alleles also showed significant associations with the expression levels of aromatase mRNA and with switching from the adipose tissue-preferred exon I.4 (1b) to the gonad-tissue-preferred

Table 2.1 SNPs in the human aromatase gene

Cancer type	SNP	Risk	Reference number
Breast cancer	rs4646	Time to progression	Colomer et al. [73]
	rs6493497	AI-effect, E2 production	Wang et al. [58]
	rs10046	CS, E2 production, OS	Zins et al. [74]
	rs700518	Recurrence, bone loss (AI)	Napoli et al. [61]
	rs727479	Recurrence, OS	Miron et al. [75]
	rs934635	MASE (AI), VMS (AI)	Fontein et al. [60]
	rs1694189	VMS (AI)	Fontein et al. [60]
	rs7176005	VMS (AI)	Fontein et al. [60]
Ovary cancer	rs727479	E2 production	Haiman et al. [76]
	rs749292	E2 production, CS	Goodman et al. [77]
Prostate cancer	rs 2470152	E2 production, CS	Kanda et al. [78]
	rs10459592	E2 production, CS	Kanda et al. [78]
	rs4775936	E2 production, CS	Kanda et al. [78]
Lung cancer	rs3764221	E2 production, CS	Kohno et al. [79]
Colorectal cancer	rs1902584	CS	Lin et al. [80]

AI aromatase inhibitor, *CS* cancer susceptibility, *MASE* musculoskeletal adverse event, *OS* overall survival, *VMS* vasomotor symptom, *E2* 17β-estradiol

exon I.3 (1c) in breast cancer tissues. Wang et al. [58] reported two SNP alleles (rs6493497/rs7176005) on the promoter region of exon I.1 (1a), which were associated with elevation of aromatase enzymatic activity, with plasma 17β-estradiol (E2) level and with the potency of aromatase inhibitors for breast cancers; however some of these findings have not been confirmed in a different patients' cohort [59]. Some SNP alleles were potentially associated with increased risk of musculoskeletal adverse events (rers934635), vasomotor symptoms (rs 934635, rs1694189, rs7176005), or bone loss (rs700518) that accompany breast cancer treatment with aromatase inhibitors [60–62]. Of note, several SNP alleles were also reported to be associated with E2 production and ovary, prostate, lung and colorectal cancer susceptibility (Table 2.1). Since genetic polymorphisms are widely distributed throughout the human genome, not all of the SNP alleles on the aromatase gene are necessarily in the translated coding region or in the promoter region and some of these alleles exist in introns or untranslated regions. Therefore, SNP alleles that display association with clinically valuable factors may not directly affect aromatase expression but may rather be in linkage disequilibrium with another genetic variant.

Post-transcriptional Regulation of Aromatase

MicroRNA (miRNA) has recently been shown to be involved in the translational regulation of various genes through control of the translational rate or of transcript stability. Estrogen production in the ovary was shown to be regulated by miR-378, a microRNA that targets aromatase [63]. Aromatase activity is also controlled by post-translational modifications such as glycosylation and phosphorylation/dephosphorylation. Post-translational glycosylation of placental aromatase increased aromatase activity by 35–40 % [64]. On the other hand, aromatase activity was down-regulated by phosphorylation and was restored by dephosphorylation in various cultured cells [65]. Furthermore, aromatase protein levels are irreversibly decreased when aromatase is in a chronically phosphorylated state [66]. Conversely, the growth factors TGFα, EGF, and FGF, whose receptors are linked to a tyrosine kinase, enhanced aromatase activity in breast cancer cells [67]. Moreover, insulin-like growth factor-1 (IGF-1) was also found to increase aromatase activity in breast cancer cells by phosphorylation that was mediated through both the PI3K/Akt and the MAPK intracellular signaling pathways [68]. Barone et al. [69] suggested a critical role for estrogens in the regulation of aromatase in breast cancer cells. Interestingly, Zhang et al. [70] reported that IGF-1 enhanced both aromatase protein levels and its activity by inhibiting autophagy through activation of mTOR. As shown in Fig. 2.4, E2 may rapidly enhance aromatase activity through tyrosine phosphorylation of aromatase by the E2-activated c-Src kinase and through the suppression of protein tyrosine phosphatase 1B (PTP1B), which can dephosphorylate tyrosine-phosphorylated aromatase, by E2-activated PI3K/Akt kinases [69].

Other Possible Factors that Affect Aromatase

Non-steroidal aromatase inhibitors were reported to increase aromatase protein levels in cultured cells, probably through the formation of a stable aromatase protein-inhibitor complex that prevents its proteolytic degradation [71]. Aromatase catalyzes the aromatization reaction of androgens by supplying electrons from the NADPH-cytochrome P450 reductase. Therefore, estrogen-synthesizing activity is also dependent on the level of NADPH-cytochrome P450 reductase in the electron transport system of the endoplasmic reticulum. However, there have been few reports regarding changes in the expression level of NADPH-cytochrome P450 reductase in breast cancer tissues. In malignant liver tumors, the level of this reductase was observed to decrease at tumor sites compared with its level at distal sites, whereas the aromatase level increased locally at tumor proximal sites [7]. A number of polymorphisms have been reported for HADPH cytochrome reductase [72]. Given the important role of this cytochrome reductase in aromatization, mutations in this gene may have implications for AI response and resistance in breast cancer. More investigation is needed to evaluate the contribution of NADPH-cytochrome P450 reductase to estrogen production and to breast cancer progression.

Conclusion

The aromatase gene has an exceptionally complex organization. It consists of 9 coding exons and multiple alternative 1st exons, controlled by different promoters. A body of studies has been accumulated over the last 30 years about the interplay of multiple transcriptional modulators, post-transcriptional modifications and genetic variations for the human aromatase gene. Knowledge of tissue-specific regulation of the aromatase gene is essential for understanding AI resistance mechanisms and for minimizing side effects of AIs in breast cancer.

Acknowledgments This work was supported in part by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

Conflict of Interest No potential conflicts of interest were disclosed.

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