Abstract. There is a large and compelling body of epidemiological and experimental evidence that oestrogens are the fuel behind the aetiology of breast cancer. The carcinogenic effects of oestrogen are postulated to be mediated by: the stimulation of cellular proliferation through their receptor-mediated hormonal activity. Other mechanisms include; direct genotoxic effects by increasing mutation rates through a cytochrome P450-mediated metabolic activation and induction of aneuploidy. The local biosynthesis of oestrogens, especially in postmenopausal women as a result of the interactions of various enzymes, is believed to play a very important role in the pathogenesis and development of hormone dependent breast carcinoma. The over-expression of such enzymes seems to be associated with the development of a more aggressive disease process, a poorer outcome and increased local and distant recurrences.

This article focuses on CYP19 gene expression and aromatase enzyme activity and discusses their role in mammary carcinogenesis. The oestrogen producing enzymes such as 17-β-hydroxysteroid dehydrogenase Type 1 (17-β-HSD), 2 and steroid sulphatase (STS), and their role in breast cancer development are also discussed in detail. In addition the role of oestrogen catalyzing enzymes including: 3-β-hydroxysteroid dehydrogenase, oestrogen sulfotransferase (EST), CYP1A1, CYP1B1, CYP3A4 and CYP3A5 are discussed.

The understanding of the regulatory mechanisms controlling these enzymes is crucial to the development of new endocrine preventative and therapeutic strategies in post-menopausal females with hormone dependant breast cancer. Currently, the third generation of aromatase inhibitors has revolutionized the treatment of estrogen dependant breast cancer. However, the important role of both STS and 17-β-HSD Type 1 in local estrogen production provides novel potential targets for endocrine therapy. Such endocrine therapy is currently being explored and the development of STS inhibitors, combined aromatase steroid sulfatase and 17-β-HSD Type 1 inhibitors is underway, with promising preliminary results.

Breast cancer affects around 1 in 10 women and is the leading cause of death in females between the ages of 40 and 50 years in the Western world (1).

In the last decade, numerous studies have indicated a link between the pathogenesis of breast cancer and the expression of enzymes responsible for the local production of oestrogens.

Oestrogen Metabolism

The local production of oestrogens is mediated by a number of enzymes; aromatase catalyzes androstenedione into oestrone (E1), while steroid sulfatase (STS) hydrolyzes oestrone sulphate (E1S) to oestrone (Figure 1). Oestrone is subsequently converted to oestradiol (E2) by 17-β-hydroxysteroid dehydrogenase Type 1 (17βHSD Type 1), and locally acts on breast cancer cells through oestrogen receptors. Additionally, other estrogen-metabolizing enzymes such as 3-β-hydroxysteroid dehydrogenase, oestrogen sulfotransferase (STS), CYP1A1, CYP1B1, CYP3A4 and CYP3A5 play essential roles in the metabolism of oestrogen and subsequently in mammary carcinogenesis.

Oestrogens and Mammary Carcinogenesis

There is a large and compelling body of epidemiological and experimental evidence that oestrogens are the fuel behind the aetiology of breast cancer. Some breast carcinomas require oestrogen for continued growth and progression (2, 3). Three
mechanisms have been considered to be responsible for the carcinogenicity of oestrogens: receptor-mediated hormonal activity; a cytochrome (CYP) P450 mediated metabolic activation, which elicits direct genotoxic effects by increasing mutation rates; and the induction of aneuploidy by oestrogen (4).

Breast cancer risk is associated with prolonged exposure to oestrogens, early onset of menarche, late menopause, hormone replacement therapy and post-menopausal obesity. Approximately 60% of pre-menopausal and 75% of post-menopausal patients with breast cancer have oestrogen dependent carcinomas (5).

The progression from proliferative disease without atypia (PDWA) to atypical ductal hyperplasia (ADH), from ADH to ductal carcinoma in situ (DCIS) and from DCIS to invasive carcinoma has been proposed to be a possible model for the development of human breast invasive ductal carcinoma (6, 7). In the early stages of this proposed cascade of breast cancer development, oestrogens, especially oestradiol (E2), have been considered as one of the most important factors (8).

Animal studies demonstrated that oestrogens can induce and promote mammary tumours in rodents. The removal of animals’ ovaries or administration of anti-estrogenic drugs had the opposite effect (9). Interestingly, exposure of the fetus to excess oestrogen is believed to increase the risk of developing breast cancer during adult life. Fetal exposure to low doses of the xenoestrogen, bisphenol A, resulted in long-lasting effects in the mouse mammary gland that were manifested during adult life. It enhanced sensitivity to oestradiol (10), decreased apoptosis and increased the number of progesterone receptor-positive epithelial cells at puberty. Bisphenol A exposure also increased terminal end bud density at puberty as well as increasing the number of terminal ends (11). Murray et al. reported that fetal exposure to bisphenol induces the development of ductal hyperplasias and carcinoma in situ at postnatal day 50 and 95 in rats (12).

Oestrogens induce the expression of peptide growth factors which are responsible for the proliferative responses of cancer cells (13, 14). Insulin-like growth factor-1 (IGF-1) is known to play an important role in mitogenesis, growth and differentiation mediated by the IGF-I receptor (IGF-IR). The activation of a cell-surface tyrosine kinase by serum IGF-I seems to be a key step in breast cancer initiation. Maor et al. showed that oestradiol treatment significantly activated the IGF-IR promoter in MCF-7 breast cancer cell line but not in isogenic ER-negative C4 cell lines. Therefore, dysregulated expression of the IGF-IR gene may have pathologic consequences with relevance in breast cancer aetiology (15).

Oestrogen has been shown to up-regulate oncogenes such as c-myc through binding to its receptor and through the Src/p21ras/mitogen-activated protein kinase pathway of c-fos and c-jun, leading to increased breast cancer cell proliferation (16, 17).

The role of oestrogen in the motility and invasion of breast cancer cells is still controversial. Although oestrogen receptor (ER)-positive breast tumors are considered less aggressive and more differentiated they still undergo metastasis. The epithelial-to-mesenchymal transition (EMT) process, where a loss of epithelial features and acquisition of mesenchymal properties, leading to migration of individual cells has been observed recently in ER-positive breast cancer cells (18). Planas-Silva et al. proved that in MCF-7 breast cancer cells, oestrogen promoted acquisition of mesenchymal-like features, while anti-oestrogens, such as tamoxifen prevented this transition (18).
Oestrogen Production

In women, oestradiol originates from different sources pre-and post-menopausally. In pre-menopausal women, the ovary or membrane granulosa of dominant follicles is the main source of circulating oestrogens (19, 20). Oestrogens are produced, secreted and transported through the circulation and act on their target tissues with specific oestrogen receptor expression. This system is known as the endocrine system. In classical endocrine systems, only a small amount of hormone is generally utilized in the target tissues, whilst the great majority is metabolized or converted to inactive forms. Post-menopausally most oestrogen is synthesized in peripheral tissues from abundantly present circulating precursor steroids (21), where the enzymes involved in the formation of androgens and oestrogens are expressed. Several epidemiological studies indicate that plasma oestradiol, adrenal androgens, and testosterone levels are higher in women who develop breast cancer over a period of several years, than in those who do not (22-24).

In post-menopausal women, oestrogens act in an autocrine fashion where oestrogen is synthesised in tumour epithelial cells. Moreover, neighboring stromal cells can produce oestrogen which is transported to the tumour cells without release into the circulation. This is an example of a paracrine mechanism. Furthermore, locally produced bioactive androgens and/or oestrogens exert their action in the cells where synthesis occurs, without release into the extracellular space. This phenomenon is different from the autocrine, paracrine and classical endocrine action, and is termed an ‘intracrine’ mechanism. Oestrogens are biosynthesized in peripheral tissues through the conversion of circulating inactive steroids (25). Androgens such as androstenedione of both adrenal and ovarian origin, especially that produced from the zona reticularis of the adrenal cortex (26) and oestrone sulphate, are considered major precursor substrates of local oestrogen production. The conversion of androgen to oestrogen occurs principally in peripheral tissues, including skin, muscle (27), fat (28) and bone (29). The intracrine system requires minimal amounts of biologically active hormones to exert their maximum effects. Therefore, the intracrine pathway is an efficient mode of hormone action and plays important roles, especially in the development of hormone-dependent neoplasms. It is also important to note that, in an intracrine system, serum concentrations of hormones do not necessarily reflect the local hormonal activity in the target tissues.

The relative contribution of any of the above-mentioned mechanisms is likely to vary with the physiological status of the female and possibly also with the local and systemic changes occurring during breast tumorigenesis and progression. Experimental evidence supports the potential of each mechanism to contribute to oestrogen synthesis and influence breast tumorigenesis (30).

Higher levels of oestradiol were seen in breast cancer tissue when compared with areas considered as morphologically normal (31). In addition, it has been observed that in post-menopausal patients with breast cancer, oestrogen levels in specimens were found to be several folds higher than those of plasma (32, 33). Although oestrogen levels decline sharply after the menopause, it has been reported that in some breast tumours, in situ formation of oestrogens can make an important contribution to the oestrogen content of breast cancer cells (34, 35). Experimental evidence using xenograft models provides direct proof that locally produced oestrogens can stimulate the growth of oestrogen-dependent MCF-7 human breast tumours to a greater extent than can oestrogen delivered via an endocrine mechanism (17). Oestrogen produced locally in tumours arising from these xenografted cells may exceed levels up taken from plasma. Oestrogen has also been shown to influence the clinical outcome of breast cancer patients by stimulating the proliferation of ER positive tumour epithelial cells (30). Intra-tumoral oestradiol levels were not observed to be significantly different between pre-menopausal and post-menopausal breast cancer patients, but the intratumoral oestradiol/oestrone ratio was significantly higher in post-menopausal than in pre-menopausal breast cancers (36). It is essential to examine the enzymatic mechanisms responsible for the local production of enzymes in order to understand their role in the development of breast cancer.

Aromatase

CYP19 gene expression and regulation. The human CYP19 (P450arom) gene belongs to the cytochrome P-450 superfamily comprising over 460 members in 74 families, of which cytochrome P450arom is the sole member of family 19 (37). It is localized on the long arm of chromosome 15 (15q21.21). CYP19 encodes aromatase which is the key enzyme for oestrogen biosynthesis (38, 39). It is localized in the endoplasmic reticulum of oestrogen-producing cells (40, 41). The aromatase enzyme complex is comprised of two polypeptides. The first of these is a specific cytochrome P450, namely aromatase cytochrome P450 (P450arom) which is the product of the CYP19 gene (41). The second is a flavoprotein, NADPH-cytochrome P450 reductase and is ubiquitously distributed in most cells. Thus, cell-specific expression of P450arom determines the presence or absence of aromatase activity. Aromatization of androstenedione to oestrone is achieved by sequential hydroxylation, oxidation and removal of the C-19 carbon with subsequent aromatization of the A ring of the steroid. In pre-menopausal women, the highest levels of aromatase are present in the ovaries. The placenta of pregnant women and the peripheral adipose tissues of post-menopausal women and in men, are the main sites for aromatase activity (17, 42, 43).
The CYP19 gene is found between markers stSG12786 and stSG47530 with the 3’-end of the gene centromeric to the 5’-end of the gene, showing the direction of transcription is from telomere to centromere. It spans about 123 Kb. Only the 30 kb (exon II-exon X) 3’-region encodes aromatase, where as the large 93 kb 5’-flanking region serves as the regulatory unit of the gene. The unusually large regulatory region contains 10 tissue-specific promoters that are alternatively used in various cell types. Further upstream of exon II, there are a number of alternative first exons which are differently spliced into distinct 5’-untranslated regions (40, 43, 44). In addition, up to nine different transcriptional start sides with individual promoters permitting tissue-specific regulation of expression have been described. Although each tissue expresses a unique first-exon 5’-untranslated region by splicing into a highly promiscuous splice acceptor site (AG-GACT) of the exon II, coding regions and translated products are identical in all tissue sites of expression (34, 45). This means that although transcripts in different tissues have different 5’ termini, the coding region is the same and therefore the proteins expressed in these tissues remain the same. The recently published Human Genome Project Data allowed, for the first time, to precisely locate all known promoters and elucidate the extraordinarily complex organization of the entire human CYP19 gene. Each promoter is regulated by a distinct set of regulatory sequences in DNA and transcription factors that bind to these specific sequences. The promoter I.7 was cloned by analysing P450arom mRNA levels in breast cancer tissue. P450arom mRNA with exon I.7 expression was significantly increased in breast cancer tissues and adipose tissue adjacent to tumors (45). This TATA-less promoter accounts for the transcription of 29-54% of P450arom mRNAs in breast cancer tissues. The in vivo cellular distribution and physiologic roles of promoter I.7 in healthy tissues, however, are not known.

It is now known that the aromatase gene expression is regulated in a tissue-specific manner by the use of alternative promoters (45). Normal breast adipose tissue maintains low levels of aromatase expression primarily via promoter I.4 which lies 73 kb up stream of the common coding region. Promoters I.3 and II are used only minimally in normal breast adipose tissue. Primer-specific RT-PCR analyses (46-48) have revealed that the two major exons; I.3 and PII are present in aromatase mRNAs isolated from breast tumors but are only used minimally in normal breast adipose tissue. These results suggest that promoters I.3 and II are the major promoters directing aromatase expression in breast cancer and surrounding stromal cells and fibroblasts. It appears that the prototype estrogen-dependent malignancy breast cancer takes advantage of four promoters (II, I.3, I.7 and I.4) for aromatase expression. The sum of P450arom mRNA species arising from these four promoters, markedly increases the total P450arom mRNA levels in breast cancer, compared with the normal breast that uses promoter I.4 almost exclusively.

Many studies have shown that a switch from an adipose-specific exon 1 (exon 1b or exon I.4) promoter used in non-tumour breast tissues to the ovary-specific exon 1 (exon 1c or exon I.2) occurred in breast cancer tissue (49, 50). This promoter specific aromatase expression in malignant tissue may serve as an interesting therapeutic target in the future.

Immunohistochemical studies have provided evidence for both an epithelial and stromal location for the aromatase enzyme complex (25, 51). Biochemical studies, however, have revealed a higher aromatase activity in the stromal rather than the epithelial component of breast tumors (52). Furthermore, measurements of aromatase activity in fibroblasts derived from breast tumors or MCF-7 cells have demonstrated a much higher level of aromatase activity in fibroblasts (53).

Enzyme activity. Several factors which can stimulate aromatase activity have now been identified. Using breast tumour-derived fibroblasts, in which it is possible to induce aromatase activity with dexamethasone, breast cyst fluid (BCF) and breast tumour cytosol were found to stimulate aromatase activity (54-56). The cytokine IL-6 has been shown to stimulate aromatase activity in stromal cells derived from subcutaneous adipose tissue (57). This stimulation requires the IL-6 soluble receptor (IL-6sR) which is produced by breast tumour-derived fibroblasts and acts synergistically with IL-6 to stimulate aromatase activity in these cells (58). In addition to IL-6, other cytokines such as TNF-α, IL-11, oncostatin M, leukaemia inhibitory factor and insulin-like growth factor Type I are known to stimulate aromatase activity (57, 59). Malignant breast epithelial cells produce large quantities of TNF and IL-11 (60). These two cytokines mediate the desmoplastic reaction where accumulation of fibroblasts around malignant epithelial cells happens and serve to maintain the strikingly hard consistency in many of these tumors. Desmoplastic reaction increases local concentrations of oestrogen via aromatase overexpression localized to these undifferentiated fibroblasts. The inhibition of fibroblast differentiation to mature adipocytes mediated by TNF and IL-11 is the key event responsible for a desmoplastic reaction. Blocking both TNF and IL-11 in cancer cell-conditioned media using neutralizing antibodies is sufficient to reverse this antiedifferentiative effect of cancer cells completely (60).

Cytokines, in the presence of glucocorticoids, regulate aromatase gene expression via the PI.4. In malignant breast tissue promoter switching occurs, resulting in aromatase gene expression which is regulated by PII and PL.3 to a greater extent than PI.4 (46, 61). Prostaglandin E2 (PGE2) is able to cause promoter switching from I.4 to II in adipose stromal cells and can increase aromatase activity (62) and
has been found to be the most potent factor stimulating aromatase expression via promoter II. A correlation between COX-2 and CYP19 mRNA levels has been demonstrated in human breast cancer specimens using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR). It has been also found that PGE2 may act by stimulating IL-6 production in fibroblasts derived from normal and malignant breast tissues (63).

**Role of aromatase in mammary carcinogenesis.** The regulation of aromatase activity in malignant tissues is highly complex. Miller and co workers (64) were the first to show that the location of a tumour within the breast, influenced aromatase activity in the quadrant in which the tumor was located. This finding, which was subsequently confirmed at the level of expression and activity of hormones, suggested that either tumors developed in an area of high aromatase activity within the breast or tumours were capable of producing factors that stimulated aromatase activity in adjacent tissues. Bulun et al. (65) reported that CYP19 mRNA levels were highest in tumour bearing quadrants. CYP19 mRNA levels were observed to be significantly higher in tumor bearing quadrants than in regions distal to the tumour or in non-malignant breast tissue (66). Using quantitative PCR analysis, it was confirmed that adipose stromal cells surrounding the cancer cells contained higher levels of CYP19 mRNA than adipose stromal cells in non-cancerous areas (65). Furthermore, James et al. (67) reported that *in vitro* aromatase activity was higher in breast tumours than in the fat adjacent to the tumour or in normal breast fat. Cell line experiments have confirmed the role of aromatase in stimulating the growth of breast cancer cells (32, 68-70). Aromatase over-expression has been reported to be associated with a poor clinical outcome in women with breast cancer (Figure 2) (71). Levels of aromatase mRNA levels were observed to be higher in breast cancer patients who developed local recurrence and distal metastasis, or those who died of breast cancer, compared with patients who are disease free after 10 years follow-up (71). Such a relationship was not seen with the clinicopathological parameters and other tumour characteristics. The lack of correlation between aromatase expression and these clinicopathological factors including: age, tumor size, axillary lymph node involvement, grade, and histological type was previously reported (72, 74). Interestingly, Brodie et al. (75) found that tumours with a relatively high aromatase activity tended to be ER-positive. Miller and coworkers (72) also observed a significant trend toward an association between aromatase activity and the presence of ERα, although tumors expressing active aromatase included both ERα positive and negative tumors. There is increasing evidence that aromatase inhibitors are superior to tamoxifen, in post-menopausal women with ER positive, early and advanced breast cancer. These findings are in keeping with observations that higher aromatase expression correlates with poor clinical outcome (76, 78).

**Role of aromatase inhibitors in breast cancer treatment.** Third-generation aromatase inhibitors such as anastrozole, letrozole, and exemestane are now considered the gold standard endocrine therapy in the first-line and second-line settings, for ER and/or progesterone receptor (PgR) positive, advanced breast cancer in post-menopausal women(78,79). There is a growing body of evidence for their superiority to tamoxifen in the adjuvant setting. The ATAC (Arimidex, Tamoxifen, Alone or in Combination) study (76), randomized 9,366 post-menopausal women into 3 groups: arimidex alone, tamoxifen alone or arimidex and tamoxifen. Over a 5 year period anastrozole was found to be superior to tamoxifen, in terms of efficacy and tolerability in treating post-menopausal women with ER positive breast cancer. Anastrozole significantly prolonged disease-free survival (DFS), reduced the recurrence of a contralateral carcinoma and reduced the risk of recurrence by 26% in patients with hormone-receptor positive disease. There was a 17% statistically non-significant reduction in breast cancer-related deaths in the anastrozole group, compared to tamoxifen, and a reduction of borderline significance in distant metastases.

The Breast International Group (BIG) 1-98 study (80) showed that letrozole significantly reduced the risk of recurrence by 28% with hormone-receptor positive disease, especially the risk of distant recurrence. This risk reduction of distant relapse is greater than that seen with anastrozole in the ATAC study. However, caution should be exercised when considering such a cross-trial comparison. Unlike the ATAC
study, subgroup analyses of BIG1-98 showed a significant DFS benefit in favour of letrozole among high risk groups such as patients with node positive breast cancer and/or tumours larger than 2 cm. The benefit of using letrozole extends to all ER positive cases regardless of PgR status.

The role of chemoprevention of aromatase inhibitors in high-risk women is currently being studied; the International Breast Cancer Intervention Study (IBIS-II) trial aims to evaluate the potential role of third generation aromatase inhibitors in high-risk postmenopausal women in the prevention of breast cancer development. A parallel trial IBIS II DCIS will investigate the role of anastrozole versus tamoxifen in the prevention of recurrence of excised DCIS.

17-β-Hydroxysteroid Dehydrogenase Type 1 and 2

Gene expression and regulation. Oestradiol (E2), a biologically potent oestrogen, contributes greatly to the growth and development of breast carcinoma cells. 17-β-HSD Type 1, which is associated with a high specificity for C18 steroids, primarily converts the inactive C18 steroid, oestrone (E1), to the biologically active oestradiol (81, 82).

The gene coding for 17-β-HSD Type 1 is located at 17q12-21 (83). 17-β-HSD Type 2, on the other hand, is an enzyme that converts E2 to E1 (84, 85) and plays an important role in the peripheral inactivation of androgens and oestrogens, thus maintaining the steady oestrogen levels in target tissues. IL-6 and TNF-α have been demonstrated to stimulate the activity of 17-β-HSD Type 1 (86, 87). In addition, insulin-like growth factor type I (IGF-1) and an albumin-like molecule isolated from breast tumour cytosol, were also found to regulate the conversions of oestrone to oestradiol (88). Miller et al. (89) and Perel et al. (90) demonstrated that human breast and its neoplasms could produce 17-β-oestradiol in vitro. 17-β-HSD type 1 was immunolocalized in the cytoplasm of carcinoma cells in 60% of invasive ductal carcinomas (91) whereas 17-β-HSD type 2 immunoreactivity was not detected in all cases examined.

Role in mammary carcinogenesis. In vivo studies showed that in oestrogen-dependent MCF-7 human breast cancer transfected with an expression plasmid for human 17-β-HSD. The enzyme efficiently converted E1 to E2 and enhanced the oestrogen-dependent growth of cultured MCF-7 cells, in the presence of hormonally less active E1. The 17-β-HSD expressing cells also formed oestrogen-dependent tumors in immunodeficient nude mice. After treating the mice with an appropriate dose of the substrate (E1), a marked difference in tumor growth was observed between non-transfected and 17-β-HSD-transfected MCF-7 cells (92).

A few immunohistochemical studies of 17-β-HSD Type 1 in human breast carcinoma have been reported and no clear relation to prognosis and clinical parameters has been found (34, 93, 94). Recent studies, however, have shown that 17-β-HSD Type 1 positive carcinoma cells of mammary epithelial proliferative lesions tend to be ER positive (95) and 17-β-HSD Type 1 may be an independent prognostic marker in breast cancer patients (96). It has been suggested that 17-β-HSD Type 1 plays an important role in hormone-dependent breast carcinomas (97). In a study conducted by Gunnarsson et al. (98), the authors found that a high level of 17-β-HSD Type 1 was associated with an increased risk of developing a late relapse of breast cancer. The authors suggested that abnormal expression of 17-β-HSD isoforms had prognostic significance in breast cancer and that altered expression of these enzymes could play an important role in breast cancer progression. In a recent study by Gunnarsson et al. (99) a significant correlation between the gene copy number of 17-β-HSD Type 1 and mRNA expression level was observed. ER positive patients with amplification of 17-β-HSD Type 1 gene showed lower breast cancer survival than patients without amplification. On the other hand, among ER-negative patients there was no significant correlation between increased gene copy number of 17-β-HSD and the prognosis. The amplification of the gene had a prognostic significance in multivariate analysis adjusting for other clinicopathological variables (99).

Feigelson et al. (100) found that a polymorphism in the gene for 17-β-HSD Type 1 could be used to identify women at an increased risk of developing advanced breast cancer. The study found the relative risk (RR) of developing advanced breast cancer was 2.21 in individuals carrying 4 ‘high risk alleles’.

In principle, a recent study (71) in which the mRNA from breast tumours (n=127) was analysed supports these findings. Using Kaplan-Meier survival curves a high aromatase and 17-β-HSD Type 1 expression was correlated with poor survival and highlights the significant relationship between poor survival and high expression of 17-β-HSD Type 1 in breast cancer patients (Figure 3) (71).

Potentials for 17-β-HSD Type 1 inhibitors in breast cancer treatment. Based on the reports of Feigelson et al., inhibition of intra-tumoral 17-β-HSD Type 1 activity or expression should be considered as a potential novel endocrine therapy and can contribute greatly to the suppression of oestrogen dependent proliferation of tumor cells. The development of potent inhibitors of 17-β-HSD type 1 has been attempted by many researchers. The oestrogen-dependent growth of the 17-β-HSD Type 1 expressing xenografts in the presence of E1 was markedly inhibited by administering a 17-β-HSD Type 1 inhibitor. After a 4-week treatment period, the tumor size was reduced by 59.8% as compared with the non-treated tumours. The results evidently show that the enzyme is a potential target for the pharmacological inhibition of oestrogen action (92).
Many of the reported 17-β-HSD inhibitors are based on a steroid template. Inhibition of 17-β-HSD has been undertaken by the use of various structures including E2 derivatives with a 3 carbon side chain at position 11a and 16a (151). Newer substrate co-factor hybrid compounds designed to potentially interact with two binding domains of 17-β-HSD have also been described (152). The use of C6-(N,N-butyl-methyl-heptanamide) derivative of E1 and E2 showed up to 82% inhibition of 17-β-HSD in T-47D cells (153). The new compound was also found to be less oestrogenic than the lead compound in the T-47D and MCF-7 breast cancer cell lines. More recently Potter et al. have reported a novel non-steroidal inhibitor which mimics the E1 template; biphenyl ethanone (154).

It is important however to point out that aromatase and 17-β-HSD Type 1 are differently regulated and no correlations between these two enzymes have been reported in patients with breast cancer (91). Suzuki et al. have shown that a high 17-β-HSD Type 1 in patients with invasive ductal carcinoma was correlated with a poor grade and prognosis. Therefore inhibition of 17-β-HSD Type 1 might be a much more efficacious therapy than aromatase inhibition in breast cancer patients whose tumours overexpress 17-β-HSD Type 1 but not aromatase. Furthermore, it may be feasible to employ 17-β-HSD Type 1 inhibitors as third or later lines of endocrine therapy after the development of resistance against conventional endocrine therapy including ER antagonists or aromatase inhibitors in patients with intratumoral overexpression of 17βHSD Type 1.

Steroid Sulphatase (STS)

Gene expression and regulation. STS is a member of a superfamily of 12 different mammalian sulfatases (102, 103). The gene coding for human STS is located on the distal short arm of the X-chromosome and maps to Xp22.3-Xpter. The STS gene is pseudo-autosomal and escapes X-inactivation. It has been cloned, characterized, and sequenced (104). On the Y-chromosome, there is a pseudogene for STS, which is transcriptionally inactive as the promoter and several exons have been deleted. The gene consists of 10 exons and spans 146 kb, with the intron sizes ranging from 102 bp up to 35 kb (105).

Information about the molecular regulation of STS is still limited. It was observed that both basic fibroblast growth factor and IGF-1 increase STS activity in a dose- and time-dependent manner in MCF-7 and MDAMB-231 breast cancer cells (106). Both cytokines TNFα and IL-6 up-regulate STS enzyme activity in MCF-7 breast cancer cells. This up-regulation appears to be post-translationally mediated rather than occurring via any changes in gene transcription or mRNA stability (107). Interestingly, STS mRNA levels decreased when MCF-7 breast cancer cells were treated with the progestagen Promegestone (R-5020) (108). In contrast, it was observed that exposure of MCF-7 and MDA-MB-231 breast cancer cells to the progestagen medroxyprogesterone acetate, stimulated STS activity in these cells (106).

Immunohistochemistry and STS mRNA expression of laser-captured microdissected samples were also used to examine the location of STS within breast tumours (94). STS immunoreactivity was detected in the cytoplasm of cancer cells, with STS mRNA expression being detected in microdissected carcinoma cells but not in stromal cells.

STS hydrolyzes circulating oestrone sulphate (E1-S) to E1 in various human tissues (109-113) and acts on dehydroepiandrosterone sulfate (DHEAS), which is considered the most abundant steroid secreted by the adrenal cortex, reducing it to dehydroepiandrosterone (DHEA) by the removal of the sulfate group. DHEA in turn can undergo reduction to adiol (114) which is known to have affinity for ER and therefore stimulate the growth of ER positive breast cancer cells in vitro (86, 115). This finding shows that adiol does not need to be converted to an oestrogen in order to stimulate tumor growth. Further studies have revealed that DHEA and adiol can directly activate the ER and stimulate the proliferation of breast cancer cells (116). Recent research has shown that DHEAS, DHEA and adiol can stimulate the proliferation of breast cancer cells in vitro and induce mammary tumors in vivo (117), and their ability to do so is blocked by the ER antagonist nafoxidene, but not by aromatase inhibitors. These results provide strong evidence that the stimulation of cell growth by DHEAS occurs via an aromatase-independent pathway that can be potentially blocked by an STS inhibitor.
Role in mammary carcinogenesis. The STS mRNA expression in malignant breast tissue seems to be significantly higher than in normal tissue (118). This finding is consistent with the higher STS enzymatic activity that has been detected in malignant breast tissue (30, 119). STS mRNA expression was found to be an independent prognostic indicator in predicting relapse-free survival. High levels of STS mRNA expression being associated with a larger tumour size, lymph node metastasis, increased risk of recurrence and poor prognosis (36, 94, 119, 120). Miyoshi et al. found the mean mRNA expression was significantly higher in tumours compared to normal tissue for aromatase, sulphatase and 17-β-HSD Type 1. Similarly mean sulphatase mRNA expression was high in tumours with nodal positivity, high grade and ER negativity, however not significantly for the latter. A similar study reported a significantly higher STS expression in node positive tumours with significantly shorter disease free survival with an increased recurrence rate. The was no effect on the overall survival. It was also reported that the association between STS mRNA expression and prognosis applied only to ER positive tumors.

Recently, a significant correlation between high levels of STS mRNA and poor survival was demonstrated (Figure 4) (121). In addition, STS mRNA levels were correlated with aromatase mRNA levels (121). Interestingly, high STS mRNA expression was observed to be associated with a poor prognosis in both pre- and post-menopausal women. This finding led to the suggestion that even in pre-menopausal women, intratumoral oestrogen synthesis may play an important role in the growth of breast tumours.

Role of STS inhibitors in treatment of breast cancer. Since oestrogen formation from E1-S and DHEAS (STS pathway) cannot be blocked by aromatase inhibitors, STS is thought to be a novel molecular target for the treatment of oestrogen-dependent tumours, post selective oestrogen receptor modulators and/or aromatase inhibitors (122). However, accurate determination of STS and ER levels in tumour specimens is required in order to achieve the maximum potential benefits from STS inhibitors. Phase III clinical trials will determine the usefulness of such drugs.

Both steroidal and non-steroidal STS inhibitors have been recently developed and seem to be effective in depressing the proliferation of oestrogen-dependent MCF-7 cells (123). Several potent irreversible STS inhibitors have now been identified, all of which have as their active pharmacophore an aryl ring to which a sulfamate ester is attached. The structure of the first-generation STS inhibitor, STX64 (non-steroid based), was shown to be a potent STS inhibitor in rodents and blocked the ability of E1-S to stimulate the growth of carcinoen-induced mammary tumors in ovariectomized rats (124).

Second generation STS inhibitors, such as the steroid based STX213, have now been developed which inhibit STS activity in rodents after the administration of a single dose for a much longer period of time than STX64 (125,126). These STS inhibitors are orally active with a high level of bioavailability. This results from their binding to carbonic anhydrase II in erythrocytes after absorption, which enables them to transit the liver without undergoing first-pass inactivation(127). Second generation STS inhibitors have been reported to inhibit the growth of ER negative tumours in mice and exhibit an anti-angiogenic property (155).

The first-ever phase I trial of an STS inhibitor in post-menopausal women with locally advanced or metastatic breast cancer was recently reported by Reed et al. (128). Inhibition of STS activity was associated with significant reductions in serum androstenediol and oestrogen concentrations. Unexpectedly, serum concentrations of androstenedione, the main substrate for aromatase in post-menopausal women, also decreased by up to 86%. This finding indicates that, in this group of women, androstenedione is derived mainly from the peripheral conversion of DHEAS and not, as previously thought, by direct secretion from the adrenal cortex.

Current research is concentrating on the development of dual aromatase steroid sulphatase inhibitors (DASI). Potter et al. introduced the STS inhibitory pharmacophore into anastrazole. They found the lead compound 11 to have a
potent dual inhibition in vivo (156). More recent work has used letrozole as the template to produce a chiral aromatase-steroid sulphatase inhibitor. This is a new structural class of DASI and is the first report of STS inhibition by an enatpure non-steroidal compound (157).

Other Oestrogen – Metabolizing Enzymes

3β-hydroxysteroid dehydrogenase (3-β-HSD). 3-β-HSD is a membrane-bound enzyme which catalyzes the conversion of DHEA into androstenedione, and increases the local tissue levels of androstenedione (21). Therefore, 3-β-HSD may play a part in the initial steps of the intracrine cascade of hormone transformation in breast carcinoma. Two distinct genes encode the tissue-specific expression of the two isoforms of human 3-β-HSD. Type 1 being predominantly found in the mammary gland and breast tumours. 3-β-HSD activity has been detected in breast carcinomas (131). Sasano et al. (132) reported that 3-β-HSD immunoreactivity of both isoforms (3-β-HSD Type 1 and 3-β-HSD Type 2), was localized in 33% of tissues in breast carcinoma cells. There was however no significant association with the ER or PgR status.

The regulation of 3-β-HSD in breast cancer is still unclear; cAMP and protein kinase-C play a role in the regulation of isofrom 3-β-HSD Type 1 (133). Both cytokines IL-4 and IL-13 are found to cause induction of 3-β-HSD Type 1 gene transcription in breast cancer cells (134,135). Purified 3-β-HSD uses DHEA as a substrate (158). In MCF-7 cells 3-β-HSD Type 1 isoenzyme has shown to have a higher affinity for DHEA than the type 2 enzyme. Human 3-β-HSD Type 1 enzyme was also noted to have a higher affinity for the inhibitor steroid epoestane. Therefore there is a therapeutic potential to be gained from selectively inhibiting the conversion of DHEA to E2 by 3-β-HSD Type 1 thereby slowing the growth of breast tumour cells.

Oestrogen sulfotransferase (EST). EST is a member of the superfamily of steroid-sulfotransferases; it sulfonates oestrogens to biologically inactive oestrogen sulfates (136,137). EST has also been postulated to play an important role in the conversion of DHEA into oestrogens in breast cancer tissue. EST is considered to be involved in the regulation of in situ oestrogen levels in human breast carcinoma. Enzymatic activity of EST was detected in breast cancer cell lines (138, 139), breast carcinoma tissues and normal breast tissues, and was associated with ER status in breast cancer tissue (140, 141). The concentration of oestrone sulfate was significantly higher in breast cancer tissues than in plasma (142).

EST mRNA expression was detected in breast cancer tissues (94) and was significantly associated with EST immunoreactivity (94). EST immunoreactivity was inversely correlated with tumor size or lymph node status and was significantly associated with a decreased risk of recurrence or improved prognosis (94). An American study has reported post-menopausal breast cancer to be less common in African American women as compared to Caucasians. Immunohistochemical analysis of sulfotransferase showed that African Americans had a significantly greater amount of sulfotransferase in epithelial cells of associated normal breast tissue as compared to Caucasians (159).

CYP1A1, CYP1B1, CYP3A4 and CYP3A5. The CYP superfamily are involved in the synthesis of steroids. Among the CYP superfamily, CYP1A1, CYP1B1 and CYP3A4 oxidatively metabolize oestradiol, suggesting a possible association with the regulation of local oestrogen levels in breast cancer tissues.

CYP1A1 catalyzes C2, C6a and C15α hydroxylation of oestradiol. CYP1A1 mRNA expression was detected in 25–46% of normal breast tissues (143,144) and 5–53% of breast carcinoma tissues (143-145). CYP1A1 protein was detected in 36% of breast cancer tissues (145). CYP1A1 protein level was significantly lower in breast cancer tissue compared with normal adjacent tissues (146).

CYP1B1 plays a role in the C4-hydroxylation of oestradiol. CYP1B1 expression is constitutively detected in breast carcinoma tissues (144, 145), and the level of expression was significantly higher in non-tumour tissues than in tumour tissues (146). It has been shown that there is no significant association between CYP1B1 immunoreactivity and clinicopathological factors, including tumour grade and ER status in breast carcinomas (148). However, Oyama et al. (149) reported an inverse correlation between CYP1B1 immunoreactivity and clinical stage in breast cancers.

CYP3A4 catalyzes the C2, C4, C6a, C12, C15a and C16a-hydroxylation of oestrogen. The regulation mechanism of CYP3A4 in breast cancer still remains unclear. The expression of CYP3A4 in breast carcinoma tissues have been studied, but results appear to be inconsistent. Expression levels of CYP3A4 mRNA were found to be significantly higher in non-tumour tissues than in tumour tissues (147). CYP3A4 protein levels were significantly lower in breast carcinoma tissues than in morphologically normal adjacent tissues (146). Other studies have reported no mRNA expression of CYP3A4 in breast tumor tissues or normal breast tissues (144). Also, Hellmold et al. (145) did not detect CYP3A protein in breast carcinoma tissues.

Of interest, 4-hydroxy-oestradiol, which is metabolized by CYP1B1 or CYP3A4 from oestradiol, is further converted to the 3,4-oestradiol quinone. This compound is recognized as a genotoxic mutagenic carcinogen and possibly induces breast cancer (150). Therefore, metabolism of oestradiol by CYP1B1 and CYP3A4 may not necessarily be associated with reducing the progression of breast tumors.
Over time ER positive breast cancer patients often develop resistance to tamoxifen, it has been suggested that the metabolism of tamoxifen by cytochrome enzymes may be responsible for the development of resistance. In a group of patients randomized to either 2 or 5 years treatment with tamoxifen. It was found that patients who were homozygous for CYP3A5*3 allele had an improved DFS when treated with tamoxifen for 5 years (160).

Further research is required to clarify the biological significance of CYP1A1, CYP1B1, CYP3A4 and CYP3A5 in human breast cancer tissues.

Conclusion

The local biosynthesis of oestrogens especially in post-menopausal women as a result of the interactions of various enzymes is believed to play a very important role in the pathogenesis and development of hormone dependent breast carcinoma. The over-expression of such enzymes seems to be associated with the development of a more aggressive disease and associated with a poorer outcome and increased local and distant recurrences. The understanding of the mechanisms that regulate these enzymes is crucial to the development of new endocrine, preventative and therapeutic strategies in post-menopausal females with hormone dependant breast cancer.

Currently, the third generation of aromatase inhibitors has revolutionized the treatment of oestrogen dependant breast cancer in post-menopausal women. However, the important role of both STS and 17βHSD type 1 in local oestrogen production, provides novel potential targets for endocrine therapy. The inhibition of STS and 17βHSD 1, in addition to aromatase inhibition, is believed to be very important in stopping the local production of oestrogen and therefore the inhibition of development and recurrence of breast carcinoma. Such new strategies are currently being explored and the development of STS inhibitors and 17βHSD 1 inhibitors is underway, and the initial results are promising.

References


23 Lipworth L, Adami HO, Trichopoulos D, Carlstrom K and Mantzoros C: Serum steroid hormone levels, sex hormone-binding globulin, and body mass index in the etiology of postmenopausal breast cancer. Epidemiology 7: 96-100, 1996.


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