Review

Oestrogen-Synthesising Enzymes and Breast Cancer

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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 dehydro-genase 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

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Key Words: Breast cancer, estrogen, postmenopausal, aromatase, 17 beta HSD type 1, Steroid sulphatase, carcinogenesis, review.
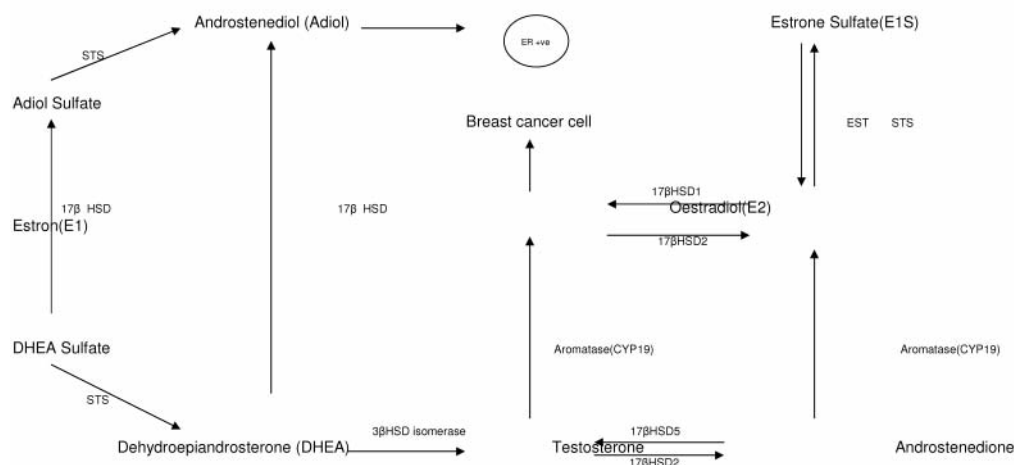
Figure 1. *The origin of estrogenic steroids in postmenopausal women with hormone dependant breast cancer. HSD, hydroxy steroid dehydrogenase; STS, steroid sulfatase; EST, estrogen sulfotransferase.*

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 androsteonedione 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 oestrone 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 (P450 arom) 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 oestrogen-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-II, 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 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 antidifferentiative 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 PI.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
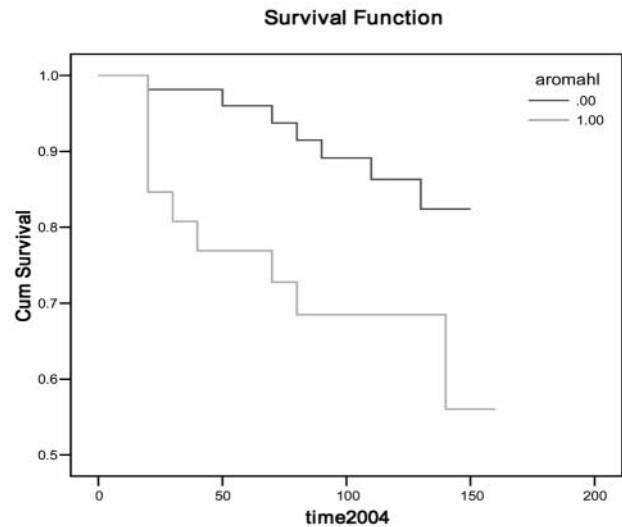

Figure 2. *Kaplan-Meier analysis of disease-free survival of breast cancer patients depending on the expression of Aromatase mRNA (p=0.0105) (71). 0=Low levels; 1.00=High levels.*

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: arimadex alone, tamoxifen alone or arimadex 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-Hydoxysteroid 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 carcinogensis. 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).
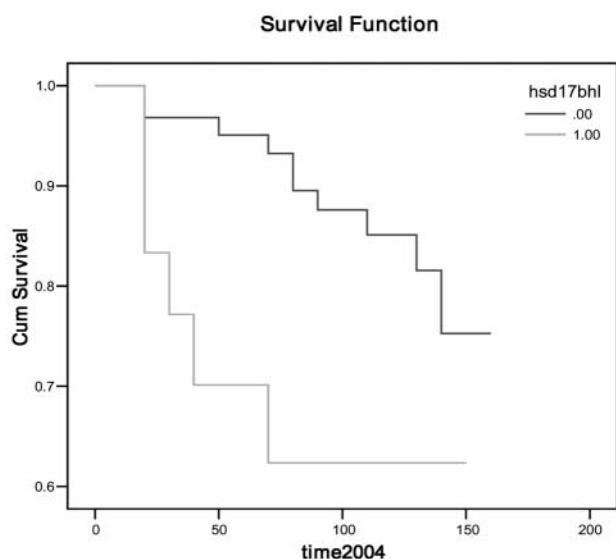
Figure 3. *Kaplan-Meier analysis of disease-free survival of breast cancer patients depending on the expression of 17 beta HSD type 1 mRNA (p=0.0182) (71). 0=Low levels; 1.00=High levels.*

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 ethananone (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 super-family 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-I 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 oestron 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 carcinogensis. 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 carcinogen-induced mammary tumors in ovariectomized rats (124).
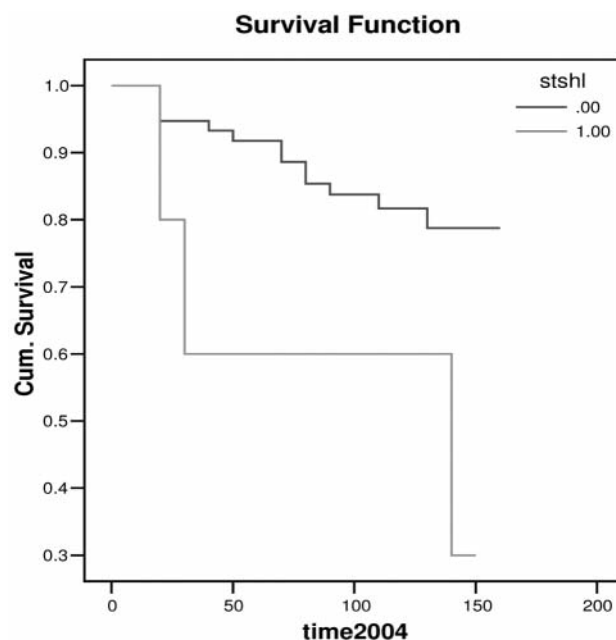

Figure 4. *Kaplan-Meier analysis of over all survival of breast cancer patients depending on the expression of STS mRNA (p=0.0452) (121). 0=low levels; 1.00=high levels.*

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 enatipure 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 in still unclear; cAMP and protein kinase-C play a role in the regulation of isoform 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 epostane. 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. Immunohisto-chemical 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 C15a 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, C6ab, C12, C15a and C16ab-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

1 Sasco AJ: Breast cancer and the environment. Horm Res 60: 50, 2003.

2 Henderson IC and Canellos GP: Cancer of the breast: The past decade. N Engl J Med 302: 17-30, 1990.

3 Henderson HS, Ross RK and Bernstein L: Estrogens as a cause of human Cancer: The Richard and Hinda Rosenthal Foundation award Lecture. Cancer Research 48: 246, 1988.

4 Page DL, Dupont WD, Rogers LW and Rados MS: Atypical hyperplastic lesions of the female breast; a longterm follow-up study. Cancer 55: 2698-2708,1985.

5 Russo J, Hasan Lareef M, Balogh G, Guo S and Russo IH: Estrogen and its metabolites are carcinogenic agents in human breast epithelial cells. J Steroid Biochem Mol Biol 87: 1-25, 2003.

6 Chen S: Aromatase and breast cancer. Front Biosci 3: 922-933, 1998.

7 London SJ, Connolly JL, Schnitt SJ and Colditz GA: A prospective study of benign breast disease and the risk of breast cancer. JAMA 267: 941-944, 1992.

8 Shekhar PVM, Werdell J and Barsrur VS: Environmental estrogen stimulation of growth and estrogen receptor function in preneoplastic and cancerous human breast cell lines. J Natl Cancer Inst 89: 1774-1782, 1997.

9 Dao TL: The role of the ovarian steroid hormones in mammary carcinogenesis. In: Hormones and Breast Cancer. (anbury repport no.8). Pike MC, Siiteri PK and Welsch CW (eds.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor. NY, pp. 281-295, 1981.

10 Wadia PR, Vandenberg LN, Schaeberle CM, Rubin BS, Sonnenschein C and Soto AM: Perinatal bisphenol A exposure increases estrogen sensitivity of the mammary gland in diverse mouse strains. Environ Health Perspect 115: 592-598, 2007.

11 Muñoz-de-Toro M, Markey CM, Wadia PR, Luque EH, Rubin BS, Sonnenschein C and Soto AM: Perinatal exposure to bisphenol-A alters peripubertal mammary gland development in mice. Endocrinology 146: 4138-4147, 2005.

12 Murray TJ, Maffini MV, Ucci AA, Sonnenschein C and Soto AM: Induction of mammary gland ductal hyperplasias and carcinoma in situ following fetal bisphenol A exposure. Reprod Toxicol 23: 383-390, 2007.

13 Pietras RJ, Marquez DC, Chen HW, Tsai E, Weinberg O and Fishbein M: Estrogen and growth factor receptor interactions in human breast and non-small cell lung cancer cells. Steroids 70: 372-381, 2005.

14 Garvin S, Nilsson UW and Dabrosin C: Effects of oestradiol and tamoxifen on VEGF, soluble VEGFR-1, and VEGFR-2 in breast cancer and endothelial cells. Br J Cancer 93: 1005-1010, 2005.

15 Maor S, Mayer D, Yarden RI, Lee AV, Sarfstein R, Werner H and Papa MZ: Estrogen receptor regulates insulin-like growth factor-I receptor gene expression in breast tumor cells: involvement of transcription factor Sp1. J Endocrinol 191: 605-612, 2006.

16 Redeuilh G, Attia A, Mester J and Sabbah M: Transcriptional activation by the oestrogen receptor alpha is modulated through inhibition of cyclin-dependent kinases. Oncogene 21: 5773-5782, 2002.

17 Yue W, Wang JP, Hamilton CJ, Demers LM and Santen RJ: In situ aromatization enhances breast tumor estradiol levels and cellular proliferation. Cancer Res 58: 927-932, 1998.

18 Planas-Silva MD and Waltz PK: Estrogen promotes reversible epithelial-to mesenchymal-like transition and collective motility in MCF-7 breast cancer cells. J Steroid Biochem Mol Biol 104: 11-21, 2007.

19 Sasano H, Okamoto M, Mason JI, Simpson ER, Mendelson CR, Sasano N and Silverberg SG: Immunolocalization of aromatase, 17 alpha-hydroxylase and side-chain-cleavage cytochromes P-450 in the human ovary. Reprod Fertil 85: 163-169, 1989.

20 Sasano H: Functional pathology of human ovarian steroidogenesis: Normal cycling ovary and steroidproducing neoplasms. Endo Pathol 5: 81-89, 1994.

21 Labrie F, Luu-The V, Labrie C, Belanger A, Simard J, Lin SX and Pelletier G: Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. Endocr Rev 24: 152-182, 2003.

22 Berrino F, Muti P, Micheli A, Bolelli G, Krogh V, Sciajno R, Pisani P, Panico S and Secreto G: Serum sex hormone levels after menopause and subsequent breast cancer. J Natl Cancer Inst *88*: 291-296, 1996.

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.

24 Dorgan JF, Stanczyk FZ, Longcope C, Stephenson Jr HE, Chang L, Miller R, Franz C, Falk RT and Kahle L: Relationship of serum dehydroepiandrosterone (DHEA), DHEA sulfate, and 5-androstene-3 beta, 17 beta-diol to risk of breast cancer in postmenopausal women. Cancer Epidemiol Biomarkers Prev *6*: 177-181, 1997.

25 Sasano H and Harada N: Intratumoral aromatase in human breast, endometrial, and ovarian malignancies. Endocrine Reviews *19*: 593-607, 1998.

26 Miller WR: Aromatase activity in breast tissue. J Steroid Biochem Mol Biol *39*: 783-790, 1991.

27 Schweikert HU, Milewich L and Wilson JD: Aromatization of androstenedione by cultured human fibroblasts. J Clin Endocrinol Metab *43*: 785-795, 1976.

28 Longcope C, Pratt JH, Schneider SN and Fineberg SE: Aromatization of androgens by muscle and adipose tissue *in vivo*. J Clin Endocrinol Metab *46*: 146- 152, 1978.

29 Sasano H, Uzuki M, Sawai T, Nagura H, Matsunaga G, Kashimoto O and Harada N: Aromatase in human bone tissue. J Bone Miner Res *12*: 1416-1423, 1997.

30 Santner SJ, Chen S, Zhou D, Korsunsky Z, Martel J and Santen RJ. Effect of androstenedione on growth of untransfected and aromatase-transfected MCF-7 cells in culture. J Steroid Biochem Mol Biol *44*: 611-616, 1993.

31 Chetrite GS, Cortes-Prieto J, Philippe JC, Wright F and Pasqualini JR: Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues. J Steroid Biochem Mol Biol *72*: 23-27, 2000.

32 Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M and Maloche C: Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and postmenopausal breast cancer patients. J Clin Endocr Metab *81*: 1460-1464, 1996.

33 Pasqualini JR: The selective estrogen enzyme modulators in breast cancer: a review. Biochimica et Biophysica Acta *1654*: 123-143, 2004.

34 Reed MJ, Owen AM, Lai LC, Coldham NG, Ghilchik MW, Shaikh NA and James VHT: *In situ* oestrone synthesis in normal breast and breast tumour tissues: effect of treatment with 4-hydroxyandrostenedione. Int J Cancer *44*: 233-237, 1989.

35 Reed MJ, Purohit A, Howarth NM and Potter BVL: Steroid sulphatase inhibitors: a new endocrine therapy. Drugs Future *19*: 673-680, 1994.

36 Miyoshi Y, Ando A, Hasegawa S, Ishitobi M, Taguchi T, Tamaki Y and Noguchi S: High expression of steroid sulfatase mRNA predicts poor prognosis in patients with estrogen receptor-positive breast cancer. Clin Cancer Res *9*: 2288-2293, 2003.

37 Nelson DR, Koymans L, Kamataki T, stegeman JJ, Feyereisen R, Waxman DJ, Waterman MR, Gotoh O, Coon MJ, Estabrook RW, Gunsalus IC and Nebert DW: P450 superfamily: Update on new sequences, gene mapping, accession numbers and nomenclature. Pharmacogenetics *6*: 1-42, 1996.

38 Chen SA, Besman MJ, Sparkes RS, Zollman S, Klisak I, Mohandas T, Hall PF and Shively JE: Human aromatase: cDNA cloning, Southern blot analysis, and assignment of the gene to chromosome 15. DNA *7*: 27-38, 1988.

39 Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Hinshelwood MM, Graham-Lorence S, Amarneh B, Ito Y, Fisher CR, Michael MD, Mendelson CR and Bulun SE: Aromatase cytochrome P450, the enzyme responsible for estrogen biosynthesis. Endocr Rev *15*: 342-355, 1994.

40 Sebastian S and Bulun SE: A highly complex organization of the regulatory region of the human CYP 19 (Aromatase) gene revealed by the Human Genome Project. J Clin Endocrinol Metab *86*: 4600-4602, 2001.

41 Simpson ER, Clyne C, Rubin G, Boon WC, Robertson K, Britt K, Speed C and Jones M: Aromatase – a brief overview. Annu Rev Physiol *64*: 93-127, 2002.

42 Simpson ER: Sources of estrogen and their importance. J Steroid Biochem Mol Biol *86*: 225-230, 2003.

43 Nelson LR and Bulun SE: Estrogen production and action. J Am Acad Dermatol *45*: 116-124, 2001.

44 Simpson ER and Davis SR: Minireview: Aromatase and the regulation of estrogen biosynthesis- some new perspectives. Endocrinology *142*: 4589-4594, 2001.

45 Sebastian S, Takayama K, Shozu M and Bulun SE: Cloning and characterization of a novel endothelial promoter of the human CYP19 (aromatase P450) gene that is up-regulated in breast cancer tissue. Mol Endocrinol *16*: 2243-2254, 2002.

46 Harada N: A unique aromatase (P-450arom) mRNA formed by alternative use of tissue-specific exons 1 in human skin fibroblasts. Biochem Biophys Res Commun *189*: 1001-1007, 1993.

47 Zhou C, Zhou D, Esteban J, Murai J, Siiteri P.K, Wilczynski S and Chen S: Aromatase gene expression and its exon I usage in human breast tumors. Detection of aromatase messenger RNA by reverse transcription-polymerase chain reaction (RT-PCR). J Steroid Biochem Mol Biol *59*: 163-171, 1996.

48 Bulun SE, Noble LS, Takayama K, Michael MD, Agarwal V, Fisher C, Zhao Y, Hinshelwood MM, Ito Y and Simpson ER: Endocrine disorders associated with inappropriately high aromatase expression. J Steroid Biochem Mol Biol *61*: 133-139, 1997.

49 Simpson ER, Michael MD, Agarwal VR, Hinshelwood MM, Bulun SE and Zhao Y: Cytochromes P450 11: expression of the CYP19 (aromatase) gene: an unusual case of alternative promoter usage. FASEB J *11*: 29-36, 1997.

50 Harada N, Utsumi T and Takagi Y: Tissue-specific expression of the human aromatase cytochrome P-450 gene by alternative use of multiple exons 1 and promoters, and switching of tissuespecific exons 1 in carcinogenesis. Proc Natl Acad Sci USA *90*: 11312-11316, 1993.

51 Esteban JM, Warsi Z, Haniu M, Hall P, Shively JE and Chen S: Detection of intratumoral aromatase in breast carcinomas. Am J Pathol *140*: 337-343, 1992.

52 Purohit A, Ghilchik MW, Duncan LJ, Wang DY, Singh A, Walker MM and Reed MJ: Aromatase activity and interleukin 6 production by normal and malignant breast tissues. J Clin Endocrinol Metab *80*: 3052-3058, 1995.

53 Singh A, Purohit A, Duncan LJ, Mokbel K, Ghilchik MW and Reed MJ: Control of aromatase in breast tumours: the role of the immune system. J Steroid Biochem Mol Biol *61*: 185-192, 1997.

54 Reed MJ, Coldham NG, Patel SR, Ghilchik MW and James VHT: Interleukin-1 and interleukin-6 in breast cyst fluid: their role in regulating aromatase activity in breast cancer cells. J Endocrinol 132: R5-R8, 1992.

55 Reed MJ and Purohit A: Sulphatase inhibitors: the rationale for the development of a new endocrine therapy. Rev Endocr Rel Cancer 45: 51-62, 1993.

56 Miller WR and Mullen P: Factors influencing aromatase activity in the breast. J Steroid Biochem Molec Biol 44: 597-604, 1993.

57 Zhao Y, Nichols JE, Bulnn SE, Mendelson CR and Simpson ER: Aromatase P450 gene expression in human adipose tissue. J Biol Chem 270: 16449-16457, 1995.

58 Singh A, Purohit A, Wang DY, Duncan LJ, Ghilchik MW and Reed MJ: IL-6sR: release from MCF-7 breast cancer cells and role in regulating peripheral oestrogen synthesis. J Endocrinol 147: R9-R12, 1995.

59 Macdiarmid F, Wang DY, Duncan LJ, Purohit A, Ghilchik MW and Reed MJ: Stimulation of aromatase activity in breast fibroblasts by tumour necrosis factor α. Molec Cell Endocr 106: 17-21, 1994.

60 Meng L, Zhou J, Hironobu S, Suzuki T, Zeitoun K and Bulun S: TNFalpha and IL-11 secreted by malignant breast epithelial cells inhibit adipocyte differentiation by selectively down-regulating C/EBPalpha and PPARgamma: mechanism of desmoplastic reaction. Cancer Res 61: 2250-2255, 2001.

61 Agarwal V, Bulun SE, Leitch M, Rohrich R and Simpson ER: Use of alternative promoters to express the aromatase P450 (CYP19) gene in breast adipose tissues of cancer-free and breast cancer patients. J Clin Endocrinol Metab 81: 3843-3849, 1996.

62 Zhao Y, Agarwal VR, Mendelsohn CR and Simpson ER: Transcriptional regulation of CYP19 (aromatase) gene expression in adipose stromal cells in primary culture. J Steroid Biochem Mol Biol 61: 203-210, 1997.

63 Singh A, Purohit A, Ghilchik MW and Reed MJ: The regulation of aromatase activity in breast fibroblasts: the role of interleukin- 6 and prostaglandin E2. Endocr-Relat Cancer 6: 139-147, 1999.

64 Miller WR and O'Neill JS: The importance of local synthesis of estrogen within the breast. Steroids 50: 537-547, 1987.

65 Bulun SE, Price TM, Aitken J, Mahendroo MS and Simpson ER: 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.

66 Utsumi T, Harada N, Maruta M and Takagi Y: Presence of alternatively spliced transcripts of aromatase gene in human breast cancer. J Clin Endocrinol Metab 81: 2344-2349, 1996.

67 James VHT, McNeill J M, Lai LC, Newton CJ, Ghilchik MW and Reed MJ: Aromatase activity in normal breast and breast tumor tissues: in vivo and in vitro studies. Steroids 50: 269-279, 1987.

68 Zhou D, Pompon D and Chen S: Stable expression of human aromatase cDNA in mammalian cells – A useful system for aromatase inhibitor screening. Cancer Res 50: 6949-6954, 1990.

69 Yue W, Zhou D, Chen S and Brodie A: A new nude mouse model for post.menopausal breast cancer using MCF-7 cells transfected with the htmmn aromatase gene. Cancer Res 54: 5092-5095, 1994.

70 Macaulay VM, Nicholls J E, GledhiU J, Rowlands MG, Dowsett M and Ashworth A: Biological effects of stable overexpression of aromatase in human hormone-dependent breast cancer ceils. Br J Cancer 69: 77-83, 1994.

71 Salhab M, Reed MJ, Al Sarakbi W, Jiang WG and Mokbel K: The role of aromatase and 17-beta hydroxysteroid dehydrogenase type 1 mRNA expression in predicting the clinical outcome of human breast cancer. Breast Cancer Res Treat 99: 155-162, 2006.

72 Miller WR, Anderson TJ and Lack WJL: Relationship between tumor aromatase activity, tumor characteristics and response to therapy, fr. Steroid Biochem. Molec Biol 37: 1055-1059, 1990.

73 Sasano H, Nagura H, Harada N, Goukon Y and Kimyra M: Immunolocalization of aromatase and other steroidogenic enzymes in human breast disorders. Hure Pathol 25: 530-535, 1994.

74 Zhang Z, Yamashita H, Toyama T, Hara Y, Omoto Y, Sugiura H, Kobayashi S, Harada N and Iwase H: Semi quantitative immunohistochemical analysis of aromatase expression in ductal carcinoma in situ of the breast. Breast Cancer Res Treat 74: 47-53, 2002.

75 Brodie A, Lu Q and Nakamura J: Aromatase in the normal breast and breast cancer. J Steroid Biochem Mol Biol 61: 281-286, 1997.

76 The ATAC (Arimidex Tamoxifen alone or in Combination) Trialists' Group, Anastrozole alone or in combination with tamoxifen versus tamoxifen alone for adjuvant treatment of postmenopausal women with early breast cancer: first results of the ATAC randomised trial, Lancet 359: 2131-2139, 2002.

77 Cunnick GH and Mokbel K: Aromatase inhibitors. Curr Med Res Opin 17: 217-22, 2001.

78 Mokbel K: The evolving role of aromatase inhibitors in breast cancer. Int J Clin Oncol 7: 279-283, 2002.

79 Mokbel R, Karat I and Mokbel K: Adjuvant endocrine therapy for postmenopausal breast cancer in the era of aromatase inhibitors: an update. Int Semin Surg Oncol 3: 31, 2006.

80 Thurlimann B, Keshaviah A, Coates AS, Mouridsen H, Mauriac L, Forbes JF, Paridaens R, Castiglione-Gertsch M, Gelber RD, Rabaglio M, Smith I, Wardly A, Price KN and Goldhirsch A: Breast International Group (BIG) 1–98 Collaborative Group. A comparison of letrozole and tamoxifen in postmenopausal women with early breast cancer. N Engl J Med 353: 2747-2757, 2005.

81 Luu-The V, Labrie C, Zhao HF, Couet J, Lachance Y, Simard J, Leblanc G, Cote J, Berube D, Gagne R and Labrie F: Characterization of cDNAs for human estradiol 17beta-dehydrogenase and assignment of the gene to chromosome 17: evidence of two mRNA species with distinct 50 -termini in human placenta. J Mol Endocrinol 3: 1301-1309, 1989.

82 Miettinen MM, Mustonen MVJ, Poutanen MH, Isomaa VV and Vihko RK: Human 17b-hydroxysteroid dehydrogenase type 1 and type 2 isozymes have opposite activities in 690 cultured cells and characteristic celland tissue-specific expression. Biochem J 314: 839-845, 1996.

83 Gunnarsson C, Ahnstrom M, Kirschner K, Olsson B, Nordenskjold B, Rutqvist LE, Skoog L and Stal O: Amplification of HSD17B1 and ERBB2 in primary breast cancer. Oncogene 22: 34-40, 2003.

84 Takeyama J, Sasano H, Suzuki T, Iinuma K, Nagura H and Andersson S: 17Betahydroxysteroid dehydrogenase types 1 and 2 in human placenta: an immunohistochemical study with correlation to placental development. J Clinic Endocrinol Metabol 83: 3710-3715, 1998.

85 Takeyama J, Suzuki T, Hirasawa G, Muramatsu Y, Nagura H, Iinuma K, Nakamura J, Kimura KI, Yoshihama M, Harada N, Andersson S and Sasano H: 17beta-hydroxysteroid dehydrogenase type 1 and 2 expression in the human fetus. J Clinic Endocrinol Metabol *85*: 410-416, 2000.

86 Adams EF, Rafferty B and White MC: Interleukin-6 is secreted by breast fibroblasts and stimulates 17 beta oxidoreductase activity in MCF-7 breast cancer cells: possible paracrine regulation of 17beta oestradiol levels. Int J Cancer *49*: 118-121, 1991.

87 Duncan LJ, Coldham NG and Reed MJ: The interaction of cytokines in regulating oestradiol 17beta hydroxysteroid dehydrogenase activity in MCF-7 cells. J Steroid Biochem Mol Biol *49*: 63-68, 1994.

88 Singh A, Blench I, Morris HR, Savoy LA and Reed MJ: Synergistic interaction of growth factors and albumin in regulating estrogen synthesis in breast cancer cells. Mol Cell Endocrinol *85*: 165-173, 1992.

89 Miller WR, Hawkins RA and Forrest APM: Significance of aromatase activity in human breast cancer. Cancer Res *42*: 3365-3368, 1982.

90 Perel E, Wilkins D and Killinger DW: The conversion of androstenedione to estrone, estradiol and testosterone in breast tissue. J Steroid Biochem *13*: 89-94, 1980.

91 Suzuki T, Moriya T, Ariga N, Kaneko C, Kanazawa M and Sasano H: 17 Beta hydroxysteroid dehydrogenase type 1 and type 2 in human breast carcinoma: a correlation to clinicopathological parameters. Br J Cancer *82*: 518-523, 2000.

92 Husen B, Huhtinen K, Saloniemi T, Messinger J, Thole HH and Poutanen M: Human hydroxysteroid (17-beta) dehydrogenase 1 expression enhances estrogen sensitivity of MCF-7 breast cancer cell xenografts. Endocrinology *147*: 5333-5339, 2006.

93 Sasano H, Frost AR, Saitoh R, Harada N, Poutanen M, Vihko R, Buhm SE, Silverberg SG and Nagura H: Aromatase and 17beta hydroxysteroid dehydrogenase type 1 in human breast carcinoma. J Clin Endocrinol Metab *11*: 4042-4046, 1996.

94 Suzuki T, Nakata T, Miki Y, Kaneko C, Moriya T, Ishida T, Akinaga S, Hirakawa H, Kimura M and Sasano H: Estrogen sulfotransferase and steroid sulfatase in human breast carcinoma. Cancer Res *63*: 2762-2770, 2003.

95 Ariga N, Moriya T, Suzuki T, Kimura M, Ohuchi N, Satomi S and Sasano H: 17 beta-Hydroxysteroid dehydrogenase type 1 and type 2 in ductal carcinoma *in situ* and intraductal proliferative lesions of the human breast. Anticancer Research *20*: 1101-1108, 2000.

96 Oduwole OO, Li Y, Isomaa VV, Mantyniemi A, Pulkka AE, Soini Y and Vihko PT: 17beta-hydroxysteroid dehydrogenase type 1 is an independent prognostic marker in breast cancer. Cancer Res *64*: 7604-7609, 2004.

97 Zhang Z, Yamashita H, Toyama T, Omoto Y, Sugiura H, Hara Y, Wu X, Kobayashi S and Iwase H: Quantitative determination, by real-time reverse transcription polymerase chain reaction, of aromatase mRNA in invasive ductal carcinoma of the breast. Breast Cancer Res *5*: 250-256, 2003.

98 Gunnarsson C, Olsson BM and Stal O: Southeast Sweden Breast Cancer GroupAbnormal expression of 17beta-hydroxysteroid dehydrogenases in breast cancer predicts late recurrence. Cancer Res *61*: 8448 -8451, 2001.

99 Gunnarsson C, Jerevall PL, Hammar K, Olsson B, Nordenskjöld B, Jansson A and Stål O: Amplification of HSD17B1 has prognostic significance in postmenopausal breast cancer. Breast Cancer Res Treat *108*: 35-41, 2008.

100 Feigelson SH, McKean-Cowdin R, Coetzee GA, Stram DO, Kolonel LN and Henderson BE: Building a multigenic model of breast cancer susceptibility: CYP 17 and HSD 17 beta 1 are two important candidates. Cancer Res *61*: 785-789, 2001.

101 Fischer DS, Allan GM, Bubert C, Vicker N, Smith A, Tutill HJ, Purohit A, Wood L, Packham G, Mahon MF, Reed MJ and Potter BV: E-ring modified steroids as novel potent inhibitors of 17 beta-hydroxysteroid dehydrogenase type 1. J Med Chem *48*: 5749-5770, 2005.

102 Bond CS, Clements PR, Ashby SJ, Collyer CA, Harrop SJ, Hopwood JJ and Guss JM: Structure of a human lysosomal sulfatase. Structure *5*: 277-289, 1997.

103 Ferrante P, Messali S, Meroni G and Ballabio A: Molecular and biochemical characterisation of a novel sulphatase gene: aryl sulphatase G (ARSG). Eur J Hum Genet *10*: 813-818, 2002.

104 Yen PH, Marsh B, Allen E, Tsai SP, Ellison J, Connoly L, Neiswanger K and Shapiro LJ: The human X-linked steroid sulfatase gene and a Y-encoded pseudogene: evidence for an inversion of the Y chromosome during primate evolution. Cell *55*: 1123-1135, 1988.

105 Reed MJ, Purohit A, Woo LW, Newman SP and Potter BV: Steroid sulfatase: molecular biology, regulation, and inhibition. Endocr Rev *26*: 171-202, 2005.

106 Purohit A and Reed MJ: Oestrogen sulphatase activity in hormone-dependent and hormone-independent breast cancer cells: modulation by steroidal and non760 steroidal therapeutic agents. Int J Cancer *50*: 901-905, 1992.

107 Newman SP, Purohit A, Ghilchik MW, Potter BVL and Reed MJ: Regulation of steroid sulphatase expression and activity in breast cancer. J Steroid Biochem Mol Biol *75*: 259-264, 2000.

108 Pasqualini JR, Maloche C, Maroni M and Chetrite G: Effect of the progestagen Promegestone (R-5020) on mRNA of the oestrone sulphatase in the MCF-7 human mammary cancer cells. Anticancer Res *14*: 1589-1594, 1994.

109 Reed MJ, Purohit A, Woo LWL and Potter BVL: The development of steroid sulphatase inhibitors. Endocr Rel Cancer *3*: 9-23, 1996.

110 Reed MJ, Topping L, Coldham NG, Purohit A, Ghilchik MW and James VHT: Control of aromatase activity in breast cancer cells: the role of cytokines and growth factors. J Steroid Biochem Molec Biol *44*: 589-596, 1993.

111 Reed MJ and Purohit A: Inhibition of steroid sulphatases. *In*: Sandler M, Smith HJ (eds.). Design of enzyme inhibitors as drugs, vol. 2. Oxford: Oxford University Press, pp. 481-494, 1994.

112 Basler E, Grompe M, Parenti G, Yates J and Ballabio A: Identification of point mutations in the steroid sulfatase gene of three patients with X-linked ichthyosis. Am J Hum Genet *50*: 483-491, 1992.

113 Alperin ES and Shapiro LJ: Characterization of point mutations in patients with Xlinked ichthyosis. Effects on the structure and function of the steroid sulfatase protein. J Biol Chem *272*: 20756-20763, 1997.

114 Poortman J, Andriesse R, Agema A, Douker GH, Schwarz F and Thijssen JHH: *In*: Adrenal androgen secretion and metabolism in postmenopausal women. Genazzani AR, Thijssen JHH, Siiteri PK (eds.). Adrenal androgens. New York: Raven Press, pp. 219-240, 1980.

115 Poulin R and Labrie F: Stimulation of cell proliferation and estrogenic response by adrenal C19- delta 5-steroids in the ZR-75–1 human breast cancer cell line. Cancer Res 46: 4933-4937, 1986.

116 Maggiolini M, Donze O, Jeannin E, Ando S and Picard D: Adrenal androgens stimulate the proliferation of breast cancer cells as direct activators of estrogen receptor alpha. Cancer Res 59: 4864-4869, 1999.

117 Morris KT, Toth-Fejel SE, Schmidt J, Fletcher WS and Pommier RF: High dehydroepiandrosterone-sulfate predicts breast cancer progression during new aromatase inhibitor therapy and stimulates breast cancer cell growth in tissue culture: a renewed role for adrenalectomy. Surgery 130: 947-953, 2001.

118 Utsumi T, Yoshimura N, Takeuchi S, Maruta M, Maeda K and Harada N: Elevated steroid sulfatase expression in breast cancer. J Steroid Biochem Mol Biol 73: 141-145, 2000.

119 Evans TR, Rowlands MG, Law M and Coombes RC: Intratumoral oestrone sulphatase activity as a prognostic marker in human breast carcinoma. Br J Cancer 69: 555-561, 1994.

120 Utsumi T, Yoshimura N, Takeuchi S, Ando J, Maruta M, Maeda K and Harada N: Steroid sulfatase expression is an independent predictor of recurrence in human breast cancer. Cancer Res 59: 377-381, 1999.

121 Al Sarakbi W, Mokbel R, Salhab M, Jiang WG, Reed MJ and Mokbel K: The Role of STS and OATP-B mRNA Expression in Predicting the Clinical Outcome in Human Breast Cancer. Anticancer Res 26: 4985-4990, 2006.

122 Nakata T, Takashima S, Shiotsu Y, Murakata C, Ishida H, Akinaga S, Li PK, Sasano H, Suzuki T and Saeki T: Role of steroid sulfatase in local formation of estrogen in post-menopausal breast cancer patients. J Steroid Biochem Mol Biol 86: 455-460, 2003.

123 Selcer KW, Hegde PV and Li PK: Inhibition of estrone sulfatase and proliferation of human breast cancer cells by nonsteroidal (p-O-sulfamoyl)-N-alkanoyl tyramines. Cancer Res 57: 702-707, 1997.

124 Purohit A, Woo LWL, Potter BVL and Reed MJ: In vivo inhibition of estrone sulfatase activity and growth of nitrosomethylurea-induced mammary tumors by 667 COUMATE. Cancer Res 60: 3394-3396, 2000.

125 Fischer DS, Chander SK, Woo, Fenton JC, Purohit A, Reed MJ and Potter BV: Novel D-ring modified steroid derivatives as potent, non-estrogenic steroid sulfatase inhibitors with in vivo activity. J Steroid Biochem Mol 84: 343-349, 2003.

126 Foster PA, Newman SP, Chander SK, Stengel C, Jhalli R, Woo LWL, Potter BVL, Reed MJ and Purohit A: In vivo efficacy of STX213, a second-generation steroid sulfatase inhibitor, for hormone-dependent breast cancer therapy. Clin Cancer Res 12: 5543-5549, 2006.

127 Ho YT, Purohit A, Vicker N, Newman SP, Robinson JJ, Leese MP, Ganeshapillai D, Woo LWL, Potter BVL and Reed MJ: Inhibition of carbonic anhydrase II by steroidal and non-steroidal sulphamates. Biochem Biophys Res Commun 305: 909-914, 2003.

128 Stanway SJ, Purohit A, Woo LWL, Sufi S, Vigushin D , Ward R, Wilson RH, Stanczyk FZ, Dobbs N, Kulinskaya E, Elliott M, Potter BVL, Reed MJ and Coombes RC: Phase I study of STX 64 (667 Coumate) in breast cancer patients: The first study of a steroid sulfatase inhibitor. Clin Cancer Res 12: 1585-1592, 2006.

129 Woo LW, Bubert C, Sutcliffe OB, Smith A, Chander SK, Mahon MF, Purohit A, Reed MJ and Potter BV: Dual Aromatase-Steroid Sulfatase Inhibitors. J Med Chem 26: 3540-60, 2007.

130 Wood PM, Woo LW, Humphreys A, Chander SK, Purohit A, Reed MJ and Potter BV: A letrozole-based dual aromatase-sulphatase inhibitor with in vivo activity. J Steroid Biochem Mol Biol 94: 123-130, 2005.

131 Gunasegaram R, Peh KL, Loganath A and Ratnam SS: Expression of 3beta-hydroxysteroid dehydrogenase-5,4-en isomerase activity by infiltrating ductal human breast carcinoma in vitro. Breast Cancer Res Treat 50: 117-123, 1998.

132 Sasano H, Nagura H, Harada N, Goukon Y and Kimura M: Immunolocalization of aromatase and other steroidogenic enzymes in human breast disorders. Human Pathology 5: 530-533, 1994.

133 Tremblay Y and Beaudoin C: Regulation of 3beta-hydroxysteroid dehydrogenase and 17betahydroxysteroid dehydrogenase messenger ribonucleic acid levels by cyclic adenosine 30 ,50-monophosphate and phorbol myristate acetate in human choriocarcinoma cells. J Mol Endocrinol 7: 355-364, 1993.

134 Gingras S, Moriggl R, Groner B and Simard J: Induction of 3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13. J Mol Endocrinol 13: 66-81, 1999.

135 Gingras S, Cote S and Simard J: Multiple signaling pathways mediate interleukin-4-induced 3betahydroxysteroid dehydro-genase/delta5-delta4 isomerase type 1 gene expression in human breast cancer cells. J Mol Endocrinol 14: 229-240, 2000.

136 Aksoy IA, Wood TC and Weinshilboum R: Human liver estrogen sulfotransferase: identification by cDNA cloning and expression. Biochem Biophys Res Commun 200: 1621-1629, 1994.

137. Falany CN, Krasnykh V and Falany JL: Bacterial expression and characterization of a cDNA for human liver estrogen sulfotransferase. J Steroid Biochem Mol Biol 52: 529-539, 1995.

138 Falany JL and Falany CN: Expression of cytosolic sulfotransferases in normal mammary epithelial cells and breast cancer cell lines. Cancer Research 56: 1551-1555, 1996.

139 Chetrite G, Le Nestour E and Pasqualini JR: Human estrogen sulfotransferase (hEST1) activities and its mRNA in various breast cancer cell lines. Effect of the progestin, promegestone (R-5020). J Steroid Biochem Mol Biol 66: 295-302, 1998.

140 Adams JB, Pewnim T, Chandra DP, Archibald L and Foo MS: A correlation between estrogen sulfotransferase levels and estrogen receptor status in human primary breast carcinoma. Cancer Research 39: 5124-5126,1979.

141 Tseng L, Mazella J, Lee LY and Stone ML: Estrogen sulfatase and estrogen sulfotransferase in human primary mammary carcinoma. J Steroid Biochem 4: 1413-1417, 1983.

142 Pasqualini JR, Chetrite G, Blacker C, Feinstein MC, Delalonde L, Talbi M and Maloche C: Concentrations of estrone, estradiol, and estrone sulfate and evaluation of sulfatase and aromatase activities in pre- and post-menopausal breast cancer patients. J Clin Endocrinol Metab 81: 1460-1464, 1996.

143 Huang Z, Fasco MJ, Figge HL, Keyomarsi K and Kaminsky S: Expression of cytochromes P450 in human breast tissue and tumors. Drug Metab Dispos 24: 899-905, 1996.

144 Iscan M, Klaavuniemi T, Coban T, Kapucuoglu N, Pelkonen O and Raunio H: The expression of cytochrome P450 enzymes in human breast tumours and normal breast tissue. Breast Cancer Res and Treat *70*: 47-54, 2001.

145 Hellmold H, Rylander T, Magnusson M, Reihner E, Warner M and Gustafsson JA: Characterization of cytochrome P450 enzymes in human breast tissue from reduction mammaplasties. J Clin Endocrinol Metab *83*: 886-895, 1998.

146 El-Rayes BF, Ali S, Heilbrun LK, Lababidi S, Bouwman D, Visscher D and Philip PA: Cytochrome p450 and glutathione transferase expression in human breast cancer. Clin Cancer Res *9*: 705-709, 2003.

147 Modugno F, Knoll C, Kanbour-Shakir A and Romkes M: A potential role for the estrogen-metabolizing cytochrome P450 enzymes in human breast carcinogenesis. Breast Cancer Res Treat *82*: 191-197, 2003.

148 McFadyen MC, Breeman S, Payne S, Stirk C, Miller ID, Melvin WT and Murray GI: Immunohistochemical localization of cytochrome P450 CYP1B1 in breast cancer with monoclonal antibodies specific for CYP1B1. J Histochem Cytochem *47*: 1457-1464, 1999.

149 Oyama T, Morita M, Isse T, Kagawa N, Nakata S, So T, Mizukami M, Ichiki Y, Ono K, Sugaya M, Uramoto H, Yoshimatsu T, Hanagiri T, Sugio K, Kawamoto T and Yasumoto K: Immunohistochemical evaluation of cytochrome P450 (CYP) and p53 in breast cancer. Front Biosci *10*: 1156-1161, 2005.

150 Liehr JG: Is estradiol a genotoxic mutagenic carcinogen? Endocrine Reviews *21*: 40-54, 2000.

151 Sam KM, Boivin, RP, Tremblay MR, Auger S and Poirier D: C16 and C17 derivatives of estradiol as inhibitors of 17β-hydroxysteroid dehydrogenase type 1: Chemical synthesis and structure-activity relationships. Drug Des and Discov *15*: 157-180, 1998.

152 Poirier D, Boivin RP, Bérube M and Lin SX: Synthesis of a first estradiol-adenosine hybrid compound. Bioorganic and Medicinal Chemistry *15*: 714-726, 2007.

153 Cadot, C, Laplante, Y, Kamal, F, Luu-The and Poirier D: C6-(N,N-butyl-methyl-heptanamide) derivatives of estrone and estradiol as inhibitors of type 1 17β-hydroxysteroid dehydrogenase: Chemical synthesis and biological evaluation. Bioorg Med Chem *15*: 714-726, 2007.

154 Allan GM, Vicker N, Lawrence HR, Tutill HJ, Day JM, Huchet M, Ferrandis E, Reed MJ, Purohit A and Barry Potter VL: Novel inhibitors of 17β-hydroxysteroid dehydrogenase type 1: Templates for design. Bioorg Med Chem *16*: 4438-4456, 2008.

155 Purohit A, Woo LW, Chander SK, Newman SP, Ireson C, Ho Y, Grasso A, Leese MP, Potter BV and Reed MJ: Steroid sulphatase inhibitors for breast cancer therapy. J Steroid Biochem Mol Biol *86*: 423-432, 2003.

156 Jackson T, Woo LW, Trusselle MN, Chander SK, Purohit A, Reed MJ and Potter BV: Dual aromatase-sulfatase inhibitors based on the anastrozole template: synthesis, *in vitro* SAR, molecular modelling and *in vivo* activity. Org Biomol Chem *5*: 2940-2952, 2007.

157 Wood PM, Woo LWL, Labrosse JR, Trusselle MN, Abbate S, Longhi G, Ettore Castiglioni, Lebon F, Purohit A, Reed MJ and Potter BV: Chiral aromatase and dual aromatase−steroid sulfatase inhibitors from the letrozole template: synthesis, absolute configuration, and *in vitro* activity. J Med Chem *51*: 4226-4238, 2008.

158 Thomas JL, Umland TC, Scaccia LA, Boswell EL and Kacsoh B: The higher affinity of human type 1 3beta-hydroxysteroid dehydrogenase (3beta-HSD1) for substrate and inhibitor steroids relative to human 3beta-HSD2 is validated in MCF-7 tumor cells and related to subunit interactions. Endocr Res *30*: 935-941, 2004.

159 Dumas NA, He D, Frost AR and Falany CN: Sulfotransferase 2B1b in human breast: Differences in subcellular localization in African American and Caucasian women. J Steroid Biochem Mol Biol *111*: 171-177, 2008.

160 Wegman P, Elingarami S, Carstensen J, Stål O, Nordenskjöld B and Wingren S: Genetic variants of CYP3A5, CYP2D6, SULT1A1, UGT2B15 and tamoxifen response in postmenopausal patients with breast cancer. Breast Cancer Res *9*: 7, 2007.