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Preclinical study

The role of aromatase and 17-β-hydroxysteroid dehydrogenase type 1 mRNA expression in predicting the clinical outcome of human breast cancer

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Summary

Introduction. There is substantial evidence that breast cancer tissue contains all the enzymes responsible for the local biosynthesis of estrogens from circulating precursors. The cytochrome P-450 aromatase enzyme complex is responsible for the conversion of C19 androgens to estrogens and 17-β-hydroxysteroid dehydrogenase (17-β-HSD) type 1 catalyses the inter-conversion of estrone to the biologically more potent estradiol. The gene encoding for the cytochrome P-450 aromatase is known as CYP19 (15q21.2). It is well established that increased exposure to local estrogens is an important risk factor in the genesis and growth of breast cancer. The aim of this study is to investigate the relationship between CYP19 and 17-β-HSD type 1 mRNA expression and clinico-pathological parameters of human breast cancer.

Methods. One hundred and twenty seven tumor tissues and 33 normal tissues were analyzed. The levels of transcription of CYP19 and 17-β-HSD type 1 were determined using real-time quantitative PCR. The mRNA expression was normalized against CK19. Levels of expression were analyzed against tumor's stage, grade, nodal status, local relapse, distant metastasis and survival over a 120 months follow up period. In addition, the levels were analyzed against estrogen receptor (ER) and HER1-4 status.

Results. Overall, high tumor levels of mRNA expression of CYP19 and 17-β-HSD type 1 correlated with poor survival ($p = 0.0105$ and $p = 0.0182$, respectively). Increased levels of CYP19 mRNA expression positively correlated with disease progression as levels were significantly higher in samples of patients who had distant metastasis and local recurrence and/or died of breast related causes when compared to those who were disease free for > 10 years ($p = 0.0015$). We also observed higher levels of CYP19 mRNA in tumor samples compared to normal breast tissue. However, this reached statistical significance only when comparing grade 1 tumors with normal tissue ($p = 0.01$). There was no correlation between CYP19 mRNA expression and tumor stage, lymph node status and tumor grade. There was however a trend for a positive correlation between CYP19 and ER mRNA expressions ($p = 0.06$). No significant difference in 17-β-HSD type 1 expression between normal and cancerous tissues was observed. In tumor samples, we observed an increase in levels correlating with tumor's grade. This correlation was statistically significant when we compared grade 1 with grade 2 and grade 1 with grade3 ($p = 0.0031$ and 0.0251, respectively).

Conclusion. Our study shows that higher levels of the enzymes responsible for the local biosynthesis of estrogens especially aromatase are associated with a poor clinical outcome in patients with breast cancer.

Introduction

It is widely accepted that breast cancer is a hormone-dependent tumor, and endogenous estrogen plays an important role in the development and progression of breast cancer. Approximately 60% of pre-menopausal and 75% of post-menopausal patients with breast cancer have estrogen-dependent carcinomas [1].

Estrogens induce the expression of peptide growth factors which are responsible for the proliferative responses of cancer cells [2,3]. Furthermore, estrogen has been shown to upregulate 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 [4,5].

There is substantial evidence that breast cancer tissue contains all the enzymes responsible for the local biosynthesis of estrogens from circulating precursors [6,7].

The local production of estradiol is mediated by three main enzymes within the breast tissue including the breast cancerous tissues. Estradiol is biosynthesized

from androgens by the cytochrome P-450 enzyme complex known as aromatase. This enzyme converts Adione to estrone E1. A second enzyme called 17-β-HSD type 1 plays an important role by reducing E1 into E2 which is the most potent endogenous estrogen. Moreover, steroid sulfatase (STS) acts on estrone sulfate E1S, formed as a result of sulfotransferase activity, to form E1, which is subsequently reduced to E2 [8].

The formation of estrogen (C18 steroids) from C19 steroids mainly androstenedione and testosterone involves sequential hydroxylation, oxidation and removal of the C-19 carbon and aromatization of the steroid A ring which is controlled by P-450 aromatase (P450 arom). Since aromatase is a key enzyme responsible for the synthesis of estrogens, and estrogens play an important role in breast cancer development, therefore, abnormal expression of aromatase in breast cancer cells and/or surrounding tissue may exert a significant influence on breast tumor maintenance and growth in breast cancer patients [9,10]. Estrogens levels in breast cancer specimens were found to be several fold higher than those of plasma in post-menopausal patients [11]. Moreover, it is recognized that estrogen influences the clinical outcome of breast cancer patients by stimulating the proliferation of estrogen receptor (ER) positive tumor epithelial cells [12].

This could occur by an endocrine mechanism by which estradiol is taken up from plasma, by a stromal to epithelial paracrine mechanism, and/or by an autocrine mechanism with estrogen synthesis in tumor epithelial cells [13].

The relative contribution of any of these mechanisms is likely to vary with the physiological status of the female and possibly with the local and systemic changes occurring during breast tumorigenesis and progression. Experimental evidence supports the potential of each mechanism to contribute to estrogen synthesis and influence breast tumorigenesis [12].

The cytochrome P450 arom belongs to the cytochrome P-450 superfamily compromising over 460 members in 74 families, of which cytochrome P-450arom is the sole member of the family 19 [14]. P-450arom is a glycoprotein located intracellularly in the endoplasmic reticulum. The human CYP19 gene (15q21.2) encoding for P450 arom spans about 123 kb with a coding region of 9 exons (about 30 kb, exon II–exon X). Further upstream of exon II, there are a number of alternative first-exons which are differently spliced into distinct 5′-untranslated regions [15–17]. In addition, up to nine different transcriptional start sides with individual promoters permitting tissue-specific regulation of expression have been described [17]. However, even though 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 [16,17].

This means that although transcripts in different tissues have different 5′ termini, the coding region and therefore the protein expressed in these tissues remain the same. Tissue specific regulation of expression has been studied by several investigators, and a switch from the adipose-specific exon 1 (exon 1b or exon I.4) promoter used in non-tumor breast tissues to the ovary-specific exon 1 (exon 1c or exon I.2) has been observed in breast cancer specimens [18,19].

The 17-β-HSD type 1 enzyme catalyzes the final conversion of estrone to estradiol and its expression was observed in human breast cancer and non-cancerous breast tissue [20,21]. Since estradiol is the most potent estrogenic end product and since this reaction controls several pathways by which estrone can be created, a possible difference in the activity of the enzyme could be of importance for estrogen levels. The gene coding for 17-β-HSD type 1 is located at 17q12-21 [22].

There are limited and conflicting data in the literature regarding the correlation between the expression of the genes coding for the enzymes responsible for the local production of estrogens and the clinical outcome as well as other clinical and pathological parameters in human breast cancer [23–25]. Intratumoral mRNA expression of the enzymes involved in the estradiol metabolism has been studied previously in separate reports on different materials for single genes such as CYP19 [23], steroid sulfatase (STS) [26] and 17-β-hydroxysteroid dehydrogenase type1 (HSD1) [27].

The aim of our study was to determine the mRNA expression levels of CYP19 and 17-β-HSD type 1 genes in malignant and non-cancerous breast tissue and correlate this with the clinical outcome and several other tumor prognostication parameters including nodal status, stage, grade, Nottingham prognostic index (NPI) score, ER status, and HER1–4 status. Here, we report that higher levels of the enzymes responsible for the local biosynthesis of estrogens and that aromatase are especially associated with a poor clinical outcome in patients with breast cancer.

Materials and methods

Materials

RNA extraction kits and reverse transcription kits were obtained from AbGene (Surrey, England, UK) and Sigma Ltd. (Poole, Dorset, UK), respectively. PCR primers were designed using Beacon Designer (version 2.3, Palo Alto, CA) and synthesized in house by Sigma Genosys. Custom made hot-start Master mix for quantitative PCR was from Abgene (Surrey, England, UK), [28,29]. The data were analyzed using parametric (*t*-test) and non-parametric (Mann–Whitney test) tests, using an Macro in Minitab (version 14.0) that was purpose written by the authors. Survival curves were constructed using the Kaplan–Meier method (SPSS version 12.0.0).

Sample collection

Institutional guidelines, including ethical approval and informed consent, were followed.

Breast cancer tissues ($n = 127$) and normal background tissues ($n = 33$) were collected immediately after surgery and stored at −80 °C until use. Patients were routinely followed up after surgery. The median follow up period was 120 months (as of June 2004). A consultant breast pathologist (A.D-J.) who examined H&E-stained frozen sections verified the presence of tumor cells in the collected tissues.

Details of histology were obtained from pathology reports. Follow-up data were recorded in a custom database. Table 1 shows the tumor characteristics and clinical follow-up data.

Tissue processing, RNA extraction and cDNA synthesis

Frozen sections of tissue were cut at a thickness of 5–10 μm and were kept for routine histology. An additional 15–20 sections were mixed and homogenized using a hand held homogeniser, in ice-cold RNA extraction solution. The concentration of RNA was determined using a UV spectrophotometer. Reverse transcription was carried out using a reverse transcription kit with an anchored olig(dT) primer supplied by Abgene, using 1 μg of total RNA in a 96-well plate. The quality of cDNA was verified using ß-actin primers (primers 5′-ATGATATCGCCGCGCTCGTC-3′ and 5′-CGCTCGGTGAGGATCTTCA-3′).

Table 1. Clinical data

Parameter	Category	Number
Node status	Node positive	54
	Node negative	73
Tumor grade	1	24
	2	43
	3	58
Tumor type	Ductal	98
	Lobular	14
	Medullary	2
	Tubular	2
	Mucinous	4
	Others	7
TNM staging	1	70
	2	40
	3	7
	4	4
Clinical outcome	Disease free	90
	Alive with metastasis	7
	With local recurrence	5
	Died of breast cancer	16
	Died of unrelated disease	9

Quantitative analysis of aromatase and 17-β-HSD type 1

The level of aromatase and 17-β-HSD type 1 transcripts from the above prepared DNA was determined using real time quantitative PCR based on the Amplifluor^{TM} technology, modified from a method reported previously [29].

PCR primers were designed using Beacon Designer software but to the reverse primer an additional sequence, known as the Z sequence (5′-actgaacctgaccgtaca-3′) which is complementary to the universal Z probe (Intergen Inc., Oxford, UK) was added. The product expands one intron. The reaction was carried out using the following: Hot-start Q-master mix (Abgene), 10 pmol of specific forward primer, 1 pmol reverse primer which has the Z sequence, 10 pmol of FAM tagged probe (Intergen Inc.) and cDNA from 50 ng of RNA. The reaction was carried out using the IcyclerIQ (BioRad) which is equipped with an optic unit that allows real time detection of 96 reactions under the following conditions: 94 °C for 12 min and 50 cycles of 94 °C for 15 s, 55 °C for 40 s and 72 °C for 20 s. The levels of the transcript were generated from a standard that was simultaneously amplified with the samples. Levels of CYP19 and 17-β-HSD type 1 expression were then normalized against CK19 expression already measured in these specimens, to correct for varying amounts of epithelial tissue between samples, as we have recently reported [30].

Primer sequences

Primer sequences for CYP19F1 were: 5′-gtatgcatgagaaa ggcatc-3′ and 5′-actgaacctgaccgtacagactgtgaccatacga-acaa-3′; for hsd17b1F1: 5′-gaaggcttatgcgagagtc-3′ and 5′-actgaacctgaccgtacactcgatcaggctcaagtg-3′, and for CK19 5′-caggtccgaggttactgac-3′ and 5′-actgaacctgaccgtacacactt tctgccagtgtgtcttc-3′, as previously reported [30].

Results

Overall, higher levels of CYP19 expression correlated with poor survival $p = 0.0105$. Figure 1 illustrates the disease-free survival (DFS) curve calculated using the Kaplan–Meier method. The mean survival period in patients who expressed low level of CYP19 mRNA was 134.71 months (95%CI: 126.47–142.94) compared with 113.92 months in those patients who had high expression of CYP19 mRNA (95%CI: 91.06–136.79) (The arbitrary cutoff value was 10,000).

Similarly, shorter survival period was seen in patients ho had a high expression of 17-β-HSD type 1 mRNA when compared with those with low levels, 140.02 months (95%CI: 130.65–149.39) and 100.93 months (95%CI: 72.93–128,93), respectively $p = 0.0182$ (The arbitrary cutoff value was 1000) (Figure 2).

We observed higher levels of CYP19 mRNA in the tumor samples compared with the normal breast tissue.

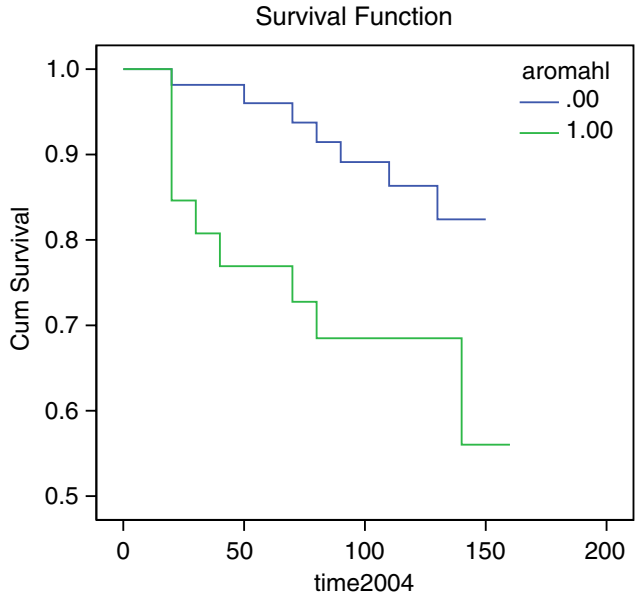

Figure 1. Kaplan–Meier analysis of disease-free survival of breast cancer patients depending on the expression of aromatase mRNA (p 0.0105). 0 = Low levels; 1.00 = High levels (cut-off point: 10,000).

But the difference did not reach statistical significance ($p = 0.16$). Normal breast samples expressed higher levels of 17-β-HSD mRNA than the breast cancerous tissue but with no significant difference ($p = 0.84$).

We observed no significant correlation or relationship between the CYP19 mRNA expression and tumor grade. However, higher levels were observed in grade 1 tumors compared to normal tissue ($p = 0.01$).

We observed an increase in levels of 17-β-HSD type 1 mRNA correlating with tumor's grade. This correlation was statistically significant when we compared grade 1 with grade 2 and grade 1 with grade 3 ($p = 0.0031$ and 0.0251, respectively, two-sample t-test).

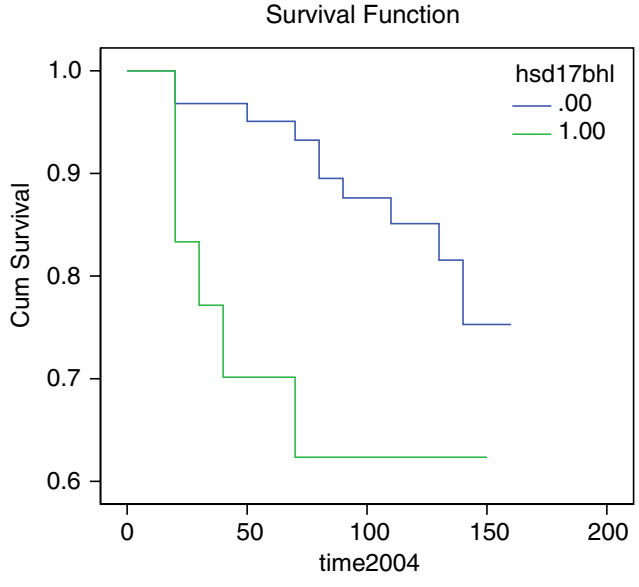

Figure 2. Kaplan–Meier analysis of disease-free survival of breast cancer patients depending on the expression of 17-β-HSD type 1 mRNA (p 0.0182). 0 = Low levels; 1.00 = High levels (cut-off point: 1000).

There was no significant correlation between the expression levels of CYP19 and 17-β-HSD type 1 and tumor stage using the TNM classification, and lymph node status. Nonetheless, higher levels of 17-β-HSD type 1 mRNA were observed in stage 2 tumors compared to stage 1 and normal tissues ($p = 0.011$ and 0.02, respectively) (Table 2).

We also examined the correlation between CYP19 and 17-β-HSD type 1 mRNA expression and NPI. We observed higher levels of CYP19 mRNA in NPI3 compared with NPI1 and NPI2. However, such observations failed to reach a statistical significance when comparing, NPI1 (NPI $<$ 3.4) with NPI3 (NPI $>$ 5.4) and NPI2 (NPI = 3.4–5.4) with NPI3 ($p = 0.35$ and 0.35, respectively). No correlation was seen between levels of 17-β-HSD type 1 and NPI.

Increased levels of CYP19 mRNA expression were positively correlated with disease progression as levels were significantly higher in samples of patients who had distant metastasis and local recurrence and/or died of breast-related causes when comparing to those who were disease free for >10 years ($p = 0.0015$). Furthermore, levels of CYP19 mRNA expression in patients who died from breast cancer were significantly higher than normal breast tissue ($p = 0.0016$). A similar trend was observed with 17-β-HSD type 1 expression. However, such a correlation did not reach statistical significance.

Levels of CYP19 and 17-β-HSD type 1 expression were seen to be higher in ductal carcinoma compared with other histopathological types of breast cancer however no significant difference was observed.

We also examined the relationship between expression levels and HER1–4 status. We observed a positive correlation between CYP19 levels and HER1 (Table 3). There was no significant correlation between 17-β-HSD type 1 levels and HER receptor status. Furthermore, there was no correlation between levels of aromatse and 17-β-HSD type 1.

Finally, there was a trend for positive correlation between CYP19 mRNA and ER positivity ($p = 0.06$). This trend was not observed with 17-β-HSD type 1 ($p = 0.82$) (Table 4).

Discussion

The formation of active steroids in peripheral tissues from inactive precursors has been proposed to play an important biological role in various sex steroid-dependent neoplasms [14]. In human breast cancer all the enzymes involved in the local biosynthesis have been identified [31,32]. The local production of estradiol is mediated by three main enzymes within the breast tissue including the breast cancerous tissues. STS mRNA expression has been shown to be an independent predictor of clinical outcome in breast cancer and a potential modality for predicting prognosis [26,31,33,34]. In this study we have focused on the correlation between the expression of the other two enzymes (aromatase and

Table 2. Levels of aromatase and 17-β-HSD type 1 mRNA and tumor stage and clinical outcome (raw data)

Parameter	Subgroups	Number of valid data	Mean ± SD Aromatse	Mean ± SD 17-β-HSD type 1
All samples	Aromatase and 17-β-HSD type 1 all tissue	150	29797 ± 216260	907 ± 2266
	Normal tissue	32	11383 ± 37040	943 ± 2247
	Tumor tissue	112	36314 ± 249447	849 ± 2211
NPI	NPI1	58	14471 ± 26666	538 ± 787
	NPI2	34	13809 ± 27079	1586 ± 3757
	NPI3	15	18277 ± 679810	634 ± 996
Grade	Grade1	19	13399 ± 13822	157.2 ± 377.9
	Grade2	36	11458 ± 21749	578 ± 712
	Grade3	55	61660 ± 355309	1282 ± 3042
TNM staging	TNM1	62	15109 ± 26165	428.2 ± 716.9
	TNM2	34	10690 ± 25221	1764 ± 3724
	TNM3	7	382053 ± 995669	526 ± 810
	TNM4	4	19270 ± 27668	1045 ± 1508
Nodal status	Node positive	49	65532 ± 376146	1295 ± 3192
Clinical outcomes	Disease free	81	9679 ± 18189	536.1 ± 831.4
	Alive with Metastasis	6	7405 ± 8049	308 ± 260
	Local recurrence	4	17441 ± 19424	722 ± 1399
	Died of breast cancer	16	188098 ± 654767	1647 ± 3377

17-β-HSD type 1) and the clinical outcome in patients with breast cancer.

It is well established that the highest levels of aromatase are present in the ovaries of pre-menopausal women, in the placenta of pregnant women, and in the peripheral adipose tissues of post-menopausal women and men [5,35,36].

In the breast, aromatase is expressed in different cell types of the breast tissue. Earlier studies reported that CYP19 mRNA levels were highest in tumor-bearing quadrants [37], and were significantly higher than in those regions distal to the tumor or in non-malignant breast tissue [38]. More recent studies using quantitative polymerase chain reaction (PCR) analysis have shown that adipose stromal cells surrounding the cancer cells contain higher levels of CYP19 mRNA than adipose stromal cells in non-cancerous areas [37]. Furthermore, James et al. [39] reported that *in vitro* aromatase activity was higher in breast tumors than in the fat adjacent to the tumor or in normal breast fat. Other investigators found a highly significant correlation between aromatase activity and the presence of tumors in the breast tissue [40]. In our study we observed higher levels of aromatse expression in cancerous tissue compared with normal breast tissue and these observations are consistent with previous reports [37–40].

Since aromatase plays an essential role in the local biosynthesis of estradiol in post-menopausal women and because estrogen is known to influence the clinical outcome in breast cancer patients by stimulating the proliferation of ER positive tumor epithelial cells [12], we hypothesized that aromatase over-expression may be associated with a poor clinical outcome in women with breast cancer.

Although estrogens levels decline sharply after the menopause, we have reported that in some breast tumors, *in situ* formation of estrogens can make an important contribution to the estrogen content of breast cancer cells [13,41]. Furthermore, experimental evidence using xenograft models provides a direct proof that locally produced estrogen can stimulate the growth of estrogen-dependent MCF-7 human breast tumors to a greater extent than can estrogen delivered via an endocrine mechanism [5]. Moreover, estrogen produced locally in tumors arising from these xenografted cells may exceed the amount taken up from plasma. Cell line experiments have confirmed the role of aromatase in stimulating the growth of breast cancer cells [11,42–45]. Consistent with these experimental findings we have observed in the present study that higher levels of aromatase expression were correlated with a poor clinical outcome in women with breast cancer. However, our findings contradict the results of three previously published studies [22–24].

Firstly, Lipton et al. [23] demonstrated that there was no relationship between aromatase activity and disease-free interval or survival in 127 patients with breast cancer.

Secondly, Yoshimura et al. [31] reported that patients with tumors expressing aromatase had a better prognosis than patients with no expression of this transcript and the lack of expression of aromatase was a strong prognostic factor in early stage breast cancer. Furthermore, the authors suggested that the poor prognosis of patients with null expression of aromatase could reflect prior treatment with drugs such as tamoxifen or aromatase inhibitors. Moreover, patients with aromatase gene using the normal adipose tissue

Table 3. Pearson correlation of levels of mRNA expression and ER and HER receptor status

	Aromatase	17-β-HSD type 1	Aromatase CK	17-β-HSD type 1CK	ERα	ERβ	ERαCK19	ERβCK19	Her1	Her1CK19	Her2	Her2CK19	Her3	Her4	Her3CK19
ERα	0.029	−0.061	0.068	0.044											
ERβ	−0.037	−0.048	−0.053	0.045	0.241										
ERαCK19	0.047	−0.023	0.688	0.187	0.307	−0.034									
ERβCK19	−0.051	−0.040	−0.019	0.018	−0.032	0.512	−0.034								
Her1	0.390	0.071	0.001	−0.012	−0.004	0.066	0.076	0.088							
Her1CK19	−0.052	0.007	0.130	0.065	−0.025	0.005	0.109	0.103	0.736						
Her2	−0.000	0.082	0.014	−0.008	−0.055	−0.020	−0.028	−0.018	0.111	0.207					
Her2CK19	0.005	−0.020	0.112	0.028	−0.049	−0.027	0.057	−0.017	0.342	0.468	0.653				
Aromatase CK19	0.382	0.011	1.000	0.028	0.068	−0.053	0.688	−0.019	0.001	0.130	0.014	0.112			
17-β-HSD 1CK19	0.009	0.199	0.028	1.000	0.044	0.045	0.187	0.018	−0.012	0.065	−0.008	0.028			
Her3	0.114	0.010	−0.015	−0.046	−0.041	0.031	−0.013	0.028	0.311	0.027	−0.023	−0.026			
Her4	−0.029	−0.028	−0.023	0.023	0.071	0.039	−0.025	0.027	−0.055	0.013	−0.032	−0.009	−0.001		
17-β-HSD 1	−0.005	1.000	0.011	0.199	−0.061	−0.048	−0.023	−0.040	0.071	0.007	0.082	−0.020	0.010	0.028	
Her3CK19	−0.020	−0.060	0.186	−0.042	−0.017	0.029	0.049	0.117	0.112	0.170	−0.030	−0.020	0.348	0.073	
Her4CK19	−0.059	−0.038	−0.004	−0.003	0.233	−0.020	−0.015	−0.015	−0.021	0.040	−0.017	0.005	−0.003	0.851	0.025

Table 4. Correlation between levels of aromatase and 17-β-HSD type 1 mRNA and ER status

	ER negative Mean ± SD	ER positive Mean ± SD	*p*-value
Aromatase	9336 ± 2507	17515 ± 5628	0.0621
17-β-HSD type 1	953 ± 282	836 ± 418	0.82

promoter had longer survival than those patients with a switch of promoters. However, they admitted that this result was somewhat paradoxical since their data and other previous data suggest that, in the course of the disease, a switch of promoter occurs and results in higher expression levels [31].

Thirdly, one study examined the correlation between mRNA and the clinicopathological parameters and showed that patients with high levels of expression of aromatase mRNA tended to have a better prognosis than did those patients with low expression levels [25]. However, their follow up period was limited to a median of 58 month compared to a median of 120 months in our study.

Although we observed a positive correlation between CYP19 expression and clinical outcome such a relationship was not seen with the clinicopathological parameters and other tumor characteristics. The lack of correlation between aromatase expression and these clinicopathological factors including age, tumor size, axillary lymph node involvement, grade, ERα and PgR status and histological type was previously reported [46–48].

The present study shows a trend towards a positive correlation between CYP19 expression and ER. This finding was also reported by Brodie et al. [49] who found that tumors with a relatively high aromatase activity tended to be ER-positive. Miller and co-workers [46] also observed a significant trend toward an association between aromatase activity and the presence of ERα, although tumors expressing active aromatase included both ER-α positive and negative tumors. It is difficult to postulate a role for estrogens produced *in situ* in tumors that lack ERs [50].

In the present study, we found that normal breast samples expressed higher levels of 17-β-HSD mRNA with no significant difference ($p = 0.84$). This is consistent with previous studies which showed that the level of 17-β-HSD type 1 expression in human breast carcinoma was variable and was not necessarily higher than in non-neoplastic breast tissue [20].

A few immunohistochemical studies of 17-β-HSD type 1 in human breast carcinoma have been reported, however no clear relation to prognosis and clinical parameters has been found [27,41,51]. It was suggested that 17-β-HSD type 1 played an important role in hormone-dependent breast carcinomas [25]. In a study conducted by Gunnarson et al. [52] they found that a high level of 17-β-HSD 1 indicated an increased risk to develop 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 may have importance in breast cancer progression. Feigelson et al. [53] 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. In principle, our study supports these findings and highlights the significant relationship between poor survival and high expression of 17-β-HSD 1 in breast cancer patients.

We found no correlation between the levels of 17-β-HSD 1 and ER status in the present study and this is in agreement with previous reports [53].

Our study has two potential limitations. Firstly, our analysis was focused on mRNA expression of these enzymes and did not include the measurement of protein expression or enzyme activity. However, previous studies showed that the levels of CYP19 transcripts were positively correlated with the enzyme activity [54,55].

Secondly, we did not divide our patients into subgroups such as pre- and post-menopausal women. This is because such subgroup analyses will reduce the statistical power of this study which is focused on tumor levels of mRNA. Furthermore, scientific evidence suggests that the local production of estrogen in breast tumors may exceed the amount taken up from plasma [5]. Moreover, estrogens levels in breast cancer specimens were found to be several fold higher than those of plasma in post-menopausal patients [11].

The increasing evidence that aromatase inhibitors are superior to tamoxifen in post-menopausal women with ER positive early and advanced breast cancer is in keeping with our observation that higher aromatase expression correlates with poor clinical outcome [56–58].

Conclusion

Our results showed that higher levels of the enzymes responsible for the local biosynthesis of estrogens in breast cancer patients, especially aromatase correlate with a poor clinical outcome in women with breast cancer.

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