17β-Hydroxysteroid Dehydrogenase 14 Affects Estradiol Levels in Breast Cancer Cells and Is a Prognostic Marker in Estrogen Receptor–Positive Breast Cancer

Agneta K. Jansson,[1] Cecilia Gunnarsson,[2] Maja Cohen,[1] Tove Sivik,[1] and Olle Stål[1]

[1]Division of Oncology, Department of Biomedicine and Surgery and [2]Division of Clinical Genetics, Linköping University, Linköping, Sweden

Abstract

Estrogens have an important role in the progression of breast cancer. The 17β-hydroxysteroid dehydrogenase (17HSD) family has been identified to be of significance in hormone-dependent tissues. 17HSD1 and 17HSD2 are the main 17HSD enzymes involved in breast cancer investigated this far, but it is possible that other hormone-regulating enzymes have a similar role. 17HSD5 and 17HSD12 are associated with sex steroid metabolism, and 17HSD14 is a newly discovered enzyme that may be involved in the estrogen balance. The mRNA expression of 17HSD5, 17HSD12, and 17HSD14 were analyzed in 131 breast cancer specimens by semiquantitative real-time PCR. The results were compared with recurrence-free survival and breast cancer-specific survival of the patients. The breast cancer cell lines MCF7, SKBR3, and ZR75-1 were transiently transfected with 17HSD14 to investigate any possible effect on estradiol levels. We found that high 17HSD5 was related to significantly higher risk of late relapse in estrogen receptor (ER)–positive patients remaining recurrence-free later than 5 years after diagnosis (P = 0.02). No relation to 17HSD12 expression was found, indicating that 17HSD12 is of minor importance in breast cancer. Patients with ER-positive tumors with high expression levels of 17HSD14 showed a significantly better prognosis about recurrence-free survival (P = 0.008) as well as breast cancer-specific survival (P = 0.01), confirmed by multivariate analysis (P = 0.04). Transfection of 17HSD14 in the human breast cancer cells MCF7 and SKBR3 significantly decreased the levels of estradiol, presenting an effect of high expression levels of the enzyme. (Cancer Res 2006; 66(23): 11471-7)

Introduction

Estrogens are of great importance in the development of breast cancer. After menopause, estrogen biosynthesis in peripheral tissue has an almost exclusive role and several different enzymes are of significance for the availability of active estrogens. The 17β-hydroxysteroid dehydrogenase (17HSD) enzymes catalyze oxidoreduction of hydroxyl/keto groups of androgens and estrogens and regulate intracellular availability of steroid hormones (1). There are 14 known members of the enzyme family, 12 of them are present in humans, and among them, several have been identified to be of importance in different hormone-dependent tissues and tumors (2, 3). All of these enzymes belong to the short-chain dehydrogenases/reductases (SDR) family, except 17HSD5. The SDR family consists of oxidoreductases that participate in the metabolism of steroids, prostaglandins, and retinoids (4). In breast tissue, several 17HSD family members have been reported to be present, such as 17HSD1, 17HSD2, 17HSD5, and 17HSD12 (5–11). Although 17HSD1 and 17HSD2 are the main 17HSD enzymes involved in breast cancer investigated this far (7, 8, 10, 11), it is possible that other hormone-regulating 17HSD enzymes may have a significant role in hormone-dependent tumors.

17HSD5 is an aldo-keto reductase dominantly expressed in prostate and mammary gland and is the main testosterone-forming 17HSD enzyme (12). 17HSD5 synthesizes 17β-diol (5-diol) from dehydroepiandrosterone and testosterone from andostendione (12). There is only one study that has investigated the prognostic importance of 17HSD5 in breast cancer. The authors found that patients with high 17HSD5 expression in the tumor had a worse prognosis than the group with low or no expression (8). The importance of 17HSD5 in breast cancer needs to be further clarified. 17HSD12 catalyzes the reduction of estrone to estradiol. The enzyme has been detected at high levels in ovary and mammary gland (9), and there have been suggestions that 17HSD12 may be as important as 17HSD1 in regulating the estradiol balance and involvement in hormone-dependent cancer progression. There are no studies to our knowledge that have investigated the importance of 17HSD12 in any hormone-dependent cancer. 17HSD14, known as DHRS10 and retSDR3, is a recently discovered enzyme (13) that belongs to the 17HSD family. 17HSD14 is a NAD^+-dependent estradiol dehydrogenase that may be involved in the estrogen balance by catalyzing oxidation from estradiol to estrone (2). The function and importance of 17HSD14 has not been investigated previously in any human tissue or cancer type.

The aim of this study was to investigate if mRNA expression levels of 17HSD5, 17HSD12, and 17HSD14, measured with real-time PCR, could be of prognostic importance in the context of recurrence-free survival and breast cancer survival in a series of 131 breast cancer patients. We further investigated how over expression of 17HSD14 affects the estradiol levels in the breast cancer-derived cell lines MCF7, SKBR3, and ZR75-1 by transient transfection.

Materials and Methods

Materials. We analyzed tissue from excised primary breast tumors of 131 women treated in the health care region of southeast Sweden between 1984

Note: The Southeast Sweden Breast Cancer Study Group collected data according clinical and pathologic information. Members of the Southeast Sweden Breast Cancer Study Group: L-G. Arnesson, B. Nordenskjöld, K. Nordenskjöld, A. Malmström (Linköping); H. Bång (Motala); A-C. Källström (Norrköping); E. Einarsson (Eksjö); B. Norberg (Jönköping); P. Skoog (Värnamo); Å. Henning (Oskarshamn); M. Sundquist (Kalmar); and G. Tejler (Västervik).

Requests for reprints: Agneta K. Jansson, Division of Oncology, Department of Biomedicine and Surgery, Linköping University, S-581 85 Linköping, Sweden. Phone: 46-13-22-28-40; Fax: 46-13-22-28-46; E-mail: agnja@ibk.liu.se.

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and 1991. The Southeast Sweden Breast Cancer Study Group collected data according clinical and pathologic information. All patients had primary breast cancer without distant metastasis at the time of diagnosis. The patients were participants in a randomized multicentric trial where 2 and 5 years of adjuvant postoperative tamoxifen treatment were compared among postmenopausal patient under the age of 75. The median period of follow-up was 13.9 years. After surgery, the tumor samples were stored at −70°C until RNA extraction was done. Further, RNA from normal mammary gland from four women ages 45, 56, 65, and 81 years old were analyzed.

Estrogen receptor (ER) and progesterone receptor (PgR) content was measured in clinical routine practice with isoelectric focusing before 1988 and thereafter with enzyme immunoassays (Abbott Laboratories, Chicago, IL). The tumors were classified as either ER, PgR positive or negative. Samples with concentrations ≥0.3 fmol/μg DNA were classified as positive. The regional ethics committee at Linköping University approved the study.

Cell cultures. MCF7, SKBR3, and ZR75-1 breast cancer epithelial cells (American Type Culture Collection, Manassas, VA) were cultured in phenol red–free Opti-MEM I (Invitrogen, Carlsbad, CA) supplemented with 4% fetal bovine serum (Invitrogen) and incubated at 37°C, 5% CO_2. In all experiments, charcoal/dextran-treated serum (HyClone, Logan, UT) was used to be able to control the levels of estradiol.

Cloning and transient transfection of 17HSD14. Synthesis of cDNA from human mammary gland RNA was done using the first-strand cDNA Synthesis kit with random hexameres (Roche Diagnostics Corp., Indianapolis, IN). To generate double-stranded 17HSD14 cDNA, a pair of primers 5′-GAAGTTATCAGTCGACGGTGAAAGAGGCCCAGAGT-3′ and 5′-ATGGTC-TAGAAAGCTTAGTTTGGGGTGGGAGAGTC-3′ containing *Sal*I and *Hin*dIII cleavage sites were used in a PCR with human mammary gland cDNA. The amplified PCR fragment was cloned into pDNR-Dual Donor Vector (BD Biosciences Clontech, Mountain View, CA) with BD In-Fusion Enzyme. 17HSD14 was subcloned to pLP-IRES2-EGFP acceptor vector (BD Biosciences Clontech, Mountain View, CA) using Cre recombinase. The plasmids containing the 17HSD14 cDNA were sequenced with MegaBACE 500 DNA analysis Systems (Amersham Biosciences, AB, Uppsala, Sweden) sequencing equipment according to the manufacturer's protocol.

For transfection of 17HSD14, 60,000 cells per well were seeded in 12-well plates in 1 mL/well in culture medium. Twenty-four hours after seeding, the cells were transfected by Fugene 6 transfection (Roche Diagnostics) by adding in each well 0.04 mL containing 0.4 μg plasmid, 1.2 μL Fugene 6 transfection reagent, and Opti-MEM I (Invitrogen). Twenty-four hours after transfection, the cells were washed with PBS and incubated in culture medium before harvested. Cells transfected with an empty vector were used as control. All experiments were run in triplicates and repeated twice.

Real-time PCR. RNA was extracted and cDNA was synthesized according to Gunnarsson et al. (11) from both tumors and cell lines. Taqman Gene Expression assay number Hs00366267_m1, Hs00275054_m1, and Hs00212233_m1 purchased from PE Applied Biosystems (Warrington, United Kingdom) was used to measure RNA expression of 17HSD5, 17HSD12, and 17HSD14, respectively. In the transfected cell lines, 17HSD14 mRNA expression levels were investigated. Primer and probes used to measure the endogenous control β-actin were purchased from PE Applied Biosystems. cDNA was added to the reaction mixture, which had a total volume of 25 μL containing 5 mmol/L $MgCl_2$, 1× Taqman buffer A (Applied Biosystems, Branchburg, NJ), 0.1 mmol/L deoxynucleotide triphosphate, 0.025 units/μL AmpliTaq Gold DNA polymerase (Applied Biosystems), and primer and probes for respective gene. The thermal conditions used were 95°C for 10 minutes, 95°C for 15 seconds, and 60°C for 1 minute. Steps two and three were repeated for 45 cycles. 17HSD5, 17HSD12, 17HSD14, and β-actin were amplified independently in separate reaction wells as duplicates and in all experiments samples without template were used as negative control. Standard curves for all analyzed genes were run on each plate, using serial diluted cDNA to normalize the runs. The obtained data from β-actin were used to standardize the sample variation in the amount of input cDNA.

The standard curve method. A relative kinetic method was applied, using a standard curve, which was constructed with 8-fold serial dilution of cDNA from normal breast tissue. Standard curves were produced for all four investigated genes after each run. The target messages in the unknown samples were quantified, using the standard curves, to determine a relative measure of the starting amount.

Measurement of estradiol levels. The concentrations of estradiol in the medium from MCF7, SKBR3, and ZR75-1 17HSD14-transfected cells and controls were examined. The medium was changed to medium containing 10^{-9} mol/L estradiol at 48 and 72 hours after transfection, thereafter incubated at 37°C at 5% CO_2 for 1, 4, 8, and 24 hours, and finally frozen in liquid nitrogen. The concentration of estradiol was measured using electrochemiluminescence immunoassay using the Elecsys Estradiol II reagent kit (Roche Diagnostics) analyzed with Roche Elecsys 1010/2010. All experiments were run in triplicates and repeated twice.

Statistical evaluation. The relationships between grouped variables were analyzed with the χ^2 test. Survival curves were produced according to the life table method described by Kaplan and Meier. Differences in recurrence-free survival were estimated with the log-rank test. Multivariate analysis of recurrence and mortality rates were done with Cox proportional hazard regression. The functional results from the cell lines were analyzed with Student's paired t test.

All the procedures were comprised in the statistical package Statistica 6.0 (StatSoft Scandinavia AB, Uppsala, Sweden). All P values are two sided, and $P < 0.05$ was considered to be statistically significant.

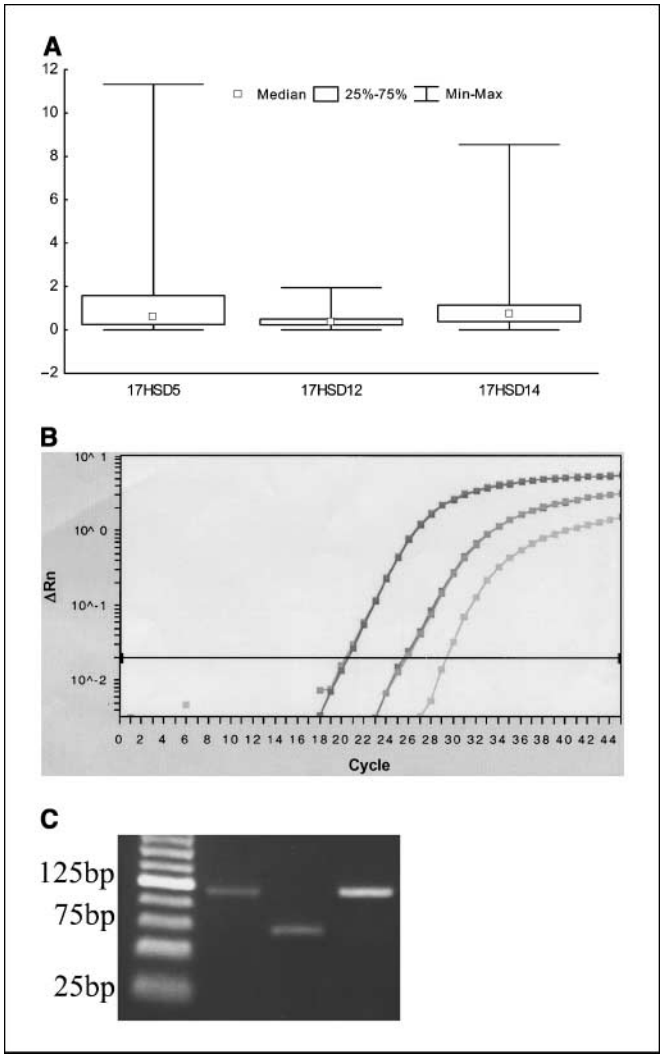

Figure 1. *A,* expression of 17HSD5, 17HSD12, and 17HSD14 in breast cancer tissue. *B,* typical pattern of the curves from real-time PCR amplification of 17HSD12, 17HSD14, and 17HSD5 (*left* to *right*). *C,* real-time PCR products were separated on agarose gel with the expected product size (*left* to *right*): 17HSD5 (112 bp), 17HSD12 (65 bp), and 17HSD14 (110 bp).

Table 1. Expression of 17HSD5, 17HSD12, and 17HSD14 in relation to tumor characteristics and tamoxifen treatment

	n	17HSD5 (%)			17HSD12 (%)			17HSD14 (%)		
		−	+	++	−	+	++	−	+	++
Nodal status*/tumor size (mm)										
N^-/>20	37	38	27	35	43	30	27	32	30	38
N^+/≤20	37	24	38	38	22	41	38	38	30	32
N^+/>20	51	35	35	29	35	29	35	33	35	31
ER status										
ER^+	103	29	35	36	32	38	30	28	38	34 †
ER^-	28	50	25	25	36	21	43	54	18	29
PgR status										
PgR^+	81	30	32	38	30	37	33	30	37	33
PgR^-	50	40	34	26	38	30	32	40	28	32
Tamoxifen (y)										
2	69	39	33	28 ‡	26	43	30	38	39	23§
5	62	27	32	40	40	24	35	29	27	44

*Data missing in six patients.
†P = 0.08.
‡P = 0.09.
§P = 0.04.

Results

mRNA expression of 17HSD5, 17HSD12, and 17HSD14. 17HSD5, 17HSD12, and 17HSD14 mRNA were examined in 131 tumors and for each enzyme the cases were divided into three groups of equal size according to expression levels using tertile values as cutoff levels. 17HSD5 was detected in 130 of the 131 analyzed tumors with a mean of 1.3. The expression levels ranged from 0 to 11.3. Forty-four cases showed low expression (<0.37), 43 showed intermediate expression (0.37-1.29), and 44 showed high expression (>1.29). 17HSD12 mRNA was detected in all tumors with a mean of 0.40. The expression levels ranged from 0.01 to 1.95. Forty-three cases showed low expression (<0.28), 45 showed intermediate expression (0.28-0.43), and 43 showed high expression (>0.43). 17HSD14 mRNA was detected in all tumors with a mean of 1.02. The expression levels ranged from 0.02 to 8.54. Forty-four cases showed low expression (<0.49), 44 showed intermediate expression (0.49-0.96), and 43 showed high expression (>0.96; Fig. 1*A*). The low, intermediate, and high groups were denoted −, +, and ++. Typical pattern of the curves from real-time PCR amplification of 17HSD5, 17HSD12, 17HSD14 and validation of the right amplicons are shown in Fig. 1*B* and *C*.

The expression levels of 17HSD5 and 17HSD12 as well as 17HSD14 and 17HSD12 were significantly correlated (P = 0.002 and 0.001, respectively).

17HSD5, 17HSD12, and 17HSD14 mRNA expression was detected in the four examined normal mammary gland samples. The mean values for 17HSD5, 17HSD12, and 17HSD14 were 0.59 (range, 0.16-1.29), 0.69 (0.12-2.11), and 0.24 (<0.01-0.51), respectively.

Prognostic value of 17HSD5, 17HSD12, and 17HSD14 mRNA expression. The mRNA expression of 17HSD5, 17HSD12, and 17HSD14 were compared with nodal status, tumor size, ER status, PgR status, and tamoxifen treatment, without any significant correlations with other tumor characteristics (Table 1). Patients with ER-positive tumors that expressed high levels of 17HSD5 compared with tumors with low and intermediate levels showed a significantly increased risk of developing recurrence in breast cancer later than 5 years after diagnosis (P = 0.03; Fig. 2*A*). However, this was not detected when all three groups were investigated individually for the whole follow-up period (P = 0.94; Fig. 2*B*). The importance of 17HSD5 for recurrence in breast cancer was not confirmed by multivariate analysis (P = 0.61).

The expression levels of 17HSD12 did not show any significant prognostic value (Fig. 2*C*). Patients with ER-positive tumors with high expression levels of 17HSD14 showed a significantly better prognosis when comparing two groups (−/+ versus ++), about recurrence-free survival (P = 0.005; Fig. 2*D*) as well as breast cancer-specific survival (P = 0.01). This was confirmed by multivariate analysis (P = 0.04; Table 2).

Transient transfection of MCF7, ZR75-1, and SKBR3 with 17HSD14. MCF7, ZR75-1, and SKBR3 were transfected with pLP-IRES2-EGFP-17HSD14 or a control vector. The endogenous expression of 17HSD14 was low in all three investigated cell lines. The mRNA expression was analyzed 24, 48, and 72 hours after transfection, and the levels of 17HSD14 increased significantly compared with the controls in each cell line at the time points examined (Table 3).

Measurements of estradiol levels. The levels of estradiol was measured in samples treated with 10^{-9} mol/L estradiol 48 and 72 hours after transfection and thereafter incubated for 1, 4, 8, and 24 hours. The concentration of estradiol decreased significantly in 17HSD14-transfected MCF7 and SKBR3 cells compared with the controls after 48 hours (P = 0.0005 and 0.04, respectively) and 72 hours (P = 0.02 and 0.02, respectively; Fig. 3*A* and *B*). The transfected ZR75-1 cells showed a trend of decreased estradiol levels after 48 hours (P = 0.11) and no differences after 72 hours were detected (P = 0.29; Fig. 3*C*).

Discussion

Estrogens are important as mitogenic stimulators in breast cancer and local production of estrogens is of significance in the

progression of the disease. Previous studies have shown the importance of 17HSD1 and 17HSD2 in breast cancer to predict late recurrence and overall survival (8, 10, 11) and it is interesting to investigate if other enzymes involved in steroid formation have the same importance. In this study, we examined mRNA expression of 17HSD5, 17HSD12, and 17HSD14 in 131 primary breast tumors. 17HSD5 and 17HSD14 showed a wide range of expression levels, whereas 17HSD12 showed a narrow range that was almost normal distributed. This might indicate the relevance of the investigated enzymes in breast cancer. Data from previous studies about 17HSD1 and 17HSD2 in breast cancer shows a wide range of expression (10, 11). If an enzyme shows small variation in a large tumor material, it may implicate that its presence is of minor importance. We found that the expression of 17HSD5 and 17HSD12 as well as 17HSD14 and 17HSD12 were significantly correlated, which may indicate that these enzymes are coregulated. It has been suggested that some cytokines may act as cofactors to regulate 17HSD expression (5, 6). Further, it is known that progesterone influences 17HSD1 and 17HSD2 expression in breast cancer cells (14). We speculate that factors that affect the expression of 17HSD1 and 17HSD2 may also affect the expression of other 17HSD enzymes.

Normal mammary gland from four post menopausal women showed a pattern of lower 17HSD5 expression than the tumors, similar 17HSD12 expression levels and lower 17HSD14 expression than the tumors. The low expression of 17HSD5 in the normal samples may show that high 17HSD5 expression is a risk factor in breast tumors and the similar expression of 17HSD12 may point out that this enzyme is not involved in tumor development. It is difficult to speculate about the low expression of 17HSD14 in the normal tissue. We do not know if the women that contributed with the normal tissue used any hormone replacement therapy. If they did, it is possible that progestin may influence the expression of more 17HSD enzymes than 17HSD1 and 17HSD2 (14). Further, because we do not have paired normal and tumor tissue from the same

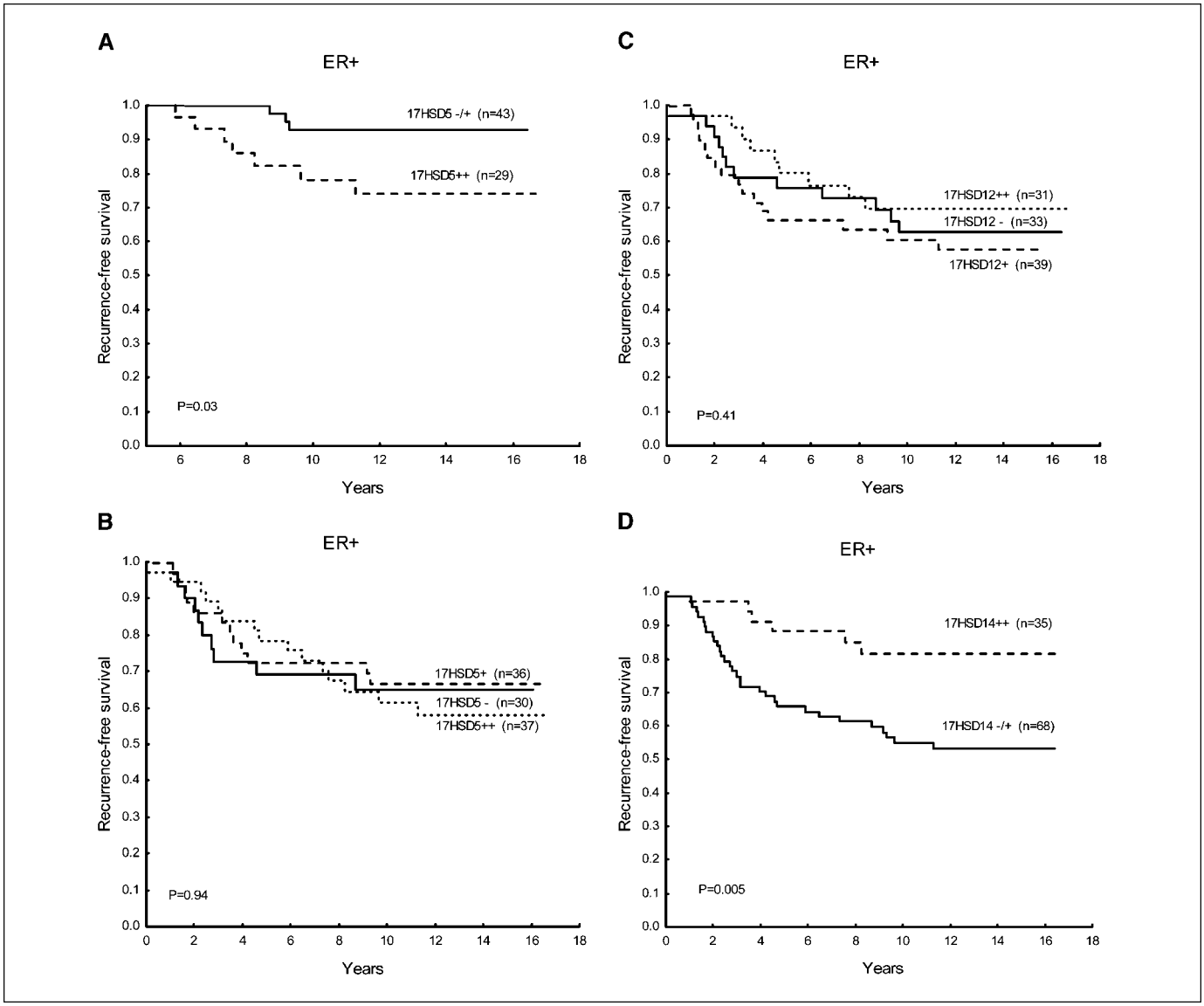

Figure 2. Kaplan-Meier curves showing recurrence-free survival in patients with ER-positive tumors (*A*) later than 5 years after diagnosis that expressed high (++) levels of 17HSD type 5 compared with tumors with intermediate (+) and low (−) levels. *B,* for the whole follow-up period about 17HSD5 expression. *C,* for the whole follow-up period about 17HSD12 expression. *D,* for the whole follow-up period about 17HSD14 expression.

Table 2. Multivariate Cox analysis of recurrence rate and breast cancer-related deaths for ER-positive patients in relation to 17HSD5, 17HSD12, and 17HSD14 and other tumor characteristics

	Recurrence		Breast cancer death	
	Rate ratio (95% CI)	Significance, *P*	Rate ratio (95% CI)	Significance, *P*
17HSD5				
−/+	1.0		1.0	
++	1.2 (0.60-2.4)	0.61	0.88 (0.41-1.9)	0.75
17HSD12				
−/+	1.0		1.0	
++	0.81 (0.36-1.8)	0.60	0.90 (0.38-2.1)	0.82
17HSD14				
−/+	1.0		1.0	
++	0.38 (0.15-0.95)	0.04	0.34 (0.12-0.94)	0.04
Nodal status				
N^-	1.0		1.0	
N^+	2.6 (1.03-6.8)	0.04	3.3 (1.2-9.0)	0.02
Tumor size (mm)				
≤20	1.0		1.0	
>20	1.5 (0.70-3.2)	0.30	1.9 (0.83-4.3)	0.13
PgR status				
PgR^-	1.0		1.0	
PgR^+	0.74 (0.36-1.5)	0.41	0.76 (0.35-1.6)	0.48
Tamoxifen (y)				
2	1.0		1.0	
5	0.88 (0.44-1.8)	0.72	1.09 (0.52-2.3)	0.82

Abbreviation: 95% CI, 95% confidence interval.

individuals, it is not possible to say if the investigated 17HSD14 levels from normal tissue are low compared with a corresponding tumor.

17HSD5 recognizes several different substrates and for instance inactivates dihydrotestosterone and catalyzes the formation of testosterone. Androgens may have an antiproliferative action on the breast epithelium (15), but in breast tumors expressing aromatase, testosterone can be converted to estradiol. There are few studies that have investigated the role of 17HSD5 in breast cancer. Lewis et al. (16) investigated paired tumor and nontumor tissue from 11 patients. They found that 17HSD5 expression was higher in normal than in tumor tissue. On the contrary, Oduwole et al. (8) found that 17HSD5 expression was higher in breast cancer specimens than in normal breast tissue. Among the breast cancer cases, the group with high 17HSD5 expression had a worse survival rate than the groups with low or no expression. This could not be confirmed in multivariate analysis. We confirm the importance of 17HSD5 in breast cancer and report that high 17HSD5 expression is related to significantly higher risk of late relapse in ER-positive patients remaining recurrence-free later than 5 years after diagnosis. However, this was not confirmed by multivariate analysis when the whole follow-up period was considered. We found previously a similar pattern for 17HSD1 (11).

In a recent study, Luu-The et al. (9) showed that 17HSD12 selectively catalyzed the transformation of estrone to estradiol. They determined the tissue distribution of 17HSD12 in some estrogen-sensitive tissues and found that 17HSD12 was highly and selectively expressed in ovary and mammary gland. In the same samples, 17HSD1 was expressed at much lower levels. Further, they suggested that 17HSD12 is the major estrogenic 17HSD responsible for estrone to estradiol conversion in women. 17HSD1 has been detected at low levels in normal breast tissue (7), and high levels of 17HSD1 have been associated to decreased survival in ER-positive breast cancer (10, 11). In this study, we did not find any support for a role of 17HSD12 mRNA in breast cancer. We did neither find any prognostic significance. 17HSD12 has a lower enzymatic activity than 17HSD1 (9) and Lukic et al. (2) suggest that the main function of 17HSD12 is

Table 3. mRNA expression of 17HSD14 24, 48, and 72 hours after transfection compared with control

	Time (h)		
	24	48	72
MCF7			
Control	0.01 ± 0.005	0.12 ± 0.002	0.01 ± 0.009
Transfected	0.43 ± 0.12	3.44 ± 0.84	1.84 ± 0.51
SKBR3			
Control	0.01 ± 0.004	0.03 ± 0.02	0.004 ± 0.002
Transfected	1.83 ± 0.48	1.19 ± 0.26	1.03 ± 0.27
ZR75-1			
Control	0.03 ± 0.02	0.03 ± 0.02	0.01 ± 0.008
Transfected	1.86 ± 1.22	0.96 ± 0.24	1.45 ± 0.36

NOTE: The results are presented as mean from triplicates in a representative experiment, ±SE.

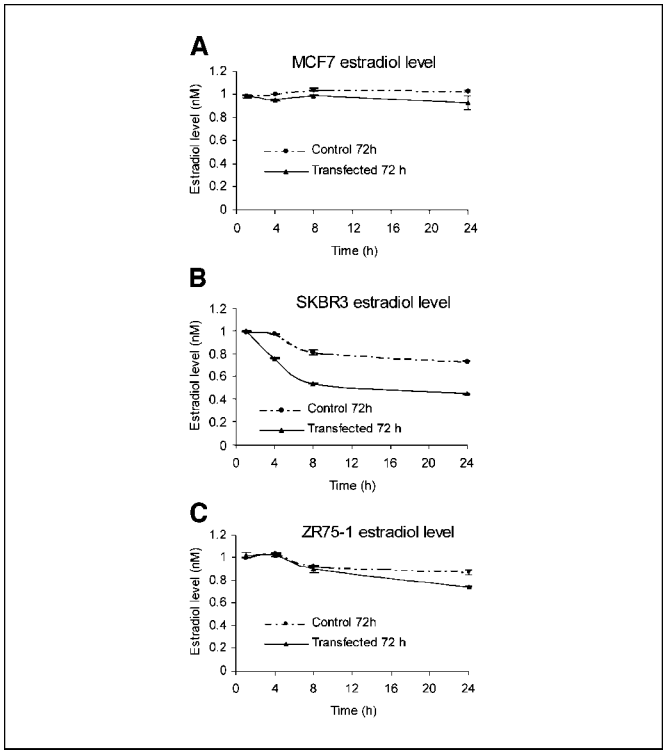

Figure 3. Estradiol levels in 17HSD14-transfected samples and controls treated with 10^{-9} mol/L estradiol 72 hours after transfection and thereafter incubated for 1, 4, 8, and 24 hours. *A,* MCF7 cells. *B,* SKBR3 cells. *C,* ZR75-1 cells. The curves are presented as mean from triplicates in a representative experiment, ±SE.

involvement in fatty acid synthesis. The importance of 17HSD1 in breast cancer has been confirmed in a large study consisting of 794 patients (8). Taken together, these results indicate that 17HSD1 is still likely to be the main enzyme responsible for the conversion of estrone to estradiol in breast cancer. However, this does not out rule the possible importance of 17HSD12 in normal breast tissue.

We investigated mRNA expression of 17HSD14 and found detectable levels in all investigated breast tumors. High expression compared with moderate and low expression was significantly associated with improved recurrence-free survival and overall breast cancer survival, which was confirmed by multivariate analysis. The results could depend on the fact that among those with high 17HSD14 expression the group that received tamoxifen for 5 years instead of 2 years was larger (43% versus 23 %), but when reanalyzing the group treated with 5 years alone, they showed the same pattern. Moreover, in the multivariate analyses, the treatment effect was adjusted for. Expression of 17HSD14 has been examined previously by Northern blot in normal samples from several human tissues and cell types (13, 17). Haeseleer et al. (13) found 17HSD14 expression at low levels in kidney and high levels in retina; no expression was detected in heart, brain, lung, or liver. On the contrary, Keller et al. (17) found high levels in lung, kidney, liver, and spleen. In both studies, single samples were investigated with one method. The expression levels of 17HSD14 in different tissues may vary among individuals.

Cultured insect cells have been transfected with human 17HSD14, but no steroid or retinol dehydrogenase activity could be detected (13). Despite that, it is possible that human recombinant proteins that are not biologically active in insect cells may be active in human cells. Lukacik et al. (2) describes NAD^+-dependent 17HSD activities using estradiol and testosterone and structure determination of the human enzyme revealed that the active site was able to accommodate steroid substrates. We found that transfection of 17HSD14 in human breast cancer cells significantly decreased the levels of estradiol in the culture medium from two cell lines. There was a trend that moderate and high 17HSD14 expression was more common among ER-positive patients. The cell lines examined represent ER-positive (MCF7 and ZR75-1) and ER-negative (SKBR3) breast cancer. However, the endogenous expression of 17HSD14 was low in all three cell lines examined. The endogenous expression of other estrogen-converting enzymes, such as sulfatase, sulfotransferase, and 17HSD enzymes, may influence the experimental results about estradiol levels, despite that we found decreased levels of estradiol. Although the decreases of estradiol concentration were small in the experiments, it may be of importance for patients with estrogen-dependent breast tumors in the long-term. Phylogenetic analysis classifies 17HSD14 closer to steroid dehydrogenases than to retinoid dehydrogenases (13), and it is also possible that other steroid hormones may be oxidated by 17HSD14. Further studies are necessary to investigate how 17HSD14 is involved in the biosynthesis of hormones.

In conclusion, we found that high 17HSD5 is related to significantly higher risk of late relapse in ER-positive patients remaining recurrence-free later than 5 years after diagnosis. Further, our results show that 17HSD12 is likely of minor importance in breast cancer, which indicates that 17HSD type 1 could be the main enzyme responsible for the conversion of estrone to estradiol. Finally, high 17HSD14 expression is significantly associated with improved recurrence-free survival and breast cancer-specific survival, confirmed by multivariate analysis. We found that transfection of 17HSD14 in the human breast cancer cells MCF7 and SKBR3 significantly decreases the levels of estradiol, presenting an effect of high expression levels of the enzyme.

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