Proliferative responses to altered 17β-hydroxysteroid dehydrogenase (17HSD) type 2 expression in human breast cancer cells are dependent on endogenous expression of 17HSD type 1 and the oestradiol receptors

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Abstract

The primary source of oestrogen in premenopausal women is the ovary but, after menopause, oestrogen biosynthesis in peripheral tissue is the exclusive site of formation. An enzyme group that affects the availability of active oestrogens is the 17β-hydroxysteroid dehydrogenase (17HSD) family. In breast cancer, 17HSD type 1 and type 2 have been mostly investigated and seem to be the principal 17HSD enzymes involved thus far. The question whether 17HSD type 1 or type 2 is of greatest importance in breast tumour development is still not clear. The aim of this study was to investigate how the loss of 17HSD type 2 expression, using siRNA in the non-tumour breast epithelial cells HMEC (human mammal epithelial cells) and MCF10A, and gain of 17HSD type 2 expression, using transient transfection in the breast cancer derived cell lines MCF7 and T47D, affect oestradiol conversion and proliferation rate measured as S-phase fraction. We further investigated how this was related to the endogenous expression of 17HSD type 1 and oestradiol receptors in the examined cell lines. The oestradiol level in the medium changed significantly in the MCF7 transfected cells and the siRNA-treated HMEC cells, but not in T47D or MCF10A. The S-phase fraction decreased in the 17HSD type 2-transfected MCF7 cells and the siRNA-treated HMEC cells. The results seemed to be dependent on the endogenous expression of 17HSD type 1 and oestradiol receptors in the examined cell lines. The oestradiol level in the medium changed significantly in the MCF7 transfected cells and the siRNA-treated HMEC cells, but not in T47D or MCF10A. The S-phase fraction decreased in the 17HSD type 2-transfected MCF7 cells and the siRNA-treated HMEC cells. The results seemed to be dependent on the endogenous expression of 17HSD type 1 and oestradiol receptors. In conclusion, we found that high or low levels of 17HSD type 2 affected the oestradiol concentration significantly. However, the response was dependent on the endogenous expression of 17HSD type 1. Expression of 17HSD type 1 seems to be dominant to 17HSD type 2. Therefore, it may be important to investigate a ratio between 17HSD type 1 and 17HSD type 2.

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Introduction

It is well documented that the mitogenic effects of oestrogens are critical in the progression of breast cancer. The effects of oestrogens are mediated by oestrogen receptors (ER)α and ERβ. Oestrogen binding to ERα induces cell proliferation whereas binding to ERβ inhibits proliferation (Paruthiyil et al. 2004, Strom et al. 2004). Various studies have shown decreased expression of ERβ in tumour versus normal tissue in the breast (Shaw et al. 2002, Roger et al. 2001). The primary source of oestrogen in premenopausal women is the ovary but, after menopause, oestrogen biosynthesis in peripheral tissue has a major role. Oestrone sulphatase and aromatase are important enzymes for the local synthesis of oestradiol. An enzyme group that affects the availability of active oestrogens is the 17β-hydroxysteroid dehydrogenase (17HSD) family (Pasqualini 2004). It is well known that 17HSD activity
is responsible for the balance between oestrone and oestradiol in breast tissue. The 17HSD family has 13 known members and several of them have been identified to be of importance in different hormone dependent tissues and tumours. There are multiple family members expressed in breast tissue (Speirs et al. 1998, Peltokeito et al. 1999, Luu-Thee et al. 2005) even though 17HSD type 1 and 2 seem to be the principal 17HSD enzymes involved in breast cancer thus far (Labrie et al. 1997, Miettinen et al. 1999). 17HSD type 1 catalyses reduction of oestrone to oestradiol with NADP(H) as a cofactor, and 17HSD type 2 catalyses oxidation from oestradiol to oestrone with NAD(H) as a cofactor (Vihko et al. 2001).

17HSD type 2 is the dominant form in normal epithelium of the breast, and protects the epithelial cells to balance the amount of oestrone against oestradiol (Speirs et al. 1998). In breast cancer, 17HSD type 1 and type 2 have been mostly investigated and high expression of 17HSD type 1 and low expression of 17HSD type 2 are associated with decreased survival in ERa positive breast cancer (Gunnarsson et al. 2001, 2005, Oduwole et al. 2004). The question whether 17HSD type 1 or type 2 is of greatest importance in tumour development is still not clear. Oxidative 17HSD type 2 activity leading to the conversion of oestradiol to oestrone has been detected to be 50 times more predominant than the reductive 17HSD type 1 activity (Vihko et al. 2001). However, Miettinen et al. (1996) suggested that, in cultured cells, 17HSD type 1 has a higher catalytic efficacy than the 17HSD type 2 enzyme.

To address this question, the aim of this study was to investigate how the loss of 17HSD type 2 expression in non-tumour breast epithelial cells and gain of 17HSD type 2 expression in breast cancer-derived cell lines affect oestradiol conversion and proliferation rate. We further investigated how this was influenced by the endogenous expression of 17HSD type 1, ERa and ERb in the examined cell lines.

**Material and methods**

**Cell culture and materials**

T47D and MCF7 breast cancer epithelial cells (American Type Culture Collection, Manassas, VA, USA) were cultured in phenol-red free Opti-Mem I (Invitrogen, Carlsbad, CA, USA), supplemented with 4% fetal bovine serum (FBS; Invitrogen) and incubated at 37 °C, 5% CO2. In all experiments, charcoal/dextran-treated serum (CTS; HyClone, UT, USA) was used to control the levels of oestradiol. The experiments with T47D were performed between passages 100 and 104 and MCF7 between passages 151 and 157.

The non-tumour breast epithelial cells MCF10A (American Type Culture Collection) and Human Mammal Epithelial Cells (HMEC; Cambrex, Walkersville, MD, USA) were cultured in serum free Mammary Epithelial Growth Medium (MEGM; Cambrex) supplemented with 100 ng/ml cholera toxin (Sigma-Aldrich), and incubated at 37 °C, 5% CO2. The experiments with MCF10A were performed between passages 99 and 101 and HMEC between passages 9 and 11.

**Cloning and transient transfection of 17HSD type 2**

Synthesis of cDNA from human mammary gland RNA was performed using the 1st strand cDNA Synthesis kit with random hexamers (Roche Diagnostics Corporation, Indianapolis, IN, USA). To generate double-stranded 17HSD type 2 cDNA, a pair of primers 5’GAAGTTATCACGTCAGCTGAAGGTGCAAGCATA- GTAC 3’ and 5’ATGGTCTGAAAGCTTTAT-TGCTAGGTCGCTTTTT 3’ containing SalI and HindIII 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. The 17HSD type 2 gene was subcloned to pLP-IRE5-EGFP Acceptor vector (BD Biosciences Clontech) using Cre recombinase. The plasmids containing the 17HSD type 2 cDNA were sequenced with MegaBACE 500 DNA analysis Systems (Amersham Biosciences) sequencing equipment according to the manufacturer’s protocol.

For transfection with 17HSD type 2, 30 000/well T47D cells and 60 000/well MCF7 cells 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 Corporation) 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 harvest. Cells transfected with an empty vector were used as control.

**siRNA**

Two 19-nucleotide specific sequences were selected in the coding region of HSD17B2 to generate 21-nucleotide sense and anti-sense strands. The sequences were submitted to BLAST (Basic Local Alignment Search Tool; http://www.ncbi.nlm.nih.gov/BLAST) to
ensure the specificity of the siRNA to the targeted sequence. The specific target sequence for the two HSD17B2 siRNA are as follows: 5′AAAGAUGCU UUACAGCAAGGUU3′; 5′AAGCCGCUACUUUGU GGAUCU3′. For transfection of the siRNA duplexes, 44 000 cells/well of MCF10A and HMEC were seeded in 12 well plates in 1 ml/well in culture medium. Twenty four hours after seeding, the cells were transfected by Lipofectamine 2000 (Invitrogen) by adding in each well 0.2 ml containing 0.5 μg siRNA, 2 μl Lipofectamine 2000 and Opti-Mem I (Invitrogen). Twenty four hours after transfection, the cells were washed with PBS and incubated in culture medium before harvest. One duplex, which does not recognise any sequence in the human genome, was used as negative control and one duplex against β-actin (Qiagen) was used as positive control in all experiments.

Real-time PCR
RNA was extracted from the siRNA-treated HMEC and MCF10A cells and controls with unspecific siRNA, and 17HSD type 2-transfected T47D and MCF7 cells and controls with an empty vector, 24, 48 and 72 h after siRNA treatment or transfection using SV Total RNA Isolation System (Promega). The RNA was stored at −70 °C. Synthesis of cDNA from total RNA was performed using the 1st strand cDNA Synthesis kit with random hexameres (Roche Diagnostics Corporation). mRNA expression of 17HSD type 1 and 17HSD type 2 was analysed according to Gunnarsson et al. (2001). Further, mRNA levels of 17HSD type 1, 17HSD type 2, ERα and ERβ were analysed in untreated HMEC, MCF10A, T47D and MCF7 cells. ERα and ERβ were analysed employing Assay on demand no Hs00174860 and no Hs00230957 respectively (Applied Biosystems, Warrington, UK) according to the manufacturer’s description. β-Actin was used as endogenous reference gene except for in the positive controls of the siRNA when phosphoglycerate kinase was used. The assays were purchased from Applied Biosystems. Standard curves for all analysed genes were run on each plate, using serial diluted cDNA to normalise the runs. The data obtained from β-actin were used to normalise the sample variation in the amount of input cDNA. All samples were run as triplicates and in all experiments samples without template were used as control. The relative quantification of 17HSD type 1, 17HSD type 2, ERα and ERβ were performed according to the manufacturer’s description (Protocol P/N 4303859, Perkin Elmer, Foster City, CA, USA).

Measurement of oestradiol levels
The concentration of oestradiol in the medium was measured in 17HSD type 2 siRNA-transfected and control HMEC and MCF10A cells, and 17HSD type 2 transfected and control T47D and MCF7 cells. The medium was changed to a medium containing 10−9 M oestradiol at 48 and 72 h after siRNA treatment or transfection and thereafter incubated at 37 °C with 5% CO2 for 1, 4, 8 and 24 h, and finally frozen in liquid nitrogen. The concentration of oestradiol was measured using electrochemiluminescence immunoassay (ELICA) employing the Elecsys Estradiol II reagent kit (Roche Diagnostics Corporation) analysed with Roche Elecsys 1010/2010.

S-Phase measurement with flow cytometry
The proportions of cells in S phase were analysed using DNA flow cytometry according to Vindelöv et al. (1983). The S-phase fraction was analysed in 17HSD type 2 siRNA-transfected and control HMEC and MCF10A cells, and in 17HSD type 2-transfected and control T47D and MCF7 cells, with or without treatment with 10−9 M oestradiol in the medium. The medium was changed at 24 and 48 h after transfection and the cells were harvested at 48 and 72 h after transfection. Ten thousand cells were analysed in each sample using a Becton Dickinson FACSCalibur (Becton Dickinson, San José, CA, USA) and ModFit LT software (Verity Software House Inc., Topsham, ME, USA).

Statistical evaluation
Student’s t-test was used for a comparison between the groups. The results are expressed as ± s.e. of the mean values obtained from three replicates repeated three times. All P-values are two sided, and P<0.05 was considered to be statistically significant. All the procedures were comprised in the statistical package Statistica 6.0 (StatSoft Scandinavia AB, Uppsala Sweden).

Results

Expression of 17HSD type 1, 17HSD type 2, ERα and ERβ
Relative quantities of mRNA expression to the endogenous reference of 17HSD type 1, 17HSD type 2, ERα and ERβ were analysed in untreated HMEC, MCF10A, T47D and MCF7 cells. MCF10A and T47D cells expressed high levels of 17HSD type 1 (Fig. 1A); MCF10A expressed high levels of 17HSD type 2 and HMEC and T47D intermediate levels (Fig 1B); MCF7
expressed high levels of ERα, T47D intermediate levels and HMEC low levels of ERα (Fig.1C); HMEC and T47D expressed high levels of ERβ (Fig 1D).

**Transfection of 17HSD type 2 and siRNA treatment**

MCF7 and T47D were transfected with pLP-IRES2-EGFP-HSD17B2 and a control vector, and MCF10A and HMEC were transfected with 17HSD type 2 siRNA and a control duplex. The mRNA expression was analysed at 24, 48 and 72 h after transfection. The levels of 17HSD type 2 increased significantly compared to the controls in MCF7 and T47D, and decreased significantly compared to the controls in MCF10A and HMEC (Table 1). The differences between the groups were greatest at 48 h after treatment. The levels of β-actin in the positive control samples decreased by 60%, 75% and 55% after 24, 48 and 72 h respectively.

**Measurements of oestradiol levels**

The concentration of oestradiol levels was measured in samples treated with 10^{-9} M oestradiol, 48 and 72 h after 17HSD type 2 siRNA transfection or 17HSD type 2 transfection and thereafter incubated at 1, 4, 8 and 24 h. The concentration of oestradiol decreased significantly more in the control compared to the 17HSD type 2 siRNA-transfected HMEC cells after 48 (Fig. 2A) and 72 h (Fig. 2B). There were no differences in oestradiol concentration in the medium from the MCF10A cells neither at 48 (Fig. 2C) nor at 72 h (Fig. 2D). The concentration of oestradiol decreased significantly in 17HSD type 2-transfected MCF7 cells compared with the control after 48 (Fig. 2E) and 72 h (Fig 2F). There were no differences in oestradiol concentration in the medium from the T47D cells at 48 (Fig 2G) or at 72 h (Fig 2H).

The levels in the medium from the HMEC cells decreased rapidly in both the controls and the siRNA transfected samples until 8 h after oestradiol treatment and thereafter the decrease declined. The same pattern was observed in the MCF10A cells, but the decreases in oestradiol level were less and the conversion of oestradiol was not detected after 8 h of incubation. The oestradiol levels in the medium from the MCF7 cells decreased in the 17HSD type 2-transfected cells after incubation, but the oestradiol level in the control did not change. In the medium from the T47D cells, the oestradiol levels were quite steady in both the controls and the transfected samples.

**Characterisation of proliferative responses**

The S-phase fraction significantly decreased in HMEC cells transfected with 17HSD type 2 siRNA and treated with oestradiol, compared with the control, 48 h after siRNA transfection. This was not detected in the samples that were not treated with oestradiol. There were no significant changes after 72 h (Fig. 3A). The MCF10A cells did not show any significant difference in S-phase fraction between any of the groups at 48 or 72 h (Fig. 3B).

The S-phase fraction decreased significantly in the MCF7 cells transfected with 17HSD type 2 and treated with oestradiol, compared with the control, 48 and 72 h after transfection. This was not detected in the samples that were not treated with oestradiol (Fig. 3C). There were no differences between any of the groups in the T47D cells at 48 or at 72 h (Fig. 3D).
Table 1 mRNA expression of 17HSD type at 24 h, 48 h and 72 h after transfection compared to control. Results are presented as mean from triplicates repeated three times, ± S.E. All samples compared to controls are significantly changed.

<table>
<thead>
<tr>
<th></th>
<th>Control MCF7</th>
<th>24 h</th>
<th>Transfected MCF7</th>
<th>48 h</th>
<th>72 h</th>
</tr>
</thead>
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<tr>
<td>Control T47D</td>
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<td>0.003 ± 0.009</td>
<td>3143 ± 14.2</td>
<td>0.005 ± 0.006</td>
<td>0.003 ± 0.006</td>
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<td>Transfected T47D</td>
<td></td>
<td>0.31 ± 0.07</td>
<td>2372 ± 15.5</td>
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<td>6.92 ± 1.97</td>
<td>2.09 ± 0.32</td>
<td>0.42 ± 0.07</td>
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<tr>
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<td>7.2 ± 3.4</td>
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</table>

Figure 2 Oestradiol levels in transfected samples treated with 10⁻⁹ M oestradiol at 48 and 72 h after transfection and thereafter incubated at 1, 4, 8 and 24 h (A) 17HSD type 2 siRNA-transfected HMEC cells at 48 h; (B) 17HSD type 2 siRNA-transfected HMEC cells at 72 h; (C) 17HSD type 2 siRNA-transfected MCF10A cells at 48 h; (D) 17HSD type 2 siRNA-transfected MCF10A cells at 72 h; (E) 17HSD type 2-transfected MCF7 cells at 48 h; (F) 17HSD type 2-transfected MCF7 cells at 72 h; (G) 17HSD type 2-transfected T47D cells at 48 h; (H) 17HSD type 2-transfected T47D cells at 72 h. Results are presented as mean from triplicates repeated three times, ± S.E.
Figure 3 S-Phase fraction shown as percentage compared to the control in oestradiol treated cells at 48 and 72 h after transfection (A) 17HSD type 2 siRNA-transfected HMEC cells; (B) 17HSD type 2 siRNA-transfected MCF10A; (C) 17HSD type 2-transfected MCF7 cells; (D) 17HSD type 2-transfected T47D cells. Results are presented as mean from triplicates repeated three times, ± s.e. E2, 17β oestradiol.
**Discussion**

Several studies have investigated the importance of 17HSD type 1 and 17HSD type 2 in different types of cancer, including breast cancer (Suzuki et al. 2000, Gunnarsson et al. 2001, 2005, Koh et al. 2002, Oduwole et al. 2002, 2004). We found that MCF7 cells express low endogenous expression of both 17HSD type 1 and 17HSD type 2, and in the medium from the control cells of MCF7, the oestradiol concentration decreased slowly. Since oestradiol is the most potent oestrogen that affects proliferation in breast cancer, we chose to only measure oestradiol levels in this study. When MCF7 was transfected with 17HSD type 2, the oestradiol level decreased significantly more than in the controls. T47D cells express relatively high endogenous expression of 17HSD type 1 and low expression of 17HSD type 2. In the medium from the T47D control cells, there were no changes in oestradiol concentration after incubation. Even though the transfected T47D cells expressed higher level of 17HSD type 2 than 17HSD type 1, we did not detect any differences in oestradiol concentration in the medium. The result from control and transfected MCF7 and T47D cells may reflect the endogenous concentration of 17HSD type 1 and 17HSD type 2. The T47D cells showed a relatively high expression of 17HSD type 1 and had an unchanged oestradiol level, but in the MCF7 cells with lower expression of 17HSD type 1 than 17HSD type 2, there was a slight decrease in concentration in medium from the control cells. This indicates that it is of importance that the expression of 17HSD type 2 is higher than that of 17HSD type 1 to protect the cells against oestradiol. In breast cancer, high expression of 17HSD type 1 and low expression of 17HSD type 2 has been correlated to bad prognosis (Gunnarsson et al. 2001, 2005, Oduwole et al. 2004). In most studies, the enzymes have been investigated individually in the tumours and not as a ratio between 17HSD type 1 and 17HSD type 2. Gunnarsson et al. (2005) determined a ratio between the two enzymes in breast cancer and found significant increase in survival rate in the tumours with a high 17HSD type 2 and low 17HSD type 1 expression, which was not found when investigating 17HSD type 1 individually. The results found on oestradiol levels in the medium in both the control and transfected cells from MCF7 and T47D support the idea to investigate a ratio between 17HSD type 1 and 17HSD type 2 instead of investigating them individually.

The oxidative pathway (oestradiol to oestrone) has been found to be predominant in normal breast epithelial cells (Speirs et al. 1998). In the non-tumour breast epithelial cells employed in this study, HMEC expressed low endogenous amounts of both 17HSD type 1 and 17HSD type 2, even though the expression of 17HSD type 1 was higher than 17HSD type 2. In the medium from the control cells of HMEC, the oestradiol concentration decreased rapidly in the first 8 h of incubation. When the 17HSD type 2 expression was decreased by siRNA, HMEC showed significantly decreased expression of oestradiol in the controls compared to the treated samples. The MCF10A expressed high endogenous amounts of both 17HSD type 1 and 17HSD type 2, and the expression of 17HSD type 2 was higher than 17HSD type 1. In the medium from the control cells of MCF10A, the oestradiol concentration decreased quickly, but the oestradiol concentration did not differ in the controls compared to the treated samples. The steep decrease in oestradiol levels in the control samples from HMEC contradicts the results from the other cell lines. However, the expression of 17HSD type 1 is low in HMEC. There may also be other members of the 17HSD family that are expressed in HMEC that work in the same direction as 17HSD type 2 and convert oestradiol to oestrone, such as 17HSD type 13. The steep decrease in oestradiol level was detected in the first 8 h of incubation in both the control and treated HMEC and MCF10A cells, but not in MCF7 and T47D. This may be a result of oestrogen sulphotransferase (EST) expression in the non-tumour breast epithelial cells that sulphonates the oestradiol. Suzuki et al. (2003) showed that there is high expression of EST in normal human mammary epithelial cells. Steroid sulphatase (STS) hydrolyses oestrone sulphate to oestrone and in MCF7 and T47D Chetrite et al. (1999a) found the activity of both EST and STS. The STS activity in the tumour cells examined may neutralise the EST activity. Ishibashi et al. (2005) found that oestradiol concentrations in human thymoma were inversely correlated to EST and positively correlated with STS and 17HSD type 1. It is likely that the same pattern can be detected in breast cancer and that 17HSD type 2 can be correlated to high expression of EST. The balance of 17HSD enzymes may be more important in breast cancer cells with both high EST and STS, than in the cells with the same expression pattern as in the thymoma, both in vitro and in vivo.

There were significant differences in the medium from the siRNA treated HMEC cells, but not from the MCF10A cells. This may be a result of more efficient siRNA transfection in the HMEC cells, but also that MCF10A has a higher endogenous expression of 17HSD type 1 than HMEC. Our results suggest that 17HSD type 1 is more dominant than 17HSD type 2, and that there must be excessive amounts of 17HSD.
type 2 to inhibit the effect of 17HSD type 1. Taken together, the results on oestradiol levels in the medium from MCF7, T47D, HMEC and MCF10A show that 17HSD type 2 is important to maintain oestrone–oestradiol balance in the cells and that factors, such as other 17HSD members can interfere with this balance. Even though changes of oestradiol levels in the medium are moderate following transfection, the influence of 17HSD type 1 and 17HSD type 2 on proliferation of ERα positive tumours in vivo may have larger effects in the long term for the patients. Progestin is administrated in contraceptives and, to prevent climacteric symptoms, has been shown to interfere with the regulation of enzymes involved in oestrogen formation, including 17HSD type 1 and 17HSD type 2 expression. In T47D cells, progestin induces 17HSD type 1 expression (Poutanen et al. 1990) and breast tumours from postmenopausal women who received lynestrenol showed higher 17HSD type 1 expression than tumours from untreated patients (Fournier et al. 1985). In contrast, medrogestrone significantly decreases 17HSD type 1 activity in both MCF7 and T47D cells in a dose-dependent manner (Chetrite et al. 1999b) and in the endometrium of oestrogen-dependent benign disease, progesterone induced 17HSD type 2 expression (Kitawaki et al. 2000). It would be interesting to investigate how the use of progestins correlates with the expression pattern of 17HSDs in breast cancer patients.

Previous in vitro studies investigating the role of 17HSD type 2 have investigated the conversion of sex steroids (Miettinen et al. 1996, Härkönen et al. 2003, Liu et al. 2005) without studying the effect on proliferation. We found that the fraction of cells in S-phase in the 17HSD type 2-transfected MCF7, as well as in the 17HSD type 2 siRNA-transfected HMEC cells significantly decreased compared to the controls. No differences were detected in the T47D cells and the MCF10A cells. The level of ERα is measured in breast cancer for the stratification of treatment, and about 70% of breast cancers show high expression. MCF7 and T47D are cell lines with high ERα expression and can be used as models for tumours with high ERα expression. Our findings indicate that tumours with high ERα expression, high 17HSD type 1 expression and low 17HSD type 2 expression are exposed to high amounts of oestradiol and have a high proliferation rate. These results are in line with our previous findings on ERα positive breast cancer when high expression of 17HSD type 1 and low expression of 17HSD type 2 were associated with decreased survival (Gunnarsson et al. 2001, 2005). The ratio of ERα/ERβ mRNA tends to increase in tumours versus normal tissue, and prompts the mitogenic effect of oestradiol (Roger et al. 2001, Shaw et al. 2002). In the MCF7 cells, the ratio of ERα/ERβ was higher than in the T47D cells. The oestradiol levels decreased between 48 and 72 h after transfection in the MCF7 cells and, as a result, we found a decrease in S-phase fraction. During this time course, only small effects on the proliferation affected by these differences were possible to detect, even though during a longer time period such decreases may be of importance. The oestradiol levels did not decrease in the T47D cells after transfection. The T47D cells express 5 times more ERβ than ERα and therefore a slight decrease in proliferation might have been expected in both the transfected cells and the controls. The proliferation in the HMEC cells decreased after siRNA transfection compared to the control, even though the oestradiol levels increased. The HMEC cells express high levels of ERβ and hardly detectable levels of ERα and exposure of oestradiol might lead to a decrease in S-phase fraction. However, there were no differences in S-phase fraction in the control cells treated with or without oestradiol, which there should have been if ERβ was the target of importance. Therefore, other mechanisms may be involved. The MCF10A cells showed no changes in oestradiol after siRNA transfection and are totally negative for both ERα and ERβ, and no differences in S-phase fraction were detected as expected.

In summary, this article examines the importance of 17HSD type 2 in breast cancer-derived cell lines and in non-tumour breast epithelial cells in relation to endogenous expression of 17HSD type 1, ERα and ERβ. We found that high or low expression levels of 17HSD type 2 affected the oestradiol concentration significantly; however, the response was dependent on the endogenous expression of 17HSD type 1. The proliferative changes induced by changes in oestradiol concentration were followed by ERα and ERβ expression. Expression of 17HSD type 1 seems to be dominant over 17HSD type 2. Therefore, it may be important to investigate a ratio between 17HSD type 1 and 17HSD type 2 instead of investigating them individually.

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References


