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Regulation of steroid sulphatase expression and activity in breast cancer

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Abstract

Steroid sulphatase (STS) catalysis the conversion of oestrone sulphate (E1S) to oestrone (E1) and its action in breast tumours makes a major contribution to in situ oestrogen production in this tissue. Although expression of STS mRNA and STS activity are increased in malignant breast tissues compared with that in non-malignant tissues, little is known about the regulation of its expression or activity. In the present study we have used a RT-PCR technique to investigate the regulation of STS mRNA expression in cultured breast tissue fibroblasts and MCF-7 cells. STS mRNA expression was readily detectable in fibroblasts derived from breast tissue proximal to tumours, breast tumour tissue and reduction mammoplasty tissue. For two pre-menopausal subjects, STS mRNA expression was similar in proximal and tumour fibroblasts whereas for a third, post-menopausal subject, expression in breast tumour fibroblasts was 2.4-fold that in proximal fibroblasts. The cytokine tumour necrosis factor α (TNFα) or the STS inhibitor, 2-methoxyoestrone-3-*O*-sulphamate, had no effect on STS mRNA expression in fibroblasts. STS mRNA was detectable in MCF-7 cells but neither TNFα nor interleukin 6 (IL-6) affected its expression. Transient transfection of COS-1 and MCF-7 cells with a STS cDNA lacking STS 5′ and 3′ sequences increased activity 17-fold and 2-fold, respectively. TNFα plus IL-6 increased STS activity in mock transfected MCF-7 cells and further increased STS activity in transfected MCF-7 cells. This indicates that activation can occur independently of STS promoter and enhancer elements. In conjunction with the lack of regulation of STS mRNA it suggest that TNFα and IL-6 may increase STS activity via a post-translational modification of the enzyme or by increasing substrate availability.

Keywords: Steroid sulphatase; Tumour fibroblasts; Proximal fibroblasts; Tumour necrosis factor α

1. Introduction

The hydrolysis of oestrone sulphate (E1S) to oestrone (E1) by steroid sulphatase (STS) is thought to make a major contribution to the production of oestrogens within breast tumours [1,2]. The activity of this enzyme in breast tumours is considerably higher than that of the aromatase enzyme complex, which can also contribute to tumour oestrogen synthesis [3]. STS activity is also higher in malignant and benign breast tumours than in adjacent non-involved tissues [4,5]. Evidence for the potential importance of STS in regulating oestrogen synthesis within breast tumours has recently been obtained. Patients with tumours containing high levels of STS mRNA were found to have a significantly shorter disease free survival as compared with those with low levels of STS mRNA [6]. Furthermore, STS mRNA levels are significantly higher in malignant than non-malignant breast tissues [7].

The highest incidence of breast cancer occurs in post-menopausal women. There is evidence that STS activity is increased in breast tumours from post-menopausal women compared with the activity in tumours from pre-menopausal women [8]. It is well established that peripheral aromatase activity is related to age and body weight [9]. Cytokines, such as interleukin 6 (IL-6) and tumour necrosis factor α (TNFα) are thought to be responsible for the increases in pe-

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ripheral aromatase activity associated with ageing and obesity [10–12].

In contrast to our understanding of the regulation of aromatase expression and activity, little is known about the control of STS expression and activity. In breast cancer cells IL-6 and TNFα are able to stimulate STS activity and act synergistically to increase enzyme activity [13]. There is also evidence that progestins may inhibit the expression and activity of E1-STS in breast cancer cells [14]. Whether this regulation occurs at the transcriptional level or post transcriptionally is not known. Studies on the promoter region of the STS gene are limited [15]. While no specific transcription binding sites in the promoter region of this gene have so far been identified the basal promoter and 5′ enhancer element activities did show tissue specificity in transient reporter gene expression studies. Both were only active in cells of placental origin where STS activity is known to be high but showed no activity in COS-1, HeLa or B82 cells. Post translationally a novel protein modification is required at the active site for full enzyme activity. An α-formylglycine residue is formed by oxidation of a cysteine residue at position 75 within the active site [16]. The regulation of, and modifying enzymes involved in this process have not so far been elucidated. It is possible that this process represents another level of regulation of STS activity.

In the present study we have employed a semi-quantitative RT-PCR technique to examine basal and cytokine stimulated STS expression in fibroblasts derived from normal and malignant breast tissues. In addition, we have also examined the ability of IL-6 and TNFα to regulate STS expression in MCF-7 breast cancer cells. We have also explored the possibility that compounds, such as 2-methoxyoestrone-3-*O*-sulphamate (2-MeOEMATE) may block the ability of cells to respond to cytokines such as TNFα. 2-MeOEMATE, while a potent inhibitor of STS activity [17], can also induce cells to undergo apoptosis and has been found to reduce the ability of TNFα to stimulate aromatase activity in fibroblasts [18,19].

2. Materials and methods

2.1. Cell culture

MCF-7 breast cancer cells and COS-1 cells were obtained from the European Collection of Cell Cultures, (Salisbury, Wilts, UK). Cells were routinely maintained in Dulbeco's minimal essential medium (DMEM) with 5% fetal calf serum (FCS) and other essential nutrients [13]. Cells were cultured at 37°C under 5% CO_2 and used when 50–60% confluent. For experiments cells were cultured in DMEM with 5% charcoal stripped FCS.

2.2. Culture of breast fibroblasts

Fibroblasts were cultured from breast tissues of women undergoing lumpectomy for removal of breast tumour or reduction mammoplasty after obtaining their informed consent. The study was approved by the Hospital Ethics Committee. Clinical details for the three subjects with breast cancer are shown in Table 1. Subject number 4, from whom reduction mammoplasty tissue was obtained, was a 28 year old pre-menopausal women. Resected breast tumour tissue, tissue proximal to the tumour and reduction mammoplasty tissue were minced with sterile scalpels and incubated in Earle's MEM for 18–24 h at 37°C with collagenase (200 μg/ml) in the presence of antibiotics (penicillin, streptomycin and amphotericin; Sigma, Poole, Dorset, UK). The dispersed cells were harvested by centrifugation and washed twice with medium to remove collagenase. Dispersed cells were seeded into T-25 culture flasks and allowed to attach. The cells were grown to confluence before passaging on a weekly basis. After the third passage cultures consisted only of fibroblasts with no contaminating epithelial cells.

2.3. RT-PCR

Cells were grown as monolayers in T-75 culture flasks and when 50–60% confluent treated with cytokines and/or drugs for up to 24 h. Total RNA was extracted using Rneasy and QIAshredder kits from QIAgen (Crawley, West Sussex, UK) in accordance with the manufacturers instructions. Briefly, cells were washed with phosphate-buffered saline (PBS), lysed with the lysis buffer provided and the sample homogenised using the QIAshredder. Total RNA was isolated by its differential binding to a silica-gel-based membrane with subsequent elution and washing with

Table 1
Clinical details of breast cancer subjects

Subject No.	Age (Yr)	Menopausal status	Tumour size (cm)	ER	PR
1	46	pre-	2.7×2.7	−	−
2	88	post-	10×7	++	+
3	37	pre-	2×2	++	+

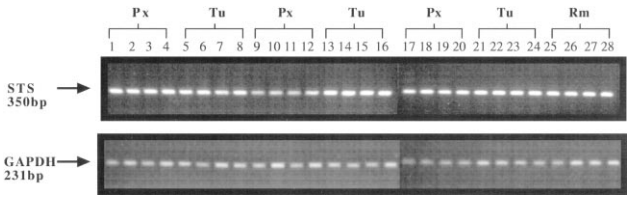

Fig. 1. Composite picture of two gels showing expression of STS and GAPDH mRNA in fibroblasts samples 1–28. Fibroblasts were derived from breast tissue proximal to tumours (Px), breast tumours (Tu) or reduction mammoplasty (Rm). STS mRNA was assessed after treating fibroblasts with vehicle (Cont), 2-methoxy-oestrone-3-*O*-sulphamate (2-ME, 10 μM) for 18 h, tumour necrosis factor α (TNFα, 20 ng/ml) for 18 h or 2-ME (10 μM for 3 h) before addition of TNFα (20 ng/ml) for a further 18 h. Lane (1–4) Subject 1 Px: Cont, 2-ME, TNFα, 2-ME + TNFα; lane (5–8) Subject 1 Tu: Cont, 2-ME, TNFα, 2-ME + TNFα; lane (9–12) Subject 2 Px: Cont, 2-ME, TNFα, 2-ME + TNFα; lane (13–16) Subject 2 Tu: Cont, 2-ME, TNFα, 2-ME + TNFα; lane (17–20) Subject 3 Px: Cont, 2-ME, TNFα, 2-ME + TNFα; lane (21–24) Subject 3 Tu: Cont, 2-ME, TNFα, 2-ME + TNFα; lane (25–28) Subject 4 Rm: Cont, 2-ME, TNFα, 2-ME + TNFα.

water. The total RNA concentration was measured spectrophotometrically and 5 μg RNA used for cDNA synthesis using a 'Ready to Go T-primed First-Strand Kit' (Amersham Pharmacia, Aylesbury, Bucks, UK) utilising oligo dT primers and monkey murine leukaemia virus reverse transcriptase. After incubating samples for 1 h at 37°C, samples were heated to 95°C for 5 min after which the sample volume was adjusted to 100 μl with water.

For the PCR reactions 1 μl of cDNA was amplified, (45 s 95°C, 45 s 60°C, 60 s 72°C) using GAPDH primers (24 ×, fGAPDH 5′tgccgtctagaaaaacctgc3′, rGAPDH 5′accctgttgctgtagccaaa3′), STS specific primers (34 ×, fSTS 5′ttggagatcctggtgctat3′ rSTS 5′aagccgtgatgtaaagggtg3′). PCR products were visualised by ethidium bromide staining on agarose gels and image analysis carried out with the Kodak 1D imaging system. Results shown are typical results of one of three experiments.

2.4. Transient transfection assays

The 2.4 kb STS cDNA (obtained from Von Figura [20]) was cloned into the mammalian expression vector pALTER MAX (Promega, Southampton, Hants, UK) and expressed in MCF-7 and COS-1 cells. Transfection efficiencies were internally controlled by including a β-galactosidase expression vector in the transfections. Monolayers of cells were grown to 30% confluence in T-75 culture flasks and the plasmids transfected into cells by calcium phosphate precipitation for 16 h (5′-3′ kit Boulder). After transfection, the cells were washed twice with sterile PBS and experiment medium added. After 24 h the cells were trypsinised, half were transferred to a T-25 cell culture flask for subsequent STS activity measurements and cell counting (Section 2.5), while the remainder were used for β-galactosidase activity measurements. All treatments were done in duplicate and the results presented are representative results of one of three experiments.

2.5. Measurements of steroid sulphatase activity

COS-1 cells and MCF-7 breast cancer cells that had previously been transfected and treated with IL-6 and TNFα (R&D Systems, Abingdon, Oxon, UK) were typsinised and reseeded into T-25 culture flasks and cells allowed to attach over a 2 h period. STS activity was assayed in the intact cells using [6,7-^{3}H(n)] E1S (90 Ci/mmol, 4×10^{5} dpm, 2 nM, NEN-Du Pont, Boston, MA) in serum free medium. After incubating cells with labelled E1S for 2 h medium was removed and STS activity quantitated. Cell numbers were determined using a Coulter counter.

3. Results

3.1. Steroid sulphatase expression in breast tumour tissue-derived fibroblasts

Having developed a RT-PCR method to examine STS mRNA expression it was used to investigate the expression of this enzyme in fibroblasts derived from samples of normal and malignant breast tissue (Fig. 1). Three matched pairs of samples of breast tumour and tissue proximal to the tumour were used to culture fibroblasts and to subsequently determine their relative levels of STS mRNA expression. For subjects 1 and 3, who were pre-menopausal, no significant difference in basal STS mRNA expression was detected in fibroblasts derived from tumour tissue and tissue proximal to the tumour. In contrast for fibroblasts cultured from a post-menopausal woman (Subject 2) STS mRNA expression in tumour-derived fibroblasts (lanes 13) was approximately 2.4-fold higher than that in fibroblasts derived from tissue proximal to the tumour (lane 9). Basal expression of STS mRNA in fibroblasts derived from reduction mammoplasty tissue (lane 25) was at the same level as in fibroblasts derived from tumour and proximal tissues of pre-menopausal women.

We also examined whether 2-MeOEMATE, a potent active site-directed inhibitor of STS activity, but also able to induce cells to undergo apoptosis, influenced STS mRNA expression. However, no evidence of any regulation of STS mRNA expression was detected for any of the tissue samples examined. The ability of

TNFα alone, or after 3 h pre-treatment with 2-MeOEMATE, to modulate STS mRNA expression was also examined but no regulatory effects were detected when used alone or after pre-treatment with 2-MeOEMATE.

3.2. E1-STS expression in MCF-7 cells

As it was originally reported that IL-6 and TNFα could act synergistically to stimulate STS activity in MCF-7 breast cancer cells, the effect of these cytokines on STS mRNA expression was examined. Using the RT-PCR method developed it was possible to readily detect STS mRNA expression in MCF-7 breast cancer cells (Fig. 2). Quantification of expression as assessed by composing the ratio of the STS: GAPDH bands by Kodak 1D gel analysis imaging system, however, did not reveal any significant changes at either 6 h or 24 h.

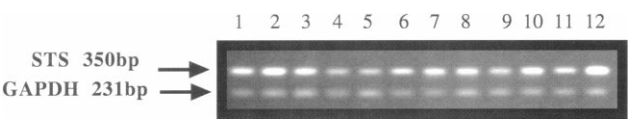

Fig. 2. STS mRNA expression in MCF-7 breast cancer cells. Cells were treated for 6 h (lanes 1–6) or 24 h (lanes 7–12) with vehicle (lanes 1 and 7); TNFα, 20 ng/ml (lanes 2 and 8); IL-6, 50 ng/ml (lanes 3 and 9); 2-MeOEMATE, 10 μM (lanes 4 and 10); TNFα, 20 ng/ml plus IL-6, 50 ng/ml (lanes 5 and 11); TNFα, 20 ng/ml plus 2-MeOEMATE, 10 μM (lanes 6 and 12).

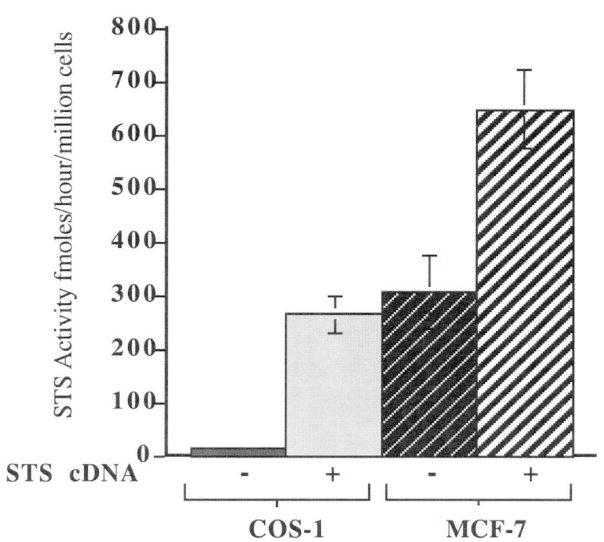

Fig. 3. Comparison of STS activities in mock transfected (−) and STS transfected (+) COS-1 and MCF-7 breast cancer cells. Cells were transfected with 2.5 μg of pALTER MAX containing the 2.4 kb STS cDNA per T-75 tissue culture flask by 16 h calcium phosphate precipitation.

3.3. Transient transfection of COS-1 and MCF-7 cells with STS cDNA

A transient transfection system was used to further investigate the regulation of STS activity by IL-6 and TNFα. The cDNA for STS was cloned into the mammalian expression vector pALTER MAX and transfected into COS-1 cells and MCF-7 cells by calcium phosphate precipitation. A β-galactosidase expression construct was also transfected into cells to determine the transfection efficiency. To allow for the basal STS activity of the cells, mock transfections were carried out and the unstimulated and stimulated E1-STS activities subtracted from the relevant STS activities after transfection.

Transient transfection of both COS-1 cells and MCF-7 cells increased STS activity by 17-fold and 2-fold, respectively (Fig. 3). In mock transfected COS-1 cells IL-6 plus TNFα had no effect on STS activity but increased STS activity in MCF-7 cells by 85% (Fig. 4a). In COS-1 cells transfected with the STS construct IL-6 plus TNFα again failed to increase STS activity. In contrast in MCF-7 cells transfected with the STS construct STS activity showed a marked (357%) increase (Fig. 4b).

4. Discussion

The formation of E1 from E1S by the action of STS is a major pathway for in situ oestrogen production in breast tumours. In the present investigation a semi-quantitative RT-PCR method has been used to start to explore the regulation of STS mRNA expression. As previously reported by Evans et al. [21], using an RT-PCR technique it was possible to detect STS mRNA expression in breast tissue-derived fibroblasts and MCF-7 breast cancer cells.

Using cultured fibroblasts derived from breast tumour and tissue proximal to the tumour, STS mRNA expression was initially compared under basal conditions. In three match pairs of fibroblasts derived from tumour and proximal tissue, STS mRNA expression was similar in both types of fibroblasts from pre-menopausal woman. In contrast, STS mRNA expression in fibroblasts derived from a tumour of a 88 year old women was approximately 2.4-times higher than in fibroblasts derived from proximal tissue. It has previously been reported that STS activity in breast tissues is increased in breast tumour compared with proximal breast tissue [4] and higher in tumour tissue from post-menopausal women [8]. STS mRNA expression in fibroblasts derived from reduction mammoplasty tissue was similar to that in tissues from other pre-menopausal women.

In a recent report STS mRNA expression was found to be significantly higher in malignant (mean 1458

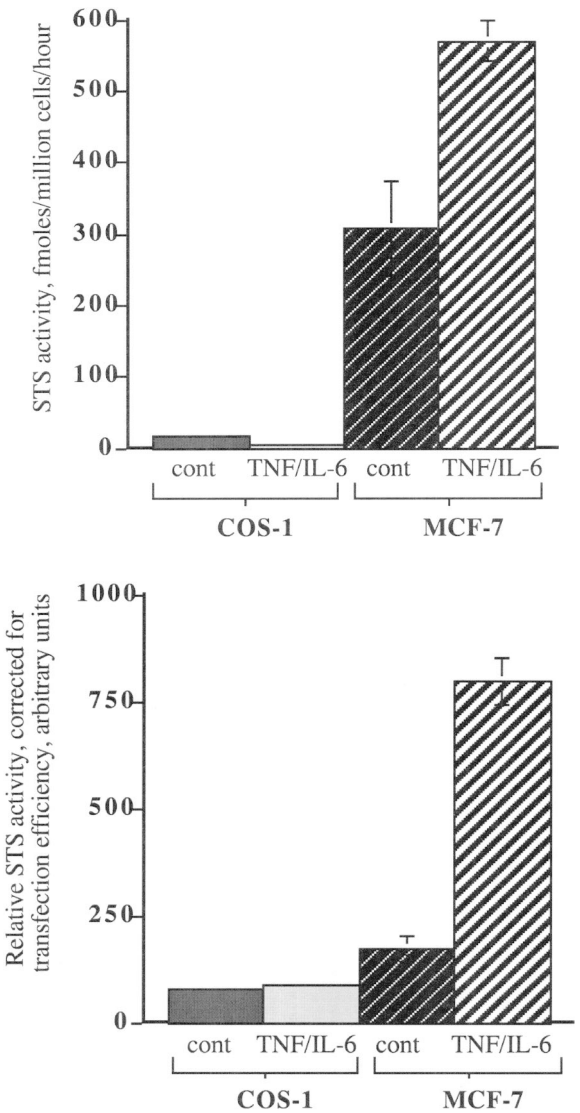

Fig. 4. (a) Effect of TNFα (20 ng/ml) plus IL-6 (50 ng/ml) on STS activity in mock transfected COS-1 and MCF-7 cells. (b) Effect of TNFα (20 ng/ml) plus IL-6 (50 ng/ml) on STS activity after transfection with a STS cDNA. The wild-type STS activities have been subtracted from the STS activities after transfection before correcting for transfection efficiency.

amole/mg/RNA) than non-malignant breast tissue (mean 536 amole/mg/RNA) [7]. Furthermore, breast cancer patients with higher levels of STS mRNA have a significantly shorter disease free survival as compared to those with low levels of STS mRNA [6]. This important finding was interpreted to suggest that in breast tumours expressing high levels of STS mRNA, tumour cells that escape surgical excision may grow faster causing patients to relapse after a shorter interval.

If STS mRNA expression is increased in malignant breast tissues it will be important to determine what factors regulate expression. In a preliminary attempt to address this question we examined whether the cytokine TNFα, which can enhance STS activity, affected STS mRNA expression in the fibroblasts derived from normal and malignant breast tissues. As yet however, no evidence was obtained for an effect of TNFα on STS mRNA expression. The apoptosis inducing agent, 2-MeOEMATE, which can reduce the ability of TNFα to stimulate aromatase activity [19], also had no effect on STS mRNA expression.

As it has previously been shown that TNFα and IL-6 can stimulate STS activity in MCF-7 cells and act synergistically to enhance activity we tested whether such effects might result from an increase in STS mRNA expression. However, exposing cells to these cytokines for 6 h or 24 h did not produce any convincing evidence for any change in STS mRNA expression. This finding suggests that the ability of TNFα and IL-6 to increase STS activity in MCF-7 cells may result from a post-transcriptional effect. Evans et al. [21], previously reported increased STS mRNA expression and activity in breast tissues and breast cancer cells. However, no correlation was found between STS mRNA levels and STS activity leading these authors to postulate that regulation might occur at a post-translational level.

Evidence to support the possible post-translational regulation of STS activity was obtained from our transient transfection studies. Transfection of the STS cDNA increased STS activity in both COS-1 and MCF-7 breast cancer cells above basal levels. A further increase in STS activity in response to TNFα/IL-6 was only seen in MCF-7 cells, where TNFα/IL-6 stimulated both endogenous and transfected STS activities. This finding suggest that IL-6 and TNFα are able to increase STS activity in the absence of any 5′ promoter sequences and supports our observation that neither IL-6 nor TNFα regulates STS mRNA expression. For STS and other sulphatases to have full activity an α-formylglycine residue is required to be formed by oxidation of a cysteine residue within the active site [16]. In fibroblasts where aryl sulphatase A (ASA) was over expressed up to 30% of ASA enzyme escaped oxidation and therefore had low catalytic activity [22]. The enzyme(s) carrying out this modification are not yet known. It is therefore likely that any increase in efficiency of this oxidation of newly synthesized or existing unmodified STS protein could result in an increase in STS activity. If TNFα or IL-6 act to increase this oxidation efficiency then it is possible that STS activity could be increased independently of mRNA levels. Alternatively the cytokines may act to increase the availability of E1S substrate to the enzyme possibly via an effect on membrane permeability. Whatever the mechanism it may be cell-type specific, as in COS-1 cells TNFα/IL-6 were not only unable to increase basal STS activity, they were also unable to up-regulate the activity in STS transfected cells.

In addition to its apoptosis inducing properties, 2-MeOEMATE is also a potent STS inhibitor. As previously discussed this drug had no effect on STS mRNA expression. Pasqualini et al. [14], have previously

demonstrated that the synthetic progestagen, Promegestrone (R5020) not only inhibits STS activity but also reduces STS mRNA expression. In other studies with a synthetic progestagen, Tibolone (Org OD14) and some of its metabolites, no evidence was found for any down-regulation of STS mRNA by these compounds [23].

These studies have revealed that while STS mRNA expression is readily detectable in cultured breast tissue fibroblasts and breast cancer cells, the mechanism by which it is regulated remains unresolved. In view of the high levels of STS mRNA in breast tumours and their increased STS activity it will be important to elucidate the mechanisms which control the expression and activity of this enzyme.

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References

[1] J.R. Pasqualini, C. Gelly, B.-L. Nguyen, C. Vella, Importance of estrogen sulfates in breast cancer, J. Steroid Biochem. 34 (1989) 155–163.

[2] M.J. Reed, A. Purohit, L.W.L. Woo, B.V.L. Potter, The development of steroid sulphatase inhibitors, Endocr.-Rel. Cancer 3 (1996) 9–23.

[3] S.J. Santer, P.D. Feil, R.J. Santen, In situ estrogen production via the estrone sulfatase pathway in breast tumours: relative importance versus the aromatase pathway, J. Clin. Endocrinol. Metab. 59 (1984) 29–33.

[4] G.S. Chetrite, J. Cortes-Prieto, J.C. Philippe, J.R. Wright, J.R. Pasqualini, Comparison of estrogen concentrations, estrone sulfatase and aromatase activities in normal, and in cancerous, human breast tissues, J. Steroid Biochem. Molec. Biol. 72 (2000) 23–27.

[5] J.R. Pasqualini, J. Cortes-Prieto, G. Chetrite, M. Talbi, A. Ruiz, Concentrations of estrone, estradiol and their sulfates and evaluation of sulfatase and aromatase activities in patients with breast fibroadenoma, Int. J. Cancer 70 (1997) 639–643.

[6] T. Utsumi, N. Yoshimura, S. Takeuchi, J. Ando, M. Maruta, K. Maeda, N. Harada, Steroid sulfatase expression is an independent predictor of recurrence in human breast cancer, Cancer Res. 59 (1999) 377–381.

[7] T. Utsumi, N. Yoshimura, S. Takeuchi, M. Maruta, K. Maeda, N. Harada, Elevated steroid sulfatase expression in breast cancer, J. Steroid Biochem. Molec. Biol., 73(2000) 141–145.

[8] J.R. Pasqualini, G. Chetrite, C. Blacker, M.-C. Feinstein, L. Delalonde, M. Talbi, C. Maloche, 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 (1996) 1460–1464.

[9] P.K. Siiteri, P.C. MacDonald, Role of extraglandular estrogen in human endocrinology, in: R.O. Greep, E.B. Astwood (Eds.), Handbook of Physiology, Washington, DC, vol. 2, part 1, Am. Physiol. Soc. (1973) 615–629.

[10] M.J. Reed, N.G. Coldham, S.R. Patel, M.W. Ghilchik, V.H.T. James, Interleukin 1 and interleukin 6 in breast cyst fluid: their role in regulating aromatase activity in breast cancer cells, J. Endocrinol. 135 (1992) R5–R8.

[11] F. Macdiarmid, D. Wang, L.J. Duncan, A. Purohit, M.W. Ghilchik, M.J. Reed, Stimulation of aromatase activity in breast fibroblasts by tumour necrosis factor α, Molec. Cell. Endocrinol. 106 (1994) 17–21.

[12] M.J. Reed, A. Purohit, Breast cancer and the role of cytokines in regulating estrogen synthesis: an emerging hypothesis, Endocr. Rev. 18 (1997) 701–715.

[13] A. Purohit, L.J. Duncan, D.Y. Wang, N.G. Coldham, M.W. Ghilchik, M.J. Reed, Paracrine control of oestrogen production in breast cancer, Endocr.-Rel. Cancer 4 (1997) 323–330.

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

[15] X.-M. Li, E.S. Alperin, E. Salido, Y. Gong, P. Yen, Shapiro, Characterisation of the promoter region of human steroid sulfatase: a gene which escapes x inactivation, Somat. Cell Molec. Genet. 18 (1996) 259–268.

[16] T. Dieks, M.R. Lecca, P. Schlotterhose, B. Schmidt, K. Von Figura, Sequence determinants directing conversion of cysteine to formylglycine in eukaryotic sulfatases, EMBO J. 18 (1999) 2084–2091.

[17] A. Purohit, K.A. Vernon, A.E. Wagenaar-Hummelinck, L.W.L. Woo, H.A.M. Hejaz, B.V.L. Potter, M.J. Reed, The development of A-ring modified analogues of oestrone-3-*O*-sulphamate as steroid sulphatase inhibitors with reduced oestrogenicity, J. Steroid Biochem. Molec. Biol. 64 (1998) 269–275.

[18] A. Purohit, H.A.M. Hejaz, L. Walden, L MacCarthy-Morrogh, G. Packham, B.V.L. Potter, M.J. Reed, The effect of 2-methoxyoestrone-3-*O*-sulphamate on the growth of breast cancer cells and induced mammary tumours, Int. J. Cancer 85 (2000) 584–589.

[19] A. Purohit, A. Singh, M.W. Ghilchik, M.J. Reed, Inhibition of tumour necrosis factor α-stimulated aromatase activity by microtubule – stabilising agents, paclitaxel and 2-methoxy estradiol, Biochem. Biophys. Res. Comm. 261 (1999) 214–217.

[20] C. Stein, A. Hille, J. Seidel, S. Rijnboutt, A. Waheed, B. Schmidt, H. Geuze, K. Vou Figura, Cloning and expression of human steroid sulfatase, J. Biol. Chem. 264 (1989) 13865–13872.

[21] T.R. Evans, M.G. Rowlands, Y.A. Luqmani, S.K. Chander, R.C. Coombes, Detection of breast cancer-associated estrone sulfatase in breast cancer biopsies and cell lines using polymerase chain reaction, J. Steroid Biochem. Molec. Biol. 46 (1993) 195–201.

[22] B. Schmidt, T. Selmer, A. Ingendoh, K. Von Figura, A novel amino acid modification in sulfatases that in defective in multiple sulphatase deficiency, Cell 82 (1995) 271–278.

[23] A. Purohit, B. Malini, S.P. Newman, Inhibition of oestrone sulphatase activity by Tibolone (Org OD14) and its metabolites, J. Steroid Biochem. Molec. Biol., submitted.