Regulation of steroid sulphotransferase and oestradiol 17β-hydroxysteroid dehydrogenase in breast cancer

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Introduction
Breast cancer remains a major cause of death for women in most Western countries, and there is an urgent need to develop new therapeutic strategies to inhibit the growth of breast tumours. The female sex hormones, the oestrogens, have a crucial role in promoting the development and growth of breast tumours, yet the highest incidence of breast cancer occurs in post-menopausal women at a time when ovarian oestrogen production has ceased. Oestrogens, however, continue to be produced at a lower rate in post-menopausal women. Androstenedione, which is secreted mainly by the adrenal cortex, is converted into oestrone by the aromatase enzyme complex (Scheme 1). In post-menopausal

Scheme 1
Origins of oestrogenic steroids in breast tumours

Abbreviations: Adione, androstenedione; E1, oestrone; E1-S, E1 sulphate; E2, oestradiol; DHA, dehydroepiandrosterone; DHA-S, DHA sulphate; Adiol, 5-androstenediol; Adiol-S, Adiol sulphate; ER, oestrogen receptor.

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women, oestrogens are produced almost exclusively by the peripheral conversion of androstenedione into oestrone [1]. Adipose tissue is a major site for the peripheral aromatization of androstenedione to oestrone, and the extent of conversion correlates with body weight [2]. This is probably the reason that obese post-menopausal women have an increased risk of developing breast cancer.

The aromatase complex is also present in normal and malignant breast tissues [3], and makes an important contribution to the high oestrogen levels found in breast tumours [4]. There is now convincing evidence that aromatase activity is higher in adipose tissue in the breast quadrant that contains a tumour [5-7]. These findings suggest either that breast tumours are producing factors that can stimulate aromatase activity in tissue proximal to the tumour or, alternatively, that tumours develop in the breast quadrant in which aromatase activity is highest.

**Steroid sulphatase**

Much of the oestrone that is formed from androstenedione is converted into the steroid conjugate oestrone sulphate (E1S) by the action of oestrone sulphotransferase (Scheme 1). Oestrogen sulphates, as such, are unable to bind to the oestrogen receptor and stimulate tumour growth. There is evidence that oestrone sulphotransferase activity is reduced in transformed cells [8]. Failure to inactivate oestrogens by sulphate formation may therefore be a mechanism by which transformed cells are exposed to increased oestrogenic stimulation.

Plasma and tissue concentrations of E1S are considerably higher than those of unconjugated oestrogens; furthermore, the half-life of E1S is much longer than that of oestrone [9-11]. It has therefore been proposed that oestrogen sulphates may act as a reservoir for the formation of unconjugated oestrogen via the action of oestrone sulphatase (Scheme 1). The activity of this enzyme is considerably higher than that of aromatase in normal and malignant breast tissues [3]. Using tumour homogenates and appropriate substrate concentrations, as much as ten times more oestrone was found to be formed from E1S, via the sulphatase pathway, as from androstenedione, via the aromatase route [12]. In ovariectomized rats the growth of carcinogen-induced mammary tumours can be markedly increased by administration of E1S [13].

While E1S therefore represents an important source of oestrogen that can be activated by oestrone sulphatase, another steroid sulphate, dehydroepiandrosterone sulphate (DHA-S), is also an important substrate for steroid sulphatase. DHA-S is produced in large amounts by the adrenal cortex and is hydrolysed by steroid sulphatase to form DHA. The importance of this sulphatase pathway (as shown in Scheme 1) is that DHA can be converted into 5-androstenediol, which, although an androgen, can bind to the oestrogen receptor and support tumour growth. Whether the sulphatase(s) responsible for the hydrolysis of E1S and DHA-S are the same or different remains controversial. However, transient transfections of COS-1 cells with a placental steroid sulphatase cDNA revealed that the expressed enzyme could hydrolyse both aryl and alkyl steroid sulphates [14]. This finding suggests that only one steroid sulphatase is responsible for the hydrolysis of both E1S and DHA-S.

5-Androstenediol can stimulate the growth of ZR-75-1 breast cancer cells in vitro and of dimethylbenz[a]anthracene-induced mammary tumours in the ovariectomized rat [15,16]. Furthermore, it is able to stimulate the growth of such tumours in vivo in the presence of an aromatase inhibitor, demonstrating that conversion of 5-androstenediol into an oestrogen is not necessary in order for this steroid to stimulate tumour growth.

The finding of increased aromatase activity in tumour-bearing breast quadrants prompted a study to examine if a similar relationship also applies to tumour location and steroid sulphatase activity [17]. Of 11 mastectomy specimens examined, 36% of the tumour-bearing quadrants had the highest sulphatase activity. While lending some support to the concept that breast tumours produce factors that are able to influence the activity of oestrone sulphatase in adjacent tissue, these results suggest that this occurs to a lesser extent than previously reported for the aromatase.

In a search to identify tumour-derived factors that stimulate steroid sulphatase activity, a large number of cytokines and growth factors have been examined for their ability to modulate the activity for this enzyme. Acidic and basic fibroblast growth factors, epidermal growth factor, transforming growth factor α and insulin-like growth factor I did not have a significant effect on sulphatase activity in MCF-7 cells [18].
Human serum albumin, which was originally found to stimulate oestradiol dehydrogenase activity [19], has a marked stimulatory effect on sulphatase activity. Interestingly, basic fibroblast growth factor was able to almost completely inhibit the ability of human serum albumin to stimulate sulphatase activity, although the reason for this remains unknown. In contrast with the lack of a stimulatory effect on sulphatase activity exhibited by most growth factors examined so far, the cytokines interleukin-6 (IL-6) and tumour necrosis factor α (TNFα) both stimulated sulphatase activity significantly in MCF-7 cells. Furthermore, these cytokines acted synergistically to enhance oestrone sulphatase activity. Breast cyst fluid (BCF), which is known to contain a number of steroids, growth factors and cytokines, was also found to influence steroid sulphatase activity in MCF-7 cells [20]. BCF tended to inhibit oestrone sulphatase activity in oestrogen-receptor-positive breast cancer cells, while stimulating activity in oestrogen-receptor-negative cells. In both cases, the extent of inhibition or stimulation was related to the electrolyte concentration of the BCF.

**Oestradiol 17β-hydroxysteroid dehydrogenase (E2DH)**

E2DH (Type I) is responsible for the reduction of steroids such as oestrone and DHA to their biologically active forms, i.e. oestradiol and 5-androstenediol respectively, which are able to interact with the oestrogen receptor. As discussed previously in relation to breast tissue aromatase activity, similar studies that examined E2DH activity in normal and malignant breast tissues also indicated that tumour-derived factors could influence activity [21]. Fractionation of breast tumour cytosol revealed the presence of a protein (66 kDa) that could stimulate E2DH activity [19]. This protein was identified as albumin, and subsequently some types of human serum albumin were found to be able to stimulate E2DH activity [22]. Several other albumin-like proteins have now been shown to possess the ability to regulate steroidogenesis in ovarian and testicular tissues.

The cytokine IL-6 was identified in conditioned medium collected from tumour-derived fibroblasts and shown to stimulate E2DH activity in MCF-7 breast cancer cells [23]. As previously noted for steroid sulphatase, the combination of IL-6 plus TNFα was also found to act synergistically to regulate E2DH activity [24].

While a number of cytokines have therefore been identified as having important roles in regulating steroid sulphatase and E2DH activities, there is evidence that prostaglandin E2 (PGE2) may be an important regulator of aromatase activity in breast tumours [25]. Using MCF-7 breast cancer cells, we therefore examined the ability of PGE2 to regulate E2DH activity. The ability of oestradiol to modulate E2DH activity was also investigated in this study. As previously reported, it was confirmed that oestradiol (1 nM) did have a modest stimulatory effect (26%) on E2DH activity (Figure 1). PGE2 (10 μM) significantly increased E2DH activity by 76% compared with untreated control cells, suggesting that, in addition to regulating aromatase, this prostaglandin may also have a role in controlling E2DH activity (Figure 1).

**Origin of steroid sulphatase and E2DH stimulatory factors**

Cytokines such as IL-6 and TNFα can be produced by stromal fibroblasts and adipocytes, but there is evidence that cells of the immune system may be the major source of cytokines that are available to stimulate tumour oestrogen synthesis [26]. Up to 50% of the volume of a breast tumour can be composed of tumour-associated macrophages and tumour-infiltrating lymphocytes. Tumour cells also produce chemokines, such as IL-8 and macrophage chemoattractant protein-1, which attract immune cells to invade tumours. In a number of studies it has also been
demonstrated that macrophages and lymphocytes produce factors that can stimulate steroid sulphatase and E2DH activities in MCF-7 breast cancer cells.

Evidence has been obtained that steroid sulphatase in macrophages within the lymphoid environment may determine whether T-helper (Th) cells develop either a Th-1 or a Th-2 phenotype, with each type producing a characteristic cytokine profile [27,28]. Whether Th cells progress to a Th-1 or Th-2 type is thought to depend on the DHA/glucocorticoid ratio. Interestingly, some years ago, Bulbrook and Hayward [29] developed the discriminant function test, based upon the ratio of urinary DHA to glucocorticoid metabolites, to predict women at risk of developing breast cancer. It has been postulated that the discriminant function test is in fact an indirect marker of Th-UTh-2 function, as some Th-2 cytokines have been shown to stimulate tumour oestrogen synthesis [30].

Development of steroid sulphatase and E2DH inhibitors

In recent years there has been considerable progress in developing a number of potent aromatase inhibitors, some of which are now undergoing clinical evaluation. Attention has now turned to the development of steroidal and non-steroidal sulphotase inhibitors incorporating the sulphamate pharmacophore have now been identified and are currently undergoing pre-clinical evaluation [31,32].

Similar potent E2DH inhibitors, such as a 6β-thiaheptamide derivative of oestradiol, have now been identified [33]. With the development of potent steroid sulphatase and E2DH inhibitors, it should soon be feasible to test the efficacy of such inhibitors for the therapeutic treatment of women with breast cancer.

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