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Review of: Proliferation of estrogen receptor-alpha-positive mammary epithelial cells is restrained by transforming growth factor-beta1 in adult mice

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Abstract of the original article:
Transforming growth factor (TGF)-beta1 is a potent inhibitor of mammary epithelial proliferation. In human breast, estrogen receptor (ER)-alpha cells rarely co-localize with markers of proliferation, but their increased frequency correlates with breast cancer risk. To determine whether TGF-beta1 is necessary for the quiescence of ER-alpha-positive populations, we examined mouse mammary epithelial glands at estrus. Approximately, 35% of epithelial cells showed TGF-beta1 activation, which co-localized with nuclear receptor-phosphorylated Smad 2/3, indicating that TGF-beta signaling is autocrine. Nuclear Smad co-localized with nuclear ER-alpha. To test whether TGF-beta inhibits proliferation, we examined genetically engineered mice with different levels of TGF-beta1. ER-alpha co-localization with markers of proliferation (i.e., Ki-67 or bromodeoxyuridine) at estrus was significantly increased in the mammary glands of TGF-beta1 C57/bl/129SV heterozygote mice. This relationship was maintained after pregnancy but was absent at puberty. Conversely, mammary epithelial expression of constitutively active TGF-beta1 via the MMTV promoter suppressed proliferation of ER-alpha-positive cells. Thus, TGF-beta1 activation functionally restrains ER-alpha-positive cells from proliferating in adult mammary gland. Accordingly, we propose that TGF-beta1 dysregulation may promote proliferation of ER-alpha-positive cells associated with breast cancer risk in humans.

Review

Transforming growth factor β (TGFβ) is a multi-functional cytokine that regulates cell proliferation, differentiation and extracellular matrix production. In the post-natal mammary gland, members of the TGFβ superfamily, their receptors, and signalling molecules are expressed and play critical roles in every phase of development (reviewed in [1–4]). The expression pattern of TGFβs in the mouse suggested that TGFβ could have roles in regulating branching morphogenesis, lactation, and involution. The growth-suppressive effects of TGFβ on the terminal end buds (TEB) were first demonstrated by implantation of slow-release pellets containing active TGFβ1 or TGFβ3 in the mammary fat pad in front of the elongating ductal tree [5,6]. This and additional data from TGFβ transgenic mice [7] suggest that TGFβ normally acts as an

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inhibitor of ductal elongation and branching. Oestrogen and progesterone, on the other hand, are critical for promoting mammary epithelial proliferation, although it is clear that mammary epithelial cells differ in their ability to respond to these signals. Furthermore, observations in the human breast epithelium that oestrogen receptor α (ERα)/progesterone receptor (PR) positive cells rarely co-localise with markers of proliferation [8,9] led several groups to propose that the steroid receptor positive cells act as sensors for adjacent proliferating cells but are themselves actively prevented from proliferating by a growth inhibitor. In this article the authors hypothesise that this growth inhibitor is TGFβ1 and that the difference in sensitivity to oestrogen or progesterone is due to the ability of TGFβ1 to restrain the proliferative response of epithelial cell populations in response to ovarian steroids.

Crosstalk between ER transcriptional activity and the TGFβ signalling pathway has already been described. ERs suppress TGFβ signalling by associating with, and acting as a transcriptional co-repressor for, Smad3 [10]. Conversely, activation of the TGFβ signalling pathway increases ER transcriptional activity. The physiological significance of TGFβ signalling-induced ER activity remains to be established. However, activation of ER by the TGFβ pathway can establish a feedback loop where oestrogen signalling would be accentuated by the TGFβ signalling itself, which in turn would be inhibited more quickly and effectively. Upon inhibition of TGFβ signalling, ER activity would return to normal levels again. In addition, oestrogen and progesterone together with TGFβ are necessary for the maintenance of p53 activity in mammary epithelium and thus the ability to sense and respond appropriately to DNA damage [11]. This crosstalk is also consistent with the observation that the action of tamoxifen is at least partially mediated through activation of TGFβ [12].

The authors examine the relationship between TGFβ1 positive and steroid hormone receptor positive epithelial subpopulations in the mammary glands of 10-week-old nulliparous and parous mice at various developmental stages. Since all cells secrete latent TGFβ and the extracellular matrix is a reservoir for this protein, the authors begin by demonstrating that active TGFβ1 co-localises with nuclear receptor activated (R) Smad indicating that TGFβ1 activation triggers TGFβ1 signalling in the same cells. They then go on to demonstrate that in nulliparous mice at oestrus, almost all ERα/PR positive cells maintain TGFβ1 activation suggesting that TGFβ1 may inhibit the cells ability to respond to ovarian hormone induced proliferation in an autocrine manner. Investigation of this possibility was conducted in TGFβ1 heterozygotes in which greater than 90% of TGFβ1 protein is depleted. TGFβ1 depletion increased proliferation overall, and the frequency of cells in which Ki-67 co-localised with ERα was increased 16-fold compared with wild type animals. Furthermore, although the origin of active TGFβ1 is unclear it was evident from these studies that the epithelial depletion of this molecule was sufficient for this effect. In line with this groups previous demonstration that endogenous TGFβ1 activation and thus activity are regulated by ovarian hormones [13], the effects of TGFβ1 depletion were also examined in hormone treated ovariectomised mice and in mice following pregnancy. In each case, an increase in the frequency of ERα positive mammary epithelial cells in cycle was observed. Conversely, the transgenic overexpression of active TGFβ1 resulted in the reduced co-localisation of ERα with markers of proliferation. Furthermore, in the pubertal mammary gland, TGFβ1 depletion did not increase the proliferation of ERα positive cells suggesting that the proliferation of ERα positive cells is differentially regulated during puberty compared with adults.

As discussed by the authors, this has important implications for understanding the biology of ERα positive cells in human breast cancers. The frequency of ERα positive cells increases with age in the human breast, which parallels increased breast cancer risk [9,14]. In addition, the proportion of ERα positive cells in cycle increases in pre-malignant disease and in invasive cancer [8,15]. Furthermore, although there is considerable evidence indicating that TGFβ functions as a tumour suppressor, there are also data pointing to a role for TGFβ in promoting the progression of cancer and metastasis (for reviews see [16,17]). In the normal epithelium, ERα positive cells have recently been proposed to comprise a putative mammary stem cell population [18]. It is possible therefore that quiescence of these putative stem cells is maintained by TGFβ1, and as suggested by the authors, decreased responsiveness to or decreased activation of TGFβ1 may be an early event that dysregulates ERα stem cells resulting in the expansion of an ERα proliferating cell population. However, it has also been suggested that cells in early-stage tumours can still respond to TGFβ1 with a growth-inhibitory response, suppressing further progression of the tumour [19,20]. It is not until later, as the tumour progresses and a different genetic or epigenetic environment exists that responsiveness to TGFβ1 is altered so that the tumour-promoting activities of TGFβ1 (increased cell motility, induction of epithelial to mesenchymal transition, extracellular matrix degradation, tumour angiogenesis and host immunosuppression) dominate. Additional information on the mechanisms by which TGFβ has potent inhibitory effects upon normal epithelial proliferation

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and how the breakdown of the autocrine and paracrine inhibitory loops in which TGFβ participates may be associated with malignant progression is clearly needed.

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