Modulation of transforming growth factor β1 gene expression in the mammary gland by insulin-like growth factor I and octreotide

HUNG HUYNH, W. BEAMER, M. POLLAK and T.W. MARK G. CHAN

Laboratory of Molecular Endocrinology, Division of Cellular and Molecular Research, National Cancer Centre of Singapore, Singapore 169610, Republic of Singapore

Abstract. Transforming growth factor β1 (TGF-β1) has been shown to exhibit anti-proliferative activity for mammary gland epithelial cells and for human breast cancer cells. Insulin-like growth factor I (IGF-I), in contrast, is a well-characterized mitogenic and anti-apoptotic factor involved in mammary gland physiology. In order to examine the hypothesis that IGF-I suppresses TGF-β1 expression in the mammary gland, we studied the effect of various manipulations of the growth hormone - IGF-I axis on TGF-β1 mRNA abundance. Administration of IGF-I to intact animal suppressed TGF-β1 mRNA levels in a dose-dependent manner to ~20% of control levels. Administration of the somatostatin analogue octreotide in a manner previously shown to acutely suppress the growth hormone - IGF-I axis increased mammary gland TGF-β1 expression ~3-fold. Transgenic mice overexpressing growth hormone expressed TGF-β1 in the mammary gland at only ~12% of the level of control animals, while mice IGF-I deficient due to the ‘lit’ mutation expressed TGF-β1 at slightly higher levels than control animals. The large differences in TGF-β1 expression between control and GH-transgenic animals were correlated with major differences in architecture of the mammary gland, while the appearance of mammary glands of normal and ‘lit’ animals was similar. These data document a previously unrecognized relationship between TGF-β1 and IGF-I physiology in the mammary gland, and suggest a novel mechanism by which somatostatin analogues influence the proliferative behaviour of breast epithelial cells.

Introduction

Several reports have suggested that transforming growth factor β1 (TGF-β1) has important negative regulatory roles in normal breast physiology and that this peptide can inhibit proliferation of breast cancer cells (1,2). Conversely, there are many reports demonstrating that insulin-like growth factor I (IGF-I) bioactivity has important stimulatory and anti-apoptotic actions on a variety of cell types, including both normal and transformed mammary epithelial cells (3-5). This evidence includes direct demonstration of mitogenic responsiveness of breast cancer cells to IGF-I in vitro (6,7) and growth-inhibitory effects of both host IGF-I deficiency (8) and blocking antibodies against the IGF-I receptor (9) on in vivo breast cancer proliferation.

Separate proposals have been made that up-regulation of TGF-β1 bioactivity (2) and reduction of IGF-I bioactivity (10-12) deserve consideration as therapeutic strategies for breast cancer prevention and/or treatment. Interestingly, data have been presented that implicate both up-regulation of TGF-β1 expression (13-15) and reduction of IGF-I bioactivity (via effects on IGF-I (16,17), IGF-I receptors (18), and IGF binding proteins (19,20)) as potential mediators of the anti-proliferative action of anti-estrogens.

Prior evidence for interactions between IGF physiology and TGF-β1 physiology include the demonstration that the anti-proliferative action of TGF-β1 in certain experimental systems involves the regulation of TGF-β1 expression. The up-regulation of TGF-β1 expression is suggested to occur in response to both IGF-I and other factors other than IGF-I (21). The demonstration of a role for the IGF-I receptor in the post-translational processing of TGF-β1 (22). We sought to evaluate the possibility that the growth stimulatory actions of IGF-I involve suppression of TGF-β1 expression.

Materials and methods

Animals. Animals were obtained from the Jackson Laboratory. Control animals were littermated heterozygotes that are indistinguishable from +/+, C57BL/6J animals homozygous for the ‘lit’ mutation (8,23,24). Transgenic C57BL/6J animals overexpressing the human growth hormone gene were used to model growth hormone - IGF-I deficiency, and transgenic C57BL/6J animals overexpressing the human growth hormone gene were used to model growth hormone - IGF-I excess (25). All experiments were performed on virgin animals approximately 8 weeks of age.

Photomicroscopy. Breast tissue from virgin mice 8 weeks of age was obtained following sacrifice, fixed in 10% formalin, and sections were stained with haematoxylin and eosin. Multiple slides from each of several animals were prepared, and photographed at x400 magnification.
Figure 1. Suppression of TGF-β1 expression in the mammary gland by systemic administration of IGF-I. IGF-I (or vehicle) was administered at indicated doses subcutaneously as described in Materials and methods. Twelve hours after the last injection, animals were sacrificed, and breast tissue was frozen for subsequent extraction of RNA and Northern blotting as described (16). Blots were hybridized with rat TGF-β1 (A) or (as loading control) 18S (B) cDNAs. Representative Northern blots are shown in (A) and (B). Independent RNA samples were obtained from 4 separate animals at each dose level, and subjected to Northern analysis and quantification by densitometric scanning. Aggregate results are summarized in (C) (mean ± SD).

Northern blotting. The TGF-β1 probe was obtained from ATCC. Messenger RNA was prepared from tissue and Northern blotting was carried out as previously described (16,26,27).

Acute responses to treatment with IGF-I or octreotide. The acute response to octreotide (kindly provided by Novartis Pharma) was determined by injecting the doses indicated on the figure legend subcutaneously at baseline, repeating this infection 12 h later, and then sacrificing animals for tissue collection and mRNA isolation 24 h after the initial infection. The acute response to IGF-I was determined by infecting recombinant human IGF-I (a kind gift from Celtrix Pharmaceuticals) at the doses indicated subcutaneously at baseline, repeating this injection 12 h later, and then sacrificing animals for tissue collection and mRNA isolation 24 h after the initial injection.

Results

As shown in the short-term experiments summarized in Fig. 1, IGF-I administration acutely down-regulated TGF-β1
expression in a dose-dependent fashion. As an initial approach to studying the impact of chronic changes in IGF-I level on mammary gland TGF-β1 expression, we compared TGF-β1 mRNA abundance in the mammary glands of transgenic mice overexpressing growth hormone (GH) (251, mice with IGF-I deficiency due to the ‘lti’ mutation (8), and control mice (Fig. 2). TGF-β1 mRNA abundance in mammary glands of transgenic mice overexpressing GH was less than 30% that of controls. On the other hand, suppression of the IGF-I - GH axis by the ‘lti’ mutation was associated with a modest but statistically significant increase in TGF-β1 mRNA abundance.

The photomicrographs shown in Fig. 3 demonstrate the effects of experimental manipulation of the GH - IGF-I axis on mammary gland morphology. Excess mitogenic stimulation is associated with hypertrophy of the epithelial ductal structures, and an abnormally high ratio of ductal to stromal tissue.

Fig. 4 demonstrates a significant acute stimulatory effect of octreotide on mammary gland TGF-β1 mRNA abundance.

Discussion

In this study, we have found that in vivo, IGF-I suppresses TGF-β1 gene expression in the mammary gland. Treatment of mice with somatostatin analogue octreotide results in up-regulation of TGF-β1 gene expression. Low levels of TGF-β1 expression in mice overexpressing growth hormone is associated with mammary epithelial cell hypertrophy.

Our animal models of IGF overexpression and under-expression each involved alterations of IGF-I expression secondary to changes in GH expression. The fact that we were able to replicate the changes in TGF-β1 levels also and suppresses GH secretion, provides evidence that it is IGF-I rather than GH that regulates TGF-β1 expression.

Somatostatin analogues such as octreotide have been shown to have anti-proliferative activity in a variety of in vivo breast cancer models (28,29). These compounds are known
to suppress GH secretion and IGF-I levels (30,31). In addition, they may have direct growth inhibitory actions related to binding to somatostatin receptors known to be present on experimental and human breast cancers (29,32-34). As prior work has shown that short-term administration of octreotide is associated with only modest (≈30%) suppression of IGF-I serum levels and IGF-I gene expression (35), we were anticipating only a modest effect of octreotide as an inducer of TGF-β1 expression in the breast, particularly in view of the small effect (Fig. 1) of the profound IGF-I deficiency associated with the "lit" mutation (8). The results therefore suggest that the stimulatory effect of octreotide on mammary gland TGF-β1 expression involves other mechanisms in addition to that related to reduction in IGF-I influence. It is tempting to speculate that octreotide may act directly by binding to somatostatin receptors present in normal breast tissue. Octreotide binds to at least three of the five cloned somatostatin receptors, which trigger various intracellular growth inhibitory signal transduction pathways (36-38). Although somatostatin receptors are frequently expressed in breast cancers, the localization of receptors among the various cell types (vascular, stromal, epithelial) comprising neoplastic breast tissue is the subject of ongoing investigations, and little is known regarding the abundance or distribution of somatostatin receptor subtypes among the cell types comprising normal breast tissue.

The data presented here document a previously unrecognized inhibitory effect of IGF-I on TGF-β1 expression. This interaction between the TGF-β1 and IGF networks of growth factors is significant in several contexts. First, as IGF-I is a potent mitogen and anti-apoptotic factor (4), it has been proposed that pharmacological strategies that target IGF-I bioactivity may be useful in breast cancer treatment and/or prevention (8,10-12). Our results suggest that such strategies will also up-regulate TGF-β1 expression, and thus may lead to a greater effect than that directly related to reduction in IGF-I bioactivity. Second, it has been previously shown that the administration of anti-estrogens is associated with reduced TGF-β1 gene expression (16,17) and increased expression of IGF binding proteins (19,20) (both of which would be expected to reduce IGF bioactivity) and on the other hand with increased TGF-β1 expression (13-15). Our results motivate further studies to determine if the up-regulation of TGF-β1 expression associated with anti-estrogen therapy in vivo is mediated in part by reduced IGF bioactivity. Third, our results demonstrate a novel action of the somatostatin analogue octreotide on mammary tissue. The potent octreotide-induced up-regulation of TGF-β1 expression may not be completely attributable to the IGF-I suppression achieved by this somatostatin analogue, and to fully characterize this effect it will be necessary to examine the cell-type specific expression of the five cloned somatostatin receptors in normal breast tissue. Finally, the results motivate studies to compare the effect of anti-estrogens and somatostatin analogue administration as single agents and in combination on TGF-β1 expression in normal and neoplastic breast tissue. This combination has been shown to have greater anti-neoplastic activity than either agent alone in the DMBa mammary tumor model (39), and is currently being studied in clinical trials (40). It has been proposed that the apparent benefit of co-administering these agents may be related in part to greater inhibition of IGF bioactivity as compared to single-agent therapy (35). Data presented here raised the possibility that the enhanced anti-neoplastic activity of the combination is in part attributable to enhanced up-regulation of TGF-β1 expression.

Acknowledgements

This work was supported by grants from the National Medical Research Council of Singapore to Dr Hung Huynh and Postgraduate scholarships to T.W. Mark G. Chan.
References


40. National Cancer Institute: Randomized trial comparing tamoxifen to the combination of tamoxifen and octreotide in the adjuvant treatment of stage I and II breast cancer. PDQ Database, 1996.