Modulation of transforming growth factor ß1 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 ß1 (TGF-ß1) 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-ß1 expression in the mammary gland, we studied the effect of various manipulations of the growth hormone - IGF-I axis on TGF-ß1 mRNA abundance. Administration of IGF-I to intact animal suppressed TGF-ß1 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-ß1 expression ~3-fold. Transgenic mice overexpressing growth hormone expressed TGF-ß1 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-ß1 at slightly higher levels than control animals. The large differences in TGF-ß1 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-ß1 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 ß1 (TGF-ß1) 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 responsivity 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-ß1 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-ß1 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-ß1 physiology include the demonstration that the anti-proliferative action of TGF-ß1 in certain experimental systems involves the reduction of responsivity to IGFs by the up-regulation of IGF binding protein expression (21), and the demonstration of a role for the IGF-II receptor in the post-translational processing of TGF-ß1 (22). We sought to evaluate the possibility that the growth stimulatory actions of IGF-I involve suppression of TGF-ß1 expression.

Materials and methods

Animals. Animals were obtained from the Jackson Laboratory. Control animals were *lit/+* heterozygotes that are indistinguishable from +/+ C57BL/6J animals homozygous for the *lit* mutation (8,23,24) 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.

Correspondence to: Dr Hung Huynh, Laboratory of Molecular Endocrinology, Division of Cellular and Molecular Research, National Cancer Centre of Singapore, 11 Hospital Drive, Singapore 169610, Republic of Singapore

Key words: TGF-ß1, mammary gland, IGF-I, octreotide

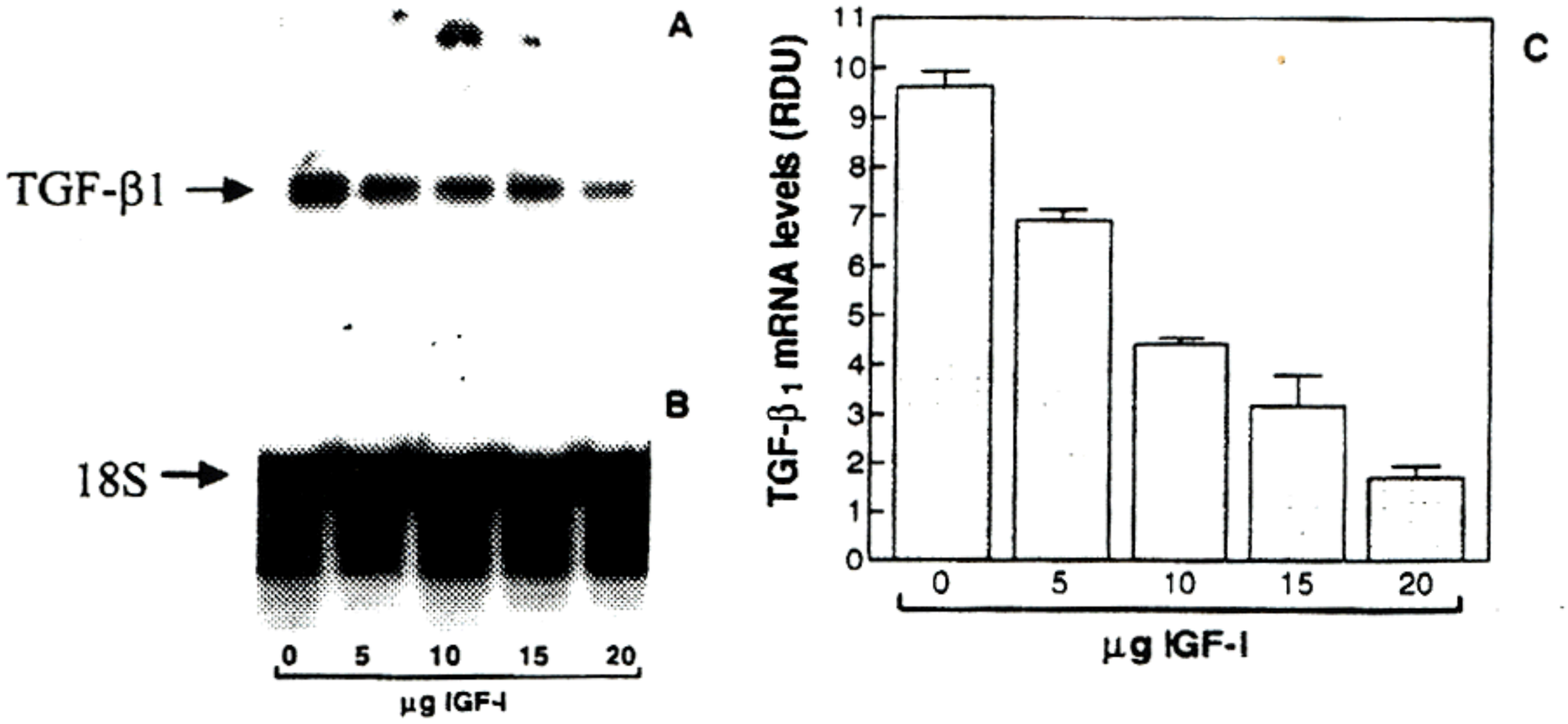

Figure 1. Suppression of TGF-ß1 expression in the mammary gland by systemic administration of IGF-I. IGF-I (or vehicle) was administered at indicated doses subcutaneously as described in Materials and methods. Twelve hours after the last injection, animals were sacrificed, and breast tissue was frozen for subsequent extraction of RNA and Northern blotting as described (16). Blots were hybridized with rat TGF-ß1 (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).

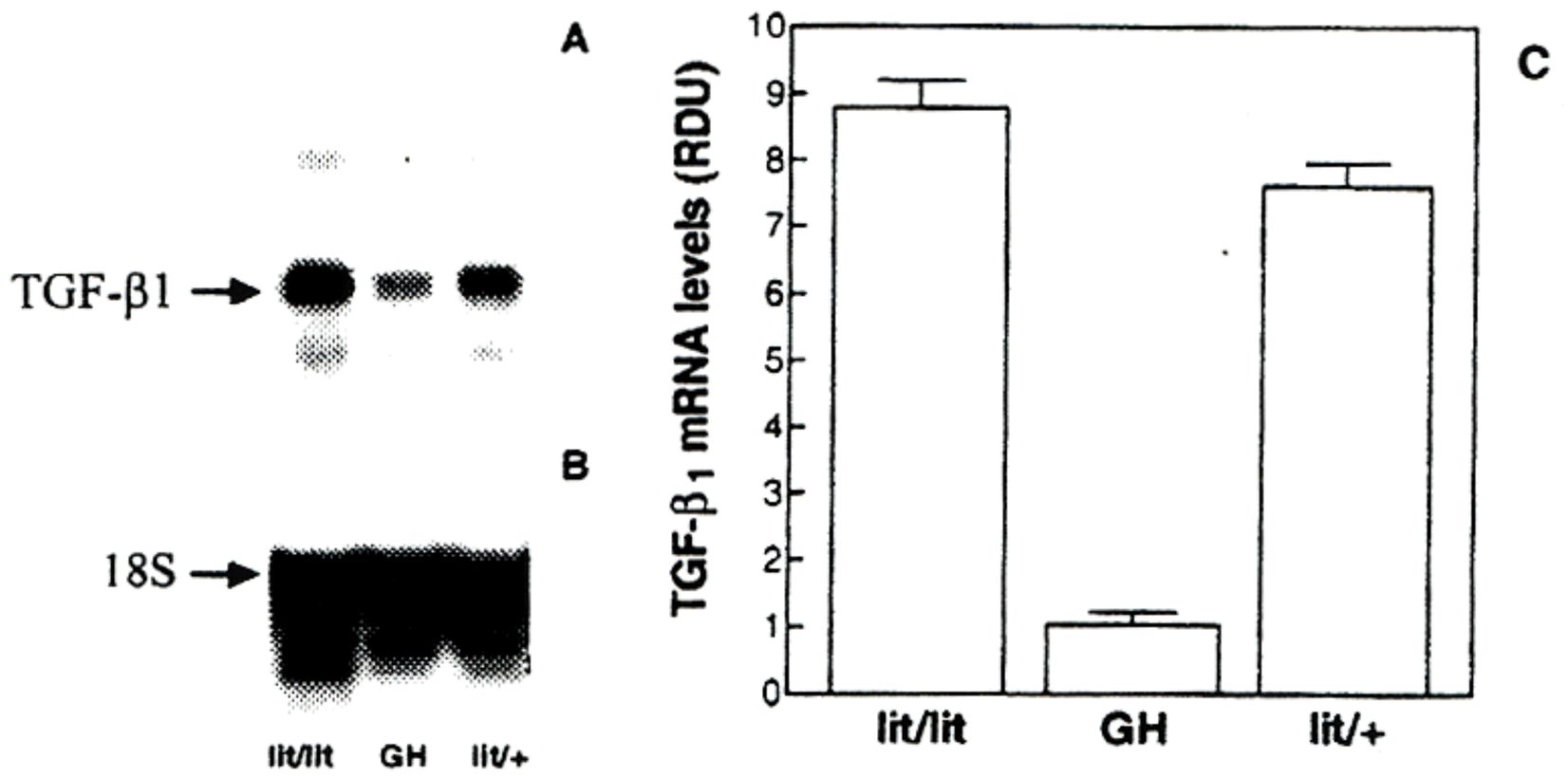

Figure 2. TGF-ß1 expression in mammary glands of *lit/lit*, GH-transgenic, and control (*lit/+*) C57BL/6J mice. Breast tissue from untreated mice was frozen for subsequent extraction of RNA and Northern blotting as described (16). Blots were hybridized with rat TGF-ß1 (A) or (as loading control) 18S (B) cDNAs. Representative Northern blots are shown in (A) and (B). Independent RNA samples were obtained from 4 separate animals of each group, and subjected to Northern analysis and quantification by densitometric scanning. Aggregate results are summarized in (C) (mean ± SD).

Northern blotting. The TGF-ß1 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-ß1

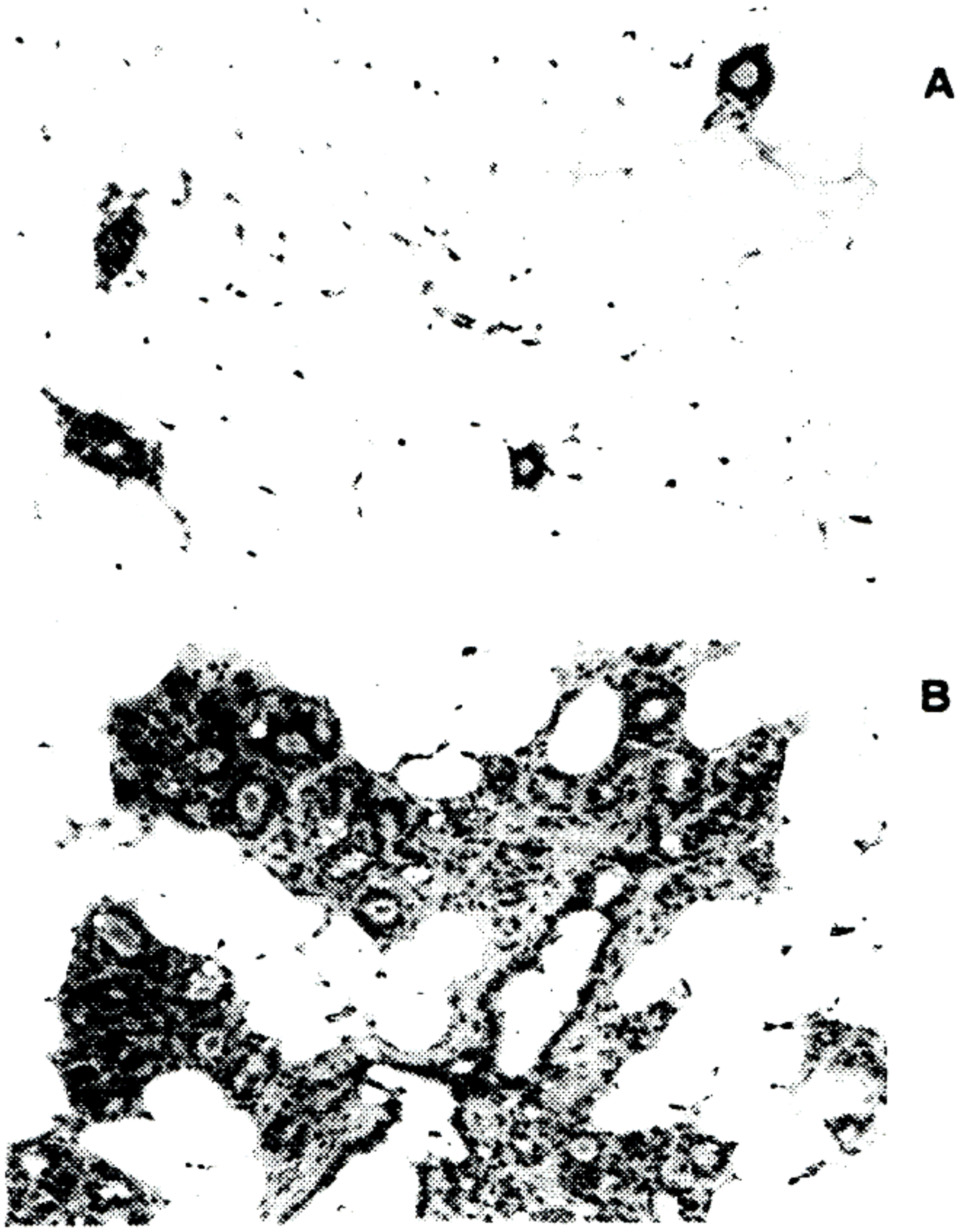

Figure 3. Histology of mammary glands of control, *lit/lit*, and GH-transgenic C57BL/6J mice. Histological sections were prepared as described in Materials and methods, and photographed at x400. Mammary tissues of control and *lit/lit* animals were indistinguishable and are shown in (A); (B), shows the ductal hyperplasia found in the GH-transgenic animals.

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-ß1 expression, we compared TGF-ß1 mRNA abundance in the mammary glands of transgenic mice overexpressing growth hormone (GH) (25), mice with IGF-I deficiency due to the '*lit*' mutation (8), and control mice (Fig. 2). TGF-ß1 mRNA abundance in mammary glands of transgenic mice overexpressing GH was less than 20% that of controls. On the other hand, suppression of the IGF-I - GH axis by the *lit* mutation was associated with a modest but statistically significant increase in TGF-ß1 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-ß1 mRNA abundance.

Discussion

In this study, we have found that *in vivo*, IGF-I suppresses TGF-ß1 gene expression in the mammary gland. Treatment of mice with somatostatin analogue octreotide results in up-regulation of TGF-ß1 gene expression. Low levels of TGF-ß1 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 in the acute experiments shown in Fig. 1 to replicate the TGF-ß1 suppressive effect of GH overexpression by the administration of IGF-I (a manipulation which raises IGF-I levels and suppresses GH secretion), provides evidence that it is IGF-I rather than GH that regulates TGF-ß1 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

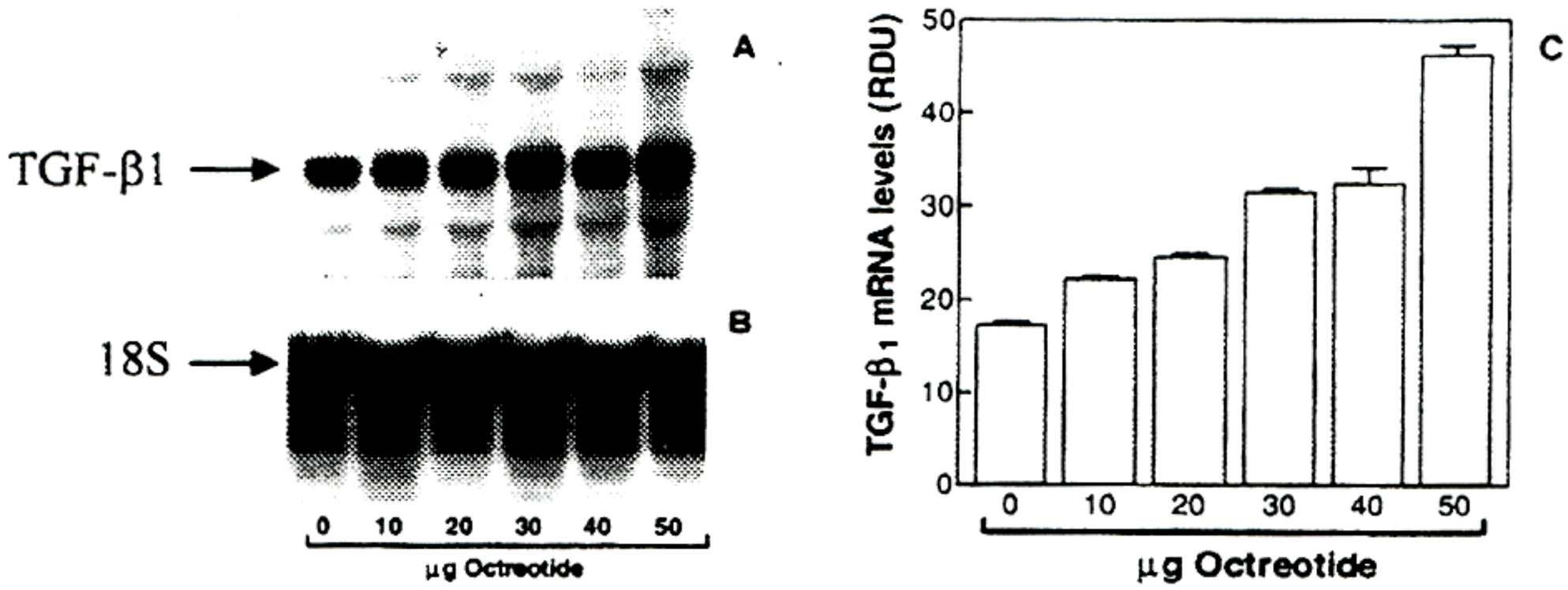

Figure 4. Effect of octreotide administration on mammary gland TGF-ß1 mRNA abundance. Octreotide (or vehicle) were administered at indicated doses subcutaneously as described in Materials and methods. Twelve hours after the last injection, animals were sacrificed, and breast tissue was frozen for subsequent extraction of RNA and Northern blotting as described (16). Blots were hybridized with rat TGF-B1 (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 of each group at each dose level, and subjected to Northern analysis and quantification by densitometric scanning. Aggregate results are summarized in (C) (mean ± SD).

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-ß1 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-ß1 expression involve 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 on-going 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-ß1 expression. This interaction between the TGF-ß1 and IGF networks of growth factors in 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-ß1 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 on the one hand with reduced IGF-I 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-ß1 expression (13-15). Our results motivate further studies to determine if the up-regulation of TGF-ß1 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-ß1 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 5 cloned somatostatin receptors in normal breast. Finally, the results motivate studies to compare the effect of anti-estrogens and somatostatin analogue administration as single agents and in combination on TGF-ß1 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-ß1 expression..

Acknowledgements

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

References

1. Arteaga CL, Tandon AK, von Hoff DD and Osborne CK: Transforming growth factor beta: potential autocrine growth inhibitor of estrogen receptor-negative human breast cancer cells. Cancer Res 48: 3898-3904, 1988.
2. Dickens TA and Colletta AA: The pharmacological manipulation of members of the transforming growth factor beta family in the chemoprevention of breast cancer (Review). Bioessays 15: 71-74, 1993.
3. Resnicoff M, Abraham D, Yutanawiboonchai W, Rotman HL, Kajstura J, Rubin R, Zoltick P and Baserga R: The insulin-like growth factor I receptor protects tumor cells from apoptosis *in vivo*. Cancer Res 55: 2463-2469, 1995.
4. Baserga R: The insulin-like growth factor I receptor: a key to tumor growth? Cancer Res 55: 249-252, 1995.
5. Rubin R and Baserga R: Insulin-like growth factor-I receptor. Its role in cell proliferation, apoptosis, and tumorigenicity (Review). Lab Invest 73: 311-331, 1995.
6. Pollak M, Polychronakos C, Yousefi S and Richard M: Characterization of insulin-like growth factor (IGF-I) receptors of human breast cancer cells. Biochem Biophys Res Commun 154: 326-331, 1988.
7. Arteaga CL and Osborne CK: Growth inhibition of human breast cancer cells *in vitro* with an antibody against the type I somatomedin receptor. Cancer Res 49: 6237-6241, 1989.
8. Yang XF, Beamer W, Huynh HT and Pollak M: Reduced growth of human breast cancer xenographs in host homozygous for the '*lit*' mutation. Cancer Res 56: 1509-1511, 1996.
9. Arteaga CL, Kitten LJ, Coronado EB, Jacobs S, Kull FC, Allred DC and Osborne CK: Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J Clin Invest 84: 1418-1423, 1989.
10. Arteaga CL: Interference of the IGF system as a strategy to inhibit breast cancer growth. Breast Cancer Res Treat 22: 101-106, 1992.
11. Pollak M, Ingle J, Suman V and Kugler J: Rationale for combined antiestrogen-somatostatin analogue therapy of breast cancer. In: Adjuvant Therapy of Cancer VIII: Proceedings of the 8th International Conference on the Adjuvant Therapy of Cancer. Salmon SE (ed). J.B. Lippicott, Philadelphia, 1996.
12. Pollak M: Enhancement of the antineoplastic effects of tamoxifen by somatostatin analogues. Digestion 57 (Suppl): 29-33, 1996.
13. Benson JR, Wakefield LM, Baum M and Colletta AA: Synthesis and secretion of transforming growth factor beta isoforms by primary cultures of human breast tumour fibroblasts *in vitro* and their modulation by tamoxifen. Br J Cancer 74: 352-358, 1996.
14. Perry RR, Kang Y and Greaves BR: Relationship between tamoxifen-induced transforming growth factor beta 1 expression, cytostasis and apoptosis in human breast cancer cells. Br J Cancer 72: 1441-1446, 1995.
15. Butta A, MacLennan K, Flanders KC, Sacks NPM, Smith I, McKinna A, Dowsett M, Wakefield LM, Sporn MB, Baum M and Coletta AA: Induction of transforming growth factor ß1 in human breast cancer *in vivo* following tamoxifen treatment. Cancer Res 52: 4261-4264, 1992.
16. Huynh HT, Tetenes E, Wallace L and Pollak M: *In vivo* inhibition of insulin-like growth factor-I gene expression by tamoxifen. Cancer Res 53: 1727-1730, 1993.
17. Pollak M, Constatino J, Polychronakos C, Blauer S, Guyda H, Redmond C, Fisher B and Margolese R: Effect of tamoxifen on serum insulin-like growth factor I levels in stage I breast cancer patients. J Natl Cancer Inst 82: 1693-1697, 1990.
18. Huynh HT, Nickerson T, Pollak M and Yang XF: Regulation of insulin-like growth factor I receptor gene expression by the pure anti-estrogen ICI 182,780. Clin Cancer Res 2: 2037-2042, 1996.
19. Huynh HT, Yang XF and Pollak M: Estradiol and antiestrogens regulate a growth inhibitory insulin-like growth factor binding protein 3 autocrine loop in human breast cancer cells. J Biol Chem 271: 1016-1021, 1996.
20. Huynh HT, Yang XF and Pollak M: A role for insulin-like growth factor binding protein 5 in the antiproliferative action of the anti-estrogen ICI 182780. Cell Growth Differ 7: 1501-1506, 1996.
21. Oh Y, Mullers HL, Ng L and Rosenfeld RG: Transforming growth factor-ß-induced cell growth inhibition in human breast cancer cells is mediated through insulin-like growth factor-binding protein-3 action. J Biol Chem 270: 13589-13592, 1995.
22. Dennis PA and Rifkin DB: Cellular activation of latent transforming growth factor beta requires binding to the cation-independent mannose 6-phosphate/insulin-growth factor type II receptor. Proc Natl Acad Sci USA 88: 580-584, 1991.
23. Godfrey P, Rahal JO, Beamer WG, Copeland NG, Jenkins NA and Mayo KE: GHRH receptor of little mice contains a missense mutation in the extracellular domain that disrupts receptor function. Nat Genet 4: 227-232, 1993.
24. Jansson JO, Downs TR, Beamer WG and Frohman LA: Receptor-associated resistance to growth hormone-releasing factor in dwarf 'little' mice. Science 232: 511-512, 1986.
25. Sweetser DA, Birkenmeier EH, Hoppe PC, McKeel DW and Gordon JI: Mechanisms underlying generation of gradients in gene expression within the intestine: and analysis using transgenic mice containing fatty acid binding protein-human growth hormone fusion genes. Genes Dev 2: 1318-1332, 1988.
26. Huynh HT and Pollak M: IGF-I gene expression in the uterus is stimulated by tamoxifen and inhibited by the pure anti-estrogen ICI 182780. Cancer Res 53: 5585-5588, 1993.
27. Huynh HT and Pollak M: Uterotrophic actions of estradiol and tamoxifen are associated with inhibition of uterine IGF binding protein 3 gene expression. Cancer Res 54: 3115-3119, 1994.
28. Schally AV: Oncological applications of somatostatin analogues. Cancer Res 48: 6877-6885, 1988.
29. Weckbecker G, Raulf F, Stolz B and Bruns C: Somatostatin analogs for diagnosis and treatment of cancer. Pharmacol Ther 60: 245-264, 1993.
30. Lamberts SWJ, Vanderlely AJ, Deherder WW and Hofland LJ: Drug therapy - octreotide. N Engl J Med 334: 246-254, 1996.
31. Pollak M, Polychronakos C and Guyda H: Somatostatin analogues SMS 201-995 reduces serum IGF-I levels in patients with neoplasms potentially dependent on IGF-I. Anticancer Res 9: 889-892, 1989.
32. Setyono-Han B, Henkelman MS, Foekens JA and Klijn GM: Direct inhibitory effects of somatostatin (analogues) on the growth of human breast cancer cells. Cancer Res 47: 1566-1570, 1987.
33. Van Eijck CH, Krenning EP, Bootsma A, Lindemans J, Jeekel J, Reubi JC and Lamberts SW: Somatostatin-receptor scintigraphy in primary breast cancer. Lancet 343: 640-643, 1994.
34. Reubi JC, Laissue J, Krenning E and Lamberts SW: Somatostatin receptors in human cancer: incidence, characteristics, functional correlates and clinical implications (Review). J Steroid Biohem Mol Biol 43: 27-35, 1992.
35. Huynh HT and Pollak M: Enhancement of tamoxifen-induced suppression of insulin-like growth factor I gene expression and serum level by a somatostatin analogue. Biochem Biophys Res Commun 203: 253-259, 1994.
36. Sharma K, Patel YC and Srikant C: Subtype specific induction of wild-type p53 and apoptosis, but not cell cycle arrest, by human somatostatin receptor 3. Mol Endocrinol 10: 1688-1696, 1996.
37. Patel YC, Greenwood MT, Panetta R, Demchyshyn L, Niznik H and Srikant CB: The somatostatin receptor family. Life Sci 57: 1249-1265, 1995.
38. Bruns C, Weckbecker G, Raulf F, Kaupmann K, Schoeffter P, Hoyer D and Lubbert H: Molecular pharmacology of somatostatin-receptor subtypes. Ann NY Acad Sci 733: 138-146, 1994.
39. Weckbecker G, Tolcsvai L, Stolz B, Pollak M and Bruns C: Somatostatin analogues octreotide enhances the antineoplastic effects of tamoxifen and ovariectomy on 7,12-dimethylbenz(a)-anthracene-induced rat mammary carcinomas. Cancer Res 54: 6334-6337, 1994.
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.