A Role for Insulin-like Growth Factor Binding Protein 5 in the Antiproliferative Action of the Antiestrogen ICI 182780[1]

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Abstract

Insulin-like growth factors (IGFs) are potent mitogens for breast cancer cells. Although IGF-binding proteins (IGFBPs) are known to regulate access of IGFs to IGF receptors, their precise biological actions are poorly defined. We observed that the potent antiestrogen ICI 182780 (ICI) increased IGFBP-5 mRNA by 2–3-fold in 9,10-dimethyl-1,2-benzanthracene-induced mammary tumors *in vivo*. *In vitro* studies showed that growth inhibition of MCF-7 human breast cancer cells induced by ICI was associated with increased transcription of the *IGFBP-5* gene, increased IGFBP-5 mRNA abundance, and increased IGFBP-5 protein accumulation in the conditioned medium. Growth stimulation following estradiol exposure was associated with opposite effects. An IGFBP-5 antisense oligodeoxynucleotide significantly decreased IGFBP-5 accumulation in conditioned media and enhanced MCF-7 cell DNA synthesis. Furthermore, this antisense oligodeoxynucleotide attenuated both antiestrogen-induced IGFBP-5 accumulation and antiestrogen-induced growth inhibition. These data demonstrate that estradiol down-regulates and ICI up-regulates an autocrine IGFBP-5 growth inhibitory pathway in MCF-7 cells and suggest that IGFBP-5 plays a role in modulation of proliferation of breast cancers by estrogens and antiestrogens.

Introduction

IGF[3]-I and IGF-II are potent mitogens and inhibitors of apoptosis for many normal and neoplastic cell types (1). Both IGF-I and IGF-II have high affinity for IGFBPs. To date, six IGFBPs have been characterized (reviewed in Ref. 2). These binding proteins are found in many physiological fluids and conditioned media of a wide variety of cell types and modulate IGF bioactivity in a complex manner. Although the IGFBPs are known to influence access of IGFs to their receptors, their precise physiological roles remain unclear, and both stimulatory and inhibitory effects on cellular proliferation have been reported under various experimental conditions (3–7).

The human *IGFBP-5* gene is divided into four exons which span 33 kb of DNA (8). IGFBP-5 was originally purified from rat serum (9), human bone (10), and medium conditioned by human osteoblast-like cells (11). IGFBP-5 has been shown to potentiate (10, 12) or inhibit (5) IGF action under specific experimental conditions. The different effects of IGFBP-5 observed in various experimental systems may be related to differences in posttranslational modification of IGFBP-5 (13) or to differences in extracellular matrix interactions (14). SV40 transformation provides an example of stimulation of proliferation correlated with decreased IGFBP-5 synthesis and increased cellular responsivity to IGFs (15).

Antiestrogens are widely used in breast cancer treatment, and it has been proposed that effects of these drugs on genes involved in regulation of IGF bioactivity may contribute to their antiproliferative activity (16–18). For example, the uterotrophic actions of tamoxifen are correlated with up-regulation of IGF-I expression and down-regulation of IGFBP-3 expression, whereas the complete antiestrogen ICI (19), which causes uterine involution, is associated with opposite effects (20).

The first example of stimulation of an autocrine growth inhibitory loop by an antiestrogen concerned tamoxifen stimulation of transforming growth factor β secretion (21). More recently, IGFBP-3 has been implicated in antiestrogen action (7). In this report, we describe experiments designed to evaluate the role of *IGFBP-5* gene expression in the modulation of proliferation of estrogen receptor-positive breast cancer cells by estradiol and antiestrogens.

Results

Effects of ICI on *IGFBP-5* Gene Expression in DMBA-induced Mammary Tumors. A prior study (19) described the antineoplastic activity of ICI on DMBA-induced mammary tumors. Fig. 1 illustrates the effect of ICI on the abundance of IGFBP-5 mRNA in this tumor model. Baseline expression of the *IGFBP-5* gene in DMBA-induced breast tumors was stimulated approximately 2–3-fold by ICI. This motivated studies of the role of IGFBP-5 in the regulation of breast cancer cell growth *in vitro*.

Effects of Estradiol and ICI on *IGFBP-5* Gene Expression in MCF-7 Breast Cancer Cells. Northern blot analysis revealed significant effects of estradiol and ICI on IGFBP-5 mRNA abundance. Densitometric scanning of Northern blots revealed that IGFBP-5 mRNA abundance was more than 7-fold higher in controls than in cells exposed to 10^{-10} M

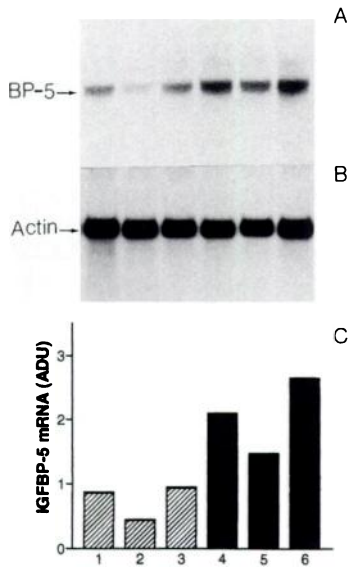

Fig. 1. Effect of ICI on IGFBP-5 mRNA abundance in DMBA-induced mammary tumors. Blots were performed using pooled total RNA from three independent groups of vehicle-treated (*Lanes 1–3*) or ICI-treated (*Lanes 4–6*) DMBA-induced mammary tumors. Each lane represented each group which consisted of four tumors. Total RNA was extracted, and Northern blot analysis was performed as described in "Materials and Methods" using the IGFBP-5 probe (*A*) and a β-actin probe as a loading control (*B*). The bands corresponding to IGFBP-5 were quantified using densitometric scanning and then corrected for equal loading using β-actin mRNA (*C*). For ICI-treated groups, all groups yielded IGFBP-5 mRNA abundance significantly different from those of controls ($P < 0.05$, Mann-Whitney *U* test).

estradiol and approximately double control values in cells exposed to 10^{-10} M ICI (Fig. 2). Estradiol inhibited *IGFBP-5* gene expression in a dose-dependent manner, with maximum inhibition at 10^{-10} M. The effect of ICI on *IGFBP-5* gene expression was opposite to that of estradiol. Equimolar ICI did not attenuate the suppressive effect of 10^{-10} M estradiol on IGFBP-5 mRNA abundance, but a 10-fold excess concentration of ICI abolished the inhibitory effects of estradiol on expression of the gene (Fig. 2). A nuclear run-off assay demonstrated that ICI increased and estradiol decreased the rate of *IGFBP-5* gene transcription relative to controls (Fig. 3), and studies of IGFBP-5 message stability using the mRNA-specific transcription inhibitor 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole demonstrated that the half-life for IGFBP-5 mRNA was approximately 12 h under control conditions and was not altered by estradiol or ICI treatments (data not shown).

Effects of Estradiol and ICI on IGFBP-5 Accumulation in MCF-7 Cell Conditioned Media. Conditioned media from cells grown in the presence of estradiol, ICI, or both were assayed for IGFBP-5 using Western blot analysis. MCF-7 cells growing in SEPF medium released the M_r 28,000–30,000 IGFBP-5 as determined by Western blotting (Fig. 4). Estradiol treatment reduced IGFBP-5 levels compared to controls, whereas ICI treatment markedly increased IGFBP-5 accumulation (Fig. 4). Thus, the direction of change in IGFBP-5 protein accumulation associated with estradiol or ICI exposure was consistent with the direction of change in

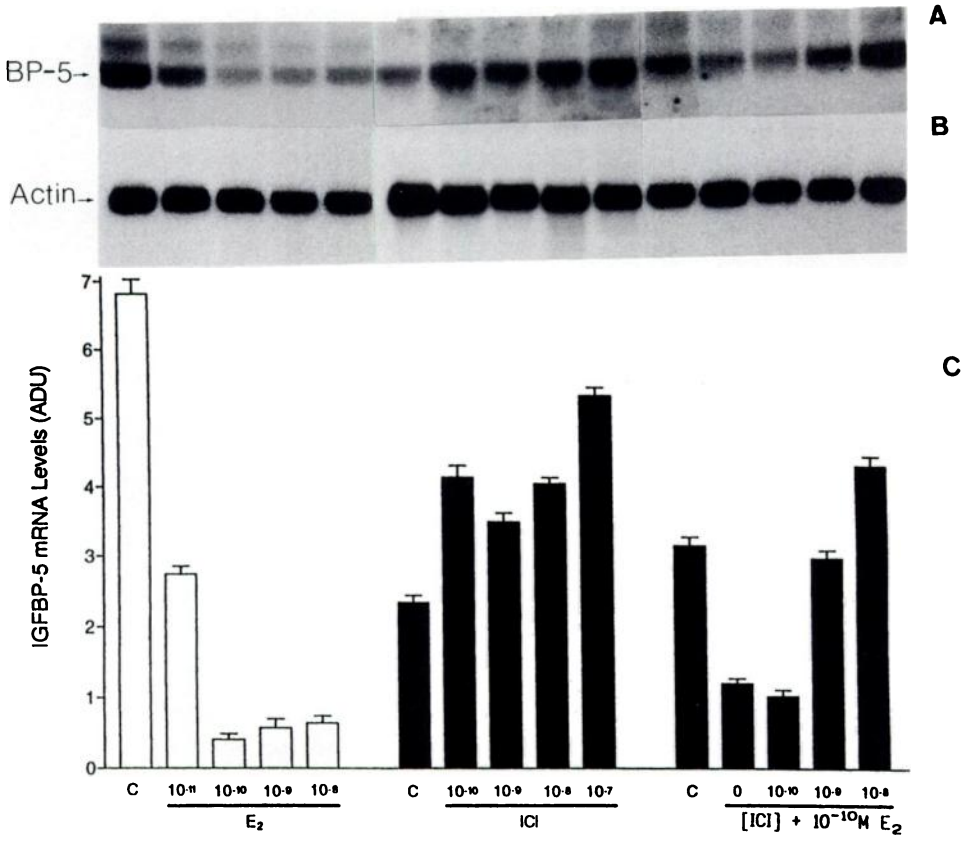

Fig. 2. Effect of estradiol and ICI on IGFBP-5 mRNA abundance in MCF-7 cells. MCF-7 cells at 85–90% confluence were grown in SEPF medium for 24 h. Cells were then treated with indicated doses of estradiol, ICI, or 10^{-10} M estradiol plus various doses of ICI for 48 h. Cytoplasmic RNA was collected, and Northern blot analysis was performed as described in "Materials and Methods" using the IGFBP-5 probe (*A*) and a β-actin probe as a loading control (*B*). The bands corresponding to IGFBP-5 were quantified densitometrically and corrected for equal loading (*C*). Means of quadruplicate experiments are plotted. Individual films were exposed for the 17β-estradiol (E_2), ICI, and ICI plus 17β-estradiol sets of data, and a control is provided for each group. Because of exposure differences, comparisons are possible only within groups. For the estradiol and ICI-treated groups, all concentrations tested yielded IGFBP-5 mRNA abundance [estimated in arbitrary density units (*ADU*)] significantly different from those of controls ($P < 0.05$, Mann-Whitney *U* test). *Bars*, SE.

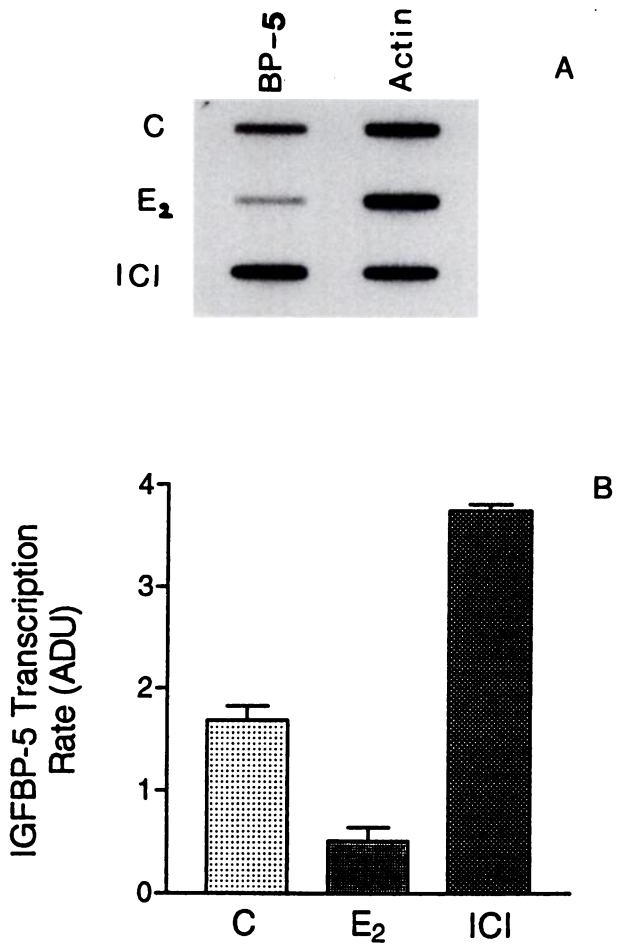

Fig. 3. Effect of estradiol and ICI on the rate of transcription of the *IGFBP-5* gene. MCF-7 cells were grown in serum-free phenol red-free medium for 24 h. Cells were treated with either serum-free phenol red-free as control (*C*) or 10^{-10} M estradiol (E_2) or 10^{-7} M ICI in serum-free phenol red-free medium for 48 h. Nuclear run-off assays were conducted as described in "Materials and Methods." *Bars*, SE.

IGFBP-5 mRNA levels. Coincubation of conditioned media from ICI-treated or estradiol-treated MCF-7 cells with ^{125}I-labeled IGFBP-5 revealed no evidence of modulation of IGFBP-5 proteolysis (data not shown) in this system, although IGFBP-5 proteolytic activity is well documented for fibroblasts (22).

Relationship of Effects of Estradiol and ICI on *IGFBP-5* Gene Expression to Effects on Proliferation. As expected, treatment of MCF-7 cells with estradiol for 48 h resulted in a dose-dependent increase in DNA synthesis (Fig. 4*C*). Of importance, even in the complete absence of estrogenic stimulation (SEPF conditions), ICI suppressed MCF-7 cell proliferation, indicating that this compound does not merely block estrogen-stimulated growth, but acts as a growth inhibitor even in the absence of estrogenic stimulation. There was a significant negative correlation (Spearman, $r = -0.71$, $P < 0.01$) between IGFBP-5 accumulation as estimated by Western blotting and proliferation as estimated by thymidine incorporation. Evidence for competition between the stimulatory effects of estradiol and the inhibitory effects of ICI was demonstrated by a series of titration experiments, exemplified by the shift to the right of the ICI dose-response curve when the experiment was performed in the presence of 0.1 nM estradiol at each ICI concentration (Fig. 4).

Effects of IGFBP-5 Antisense Oligodeoxynucleotides on MCF-7 Cell Proliferation. To determine whether the effect of ICI on IGFBP-5 expression contributes to the antineoplastic action of the drug, we used an IGFBP-5 antisense oligodeoxynucleotide to examine the consequences of reducing basal and ICI-stimulated IGFBP-5 secretion. Western blotting confirmed that this oligodeoxynucleotide reduced IGFBP-5 accumulation in MCF-7 cell-conditioned media (Fig. 5). IGFBP-3 and IGFBP-2 levels were not affected by this treatment (data not shown). Basal MCF-7 thymidine incorporation was nearly double in the presence of the antisense IGFBP-5 oligodeoxynucleotide. The scrambled antisense IGFBP-5 oligodeoxynucleotide was without significant effect on either IGFBP-5 accumulation or thymidine incorporation (Fig. 5). In the presence of 10^{-10} M ICI, thymidine incorporation by MCF-7 cells was 54 ± 11% of control values, and we observed that this antiestrogen-induced inhibition was significantly attenuated by 10 μg/ml IGFBP-5 antisense oligodeoxynucleotide, whereas an equal concentration of the scrambled antisense oligodeoxynucleotide had no significant effect (Fig. 5).

Discussion

We demonstrate that administration of ICI using the dose and route previously shown to have an inhibitory effect on DMBA-induced mammary tumors (19) induced IGFBP-5 mRNA accumulation *in vivo*. Furthermore, we show that the inhibitory action of ICI on DNA synthesis by MCF-7 cells after a 48-h exposure to the drug under serum-free conditions is related to stimulation of *IGFBP-5* gene expression. Our data therefore provide evidence for a previously unrecognized aspect of estrogen and antiestrogen action: estradiol-stimulated proliferation involves relaxation of an autocrine IGFBP-5 inhibitory loop, and the antiproliferative effect of ICI is related in part to up-regulation of *IGFBP-5* gene expression. Stimulation of *IGFBP-5* gene expression in MCF-7 cells by ICI involves increased IGFBP-5 mRNA synthesis but not changes in the IGFBP-5 mRNA half-life. Although secretion of IGFBP-5 proteolytic activity is an important determinant of IGFBP-5 accumulation in conditioned media in other experimental systems (22), this was found not to be an important variable in our experimental model.

Recent data suggest that IGFBPs in the extracellular environment may have direct (IGF-independent) actions on target cells (23), apart from classic actions related to modulation of IGF bioavailability. Intracellular actions of IGFBPs have also been proposed (24). Although our data demonstrate that *IGFBP-5* gene expression is involved in mediating the early effects of estrogens and antiestrogens on MCF-7 cell proliferation, additional data from ongoing studies will be needed to elucidate the pathway by which *IGFBP-5* expression influences proliferation. The fact that IGFBP-5 can either stimulate or inhibit cellular proliferation in different experi-

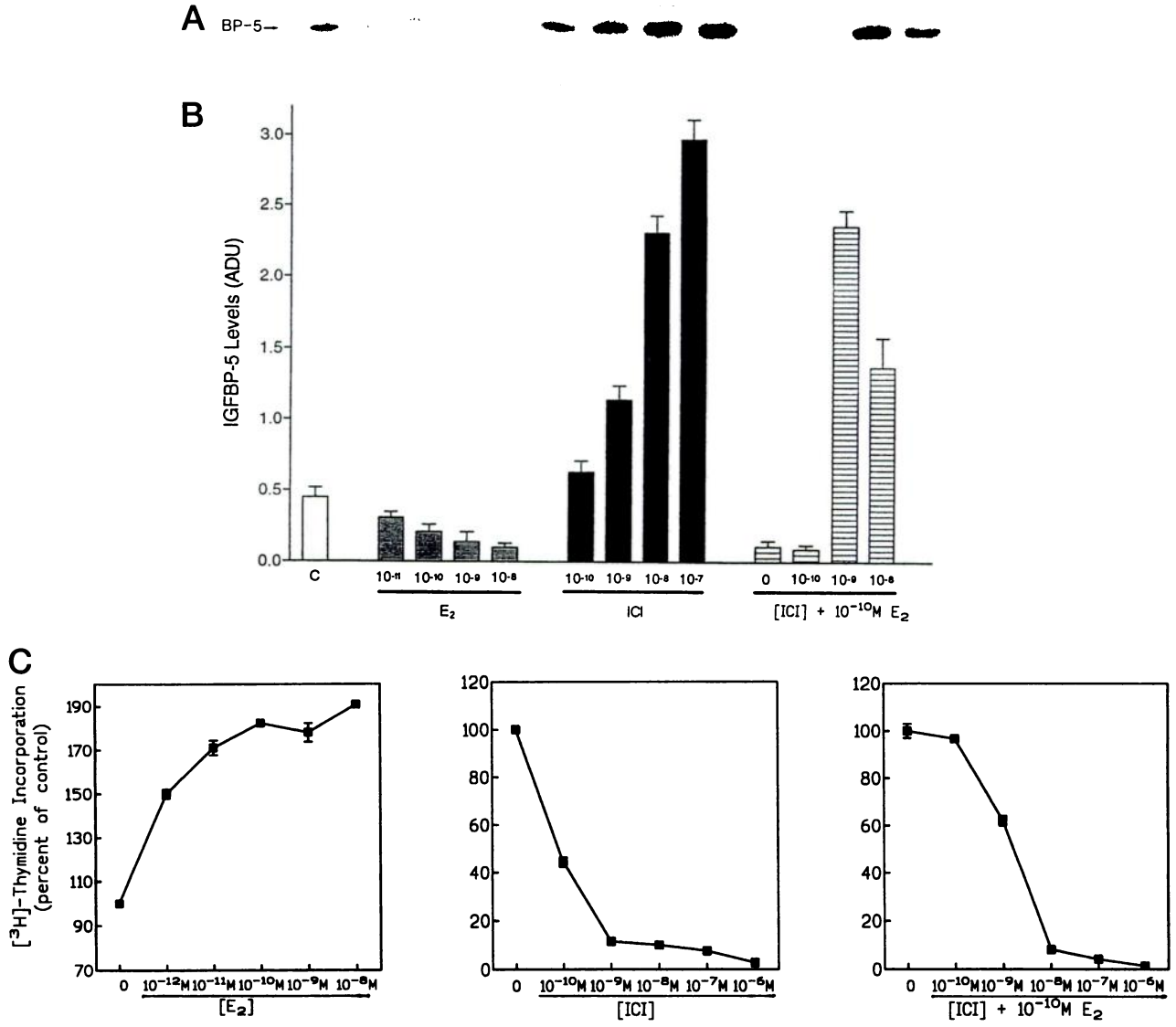

Fig. 4. Effects of estradiol and ICI on MCF-7 cell DNA synthesis and on the IGFBP-5 concentration in MCF7 cell-conditioned media. MCF-7 cells at 85–90% confluence were grown in SEPF medium for 24 h. Cells were then treated with indicated doses of estradiol (E_2), ICI, or 10^{-10} M estradiol plus various doses of ICI for 48 h. Western blotting of conditioned media (from equal cell number) was performed as described in "Materials and Methods." Representative results are shown in *A* and densitometric quantification (mean) is shown in *B*. The order of the lanes in *A* corresponds to the order of experimental conditions (*from left to right*) in *B*. *C*, proliferation rates estimated by thymidine incorporation for each experimental condition. The means of quadruplicate experiments are plotted. SEM at each point was ≤14%. *Bars*, SE.

mental systems (reviewed in Refs. 2 and 25) suggests that there are poorly characterized complexities in IGFBP-5 action and/or that *in vitro* experiments may incompletely model *in vivo* physiology. Certain systems (*e.g.*, see Refs. 5 and 15) support the view that IGFBP-5 acts as a growth inhibitor simply by reducing the availability of IGFs from autocrine, paracrine, or endocrine sources for binding to IGF-I receptors. This may be the case for certain breast cancers *in vivo*, particularly in view of data suggesting that human breast cancer is responsive to IGFs (26) and that autocrine or paracrine sources of IGF-II are involved in breast cancer pathophysiology (27, 28). The importance of the effect of ICI on IGFBP-5 physiology relative to previously described mechanisms of antiestrogen action (7, 19, 29) remains to be determined.

Materials and Methods

Animals and Drug Administration. We used the standard DMBA- induced mammary tumor experimental model (30) after approval of this study by the McGill Animal Care Committee. Rats bearing mammary tumors received s.c. injections of 5 mg ICI (Ref.19; kindly provided by Dr. A. Wakeling, Zeneka Pharmaceuticals) in 0.2 ml peanut oil once daily on 2 consecutive days. Control rats were s.c. administered 0.2 ml peanut oil. All animals were sacrificed by CO_2 exposure 7 days after the first day of treatment. The tumors were excised and frozen in liquid nitrogen and stored at −75°C for subsequent RNA isolation.

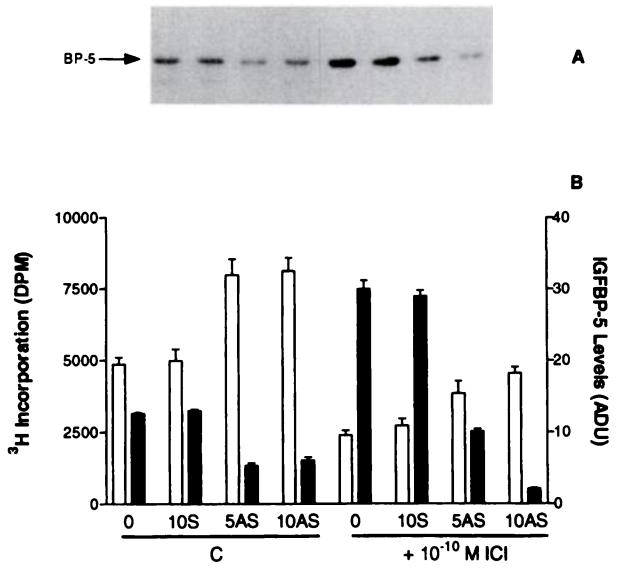

Fig. 5. Effect of IGFBP-5 antisense oligodeoxynucleotide on IGFBP-5 concentration in MCF-7 cell-conditioned media and on baseline and ICI-inhibited MCF-7 cell proliferation. Cells were cultured as described in "Materials and Methods" and then incubated for 48 h in SEPF medium with the indicated concentration (μg/ml) of antisense (*AS*) or scrambled antisense oligodeoxynucleotide (*S*) in the absence (*C*) or presence of 10^{-10} M ICI, and the thymidine incorporation and IGFBP-5 concentration in conditioned media were then determined. The viability of the cells in each treatment was >95% as determined by trypan blue exclusion. A representative Western blot of conditioned media (adjusted for equal cell number) is shown in *A*. Densitometric quantification of IGFBP-5 [■, arbitrary density units (*ADU*), *right axis*] and proliferation rates estimated by thymidine incorporation for each experimental condition (□, dpm, *left axis*) are shown in *B*. The order of the lanes in *A* corresponds to the order of experimental conditions (*from left to right*) in *B*. The means of triplicate experiments are plotted. SEM was ≤20% of the means in each case. Basal and ICI-suppressed proliferation rates were significantly increased in the presence of antisense IGFBP-5 oligodeoxynucleotide ($P < 0.01$, Mann-Whitney *U* test), and there was a negative correlation between the IGFBP-5 level and thymidine incorporation rate ($r = -0.82$, $P < 0.005$).

Cell Culture and Thymidine Incorporation Assay. 17β-Estradiol was purchased from Sigma (St. Louis, MO). MCF-7 human breast cancer cells were maintained and allowed to proliferate in serum-free conditions as described (7). Cells were cultured for 48 h in the presence or absence of various concentrations of compounds under study, as indicated in the legends to Figs. 1–5. Conditioned media were collected, concentrated, and assayed for IGFBP-5 using Western blots. The effect of scrambled antisense and antisense IGFBP-5 phosphorothioate oligodeoxynucleotides on cellular proliferation was studied under the same conditions, except that incubation prior to measurement of thymidine incorporation was for 48 h. The antisense oligodeoxynucleotide was a 21-mer (5′-CGCAGTGCACGAAGGAGCCCA-3′) corresponding to nucleotides 117–138 of the human IGFBP-5 mRNA (9). The scrambled antisense oligodeoxynucleotide was similar to the antisense sequence with a substitution of one different base per every four bases. The phosphorothioate oligodeoxynucleotides were prepared by the Oligos Etc. (Wilsonville, OR). Thymidine incorporation was measured as described previously (7). Experiments were carried out in triplicate.

Northern Blot Analysis. MCF-7 cells were treated as for measurement of thymidine incorporation. RNA extraction (from DMBA-induced mammary tumors and cells) and Northern blot analysis were performed as described previously (17). Blots were hybridized with the rat IGFBP-5 cDNA probe (9). This probe hybridizes to both human and rat IGFBP-5 mRNA. To control for equal loading of wells, blots were rehybridized with human β-actin cDNA (American Type Culture Collection).

Western Blotting. Conditioned medium (from equal cell number) was concentrated 20 times by a Centricon 10 microconcentrator (Amicon, Beverly, MA). Total proteins were resolved by electrophoresis in a 12% denaturing polyacrylamide gel, and proteins were electroblotted to nitrocellulose membranes. Immunoblot analysis with polyclonal antibodies against human IGFBP-5 (United Biomedical, Inc., Lake Placid, NY), and horseradish peroxidase-conjugated antirabbit antiserum (Amersham, Oakville, Ontario, Canada) were performed using standard protocols. Western blots were visualized by a chemiluminescence-based photoblot system (Amersham).

Nuclear Run-off Assay. MCF-7 cells were treated with either 10^{-10} M estradiol or 10^{-8} M ICI for 48 h. Nuclei were isolated, and nuclear run-off assays for IGFBP-5 and actin were performed as described (31).

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References

1. Resnicoff, M., Abraham, D., Yutanawiboonchai, W., Rotman, H. L., 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.

2. Jones, J. I., and Clemmons, D. R. Insulin-like growth factors and their binding proteins: biological actions. Endocr. Rev., *16:* 3–34, 1995.

3. Elgin, R. G., Busby, W. H., and Clemmons, D. R. An insulin-like growth factor (IGF) binding protein enhances the biological response to IGF-I. Proc. Natl. Acad. Sci. USA, *84:* 3254–3258, 1987.

4. Rutanen, E. M., Pekonen, F., and Makinen, T. Soluble 34K binding protein inhibits the binding of insulin-like growth factor I to its cell receptors in human secretory phase endometrium: evidence for autocrine/paracrine regulation of growth factor action. J. Clin. Endocrinol. Metab., *66:* 173–180, 1988.

5. Kiefer, M. C., Schmid, C., Waldvogel, M., Schlapfer, I., Futo, E., Masiarz, F. R., Green, K., Barr, P. J., and Zapf, J. Characterization of recombinant human insulin-like growth factor binding proteins 4, 5, and 6 produced in yeast. J. Biol. Chem., *267:* 12692–12699, 1992.

6. Oh, Y., Mullers, H. L., Ng, L., and Rosenfeld, R. G. Transforming growth factor-β-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.

7. Huynh, H. T., Yang, X., 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.

8. Allander, S. V., Larsson, C., Ehrenborg, E., Suwanichkul, A., Weber, G., Morris, S. L., Bajalica, S., Kiefer, M. C., Luthman, H., and Powell, D. R. Characterization of the chromosomal gene and promoter for human insulin-like growth factor binding protein-5. J. Biol. Chem., *269:* 10891–10898, 1994.

9. Shimasaki, S., Shimonaka, M., Zhang, H., and Ling, N. Identification of five different insulin-like growth factor binding proteins (IGFBPs) from adult rat serum and molecular cloning of a novel IGFBP-5 in rat and human. J. Biol. Chem., *266:* 10646–10653, 1991.

10. Bautista, C. M., Baylink, D. J., and Mohan, S. Isolation of a novel insulin-like growth factor (IGF) binding protein from human bone: a potential candidate for fixing IGF-II in human bone. Biochem. Biophys. Res. Commun., *176:* 756–763, 1991.

11. Andress, D. L., and Birnbaum, R. S. A novel human insulin-like growth factor binding protein secreted by osteoblast-like cells. Biochem. Biophys. Res. Commun., *176:* 213–218, 1991.

12. Jones, J. I., Gockerman, A., Busby, W. H. J., Camacho-Hubner, C., and Clemmons, D. R. Extracellular matrix contains insulin-like growth factor binding protein-5: potentiation of the effects of IGF-1. J. Cell Biol., *121:* 679–687, 1993.

13. Andress, D. L., Loop, S. M., Zapf, J., and Kiefer, M. C. Carboxy-truncated insulin-like growth factor binding protein-5 stimulates mitogenesis in osteoblast-like cells. Biochem. Biophys. Res. Comm., *195:* 25–30, 1993.

14. Arai, T., Arai, A., Busby, W. H., Jr., and Clemmons, D. R. Glycosaminoglycans inhibit degradation of insulin-like growth factor-binding protein-5. Endocrinology, *135:* 2358–2363, 1994.

15. Reeve, J. G., Guadano, A., Xiong, J., Morgan, J., and Bleehen, N. M. Diminished expression of insulin-like growth factor (IGF) binding protein-5 and activation of IGF-I-mediated autocrine growth in simian virus 40-transformed human fibroblasts. J. Biol. Chem., *270:* 135–142, 1995.

16. Pollak, M., Costantino, 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.

17. Huynh, H. T., 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.

18. Pollak, M. Effects of adjuvant tamoxifen therapy on growth hormone and insulin-like growth factor I (IGF-I) physiology. *In:* S. E. Salmon (ed.), Adjuvant Therapy of Cancer VII, pp. 43–54. Philadelphia: J. B. Lippincott Company, 1993.

19. Wakeling, A. E., Dukes, M., and Bowler, J. A potent specific pure antiestrogen with clinical potential. Cancer Res., *51:* 3867–3873, 1991.

20. Huynh, H. T., 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.

21. Knabbe, C., Lippman, M. E., Wakefield, L. M., Flanders, K. C., Kasid, A., Derynck, R., and Dickson, R. B. Evidence that transforming growth factor-β is a hormonally regulated negative growth factor in human breast cancer cells. Cell, *48:* 417–428, 1987.

22. Nam, T. J., Busby, W. H., Jr., and Clemmons, D. R. Human fibroblasts secrete a serine protease that cleaves insulin-like growth factor-binding protein-5. Endocrinology, *135:* 1385–1391, 1994.

23. Mohan, S., Nakao, Y., Honda, Y., Landale, E., Leser, U., Dony, C., Lang, K., and Baylink, D. J. Studies on the mechanisms by which insulin-like growth factor (IGF) binding protein-4 (IGFBP-4) and IGFBP-5 modulate IGF actions in bone cells. J. Biol. Chem., *270:* 20424–20431, 1995.

24. Jaques, G., Wegmann, B., Noll, K., Radulescu, R., and Havemann, K. Nuclear localization of IGFBP-3 in lung cancer cell lines. Third International Symposium on Insulin-like Growth Factor Binding Proteins (Tübingen, Germany), Abstract 3–28, 1995.

25. Conover, C. A., Bale, L. K., Clarkson, J. T., and Torring, O. Regulation of insulin-like growth factor binding protein-5 messenger ribonucleic acid expression and protein availability in rat osteoblast-like cells. Endocrinology, *132:* 2525–2530, 1993.

26. Arteaga, C. L., Kitten, L. J., Coronado, E. B., Jacobs, S., Kull, F. C., Allred, D. C., and Osborne, C. K. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J. Clin. Invest., *84:* 1418–1423, 1989.

27. Osborne, C. K., Coronado, E. B., Kitten, L. J., Arteaga, C. L., Fuqua, S. A., Ramasharma, K., Marshall, M., and Li, C. H. Insulin-like growth factor-II (IGF-II): a potential autocrine/paracrine growth factor for human breast cancer acting via the IGF-I receptor. Mol. Endocrinol., *3:* 1701–1709, 1989.

28. Cullen, K. J., Lippman, M. E., Chow, D., Hill, S., Rosen, N., and Zwiebel, J. A. Insulin-like growth factor-II overexpression in MCF-7 cells induces phenotypic changes associated with malignant progression. Mol. Endocrinol., *6:* 91–100, 1992.

29. Jordan, V. C., and Murphy, C. S. Endocrine pharmacology of antiestrogens as antitumor agents. Endocr. Rev., *11:* 578–611, 1990.

30. Welsch, C. W. Host factors affecting the growth of carcinogen-induced rat mammary carcinomas: a review and tribute to Charles Brenton Huggins. Cancer Res., *45:* 3415–3443, 1985.

31. Ausubel, E. F., Brent, R., Kingston, R. E., Moore, D. D., Seidman, J. G., Smith, J. A., and Struhl, K. Current Protocols in Molecular Biology. Current Protocols, Green Publishing Associates, Inc. and John Wiley & Sons, Inc., 1993.