Inhibition of insulin-like growth factor I receptor signaling by the vitamin D analogue EB1089 in MCF-7 breast cancer cells: A role for insulin-like growth factor binding proteins

FLORENCE ROZEN and MICHAEL POLLAK

Lady Davis Institute for Medical Research of the Jewish General Hospital and Departments of
Medicine and Oncology, McGill University, Montreal, Quebec H3T 1E2, Canada

Abstract. Insulin-like growth factors I and II (IGF-I and IGF-II) are potent mitogens involved in growth regulation of breast epithelial cells and are implicated in the pathophysiology of breast cancer. Their bioactivity is enhanced or inhibited by specific IGF-binding proteins (IGFBPs). Vitamin D-related compounds (VDRCs) have been shown to inhibit proliferation and induce apoptosis of MCF-7 breast carcinoma cells. We have previously demonstrated that VDRCs antagonize the growth-promoting activity of IGF-I by stimulating autocrine production of IGFBP-5 in MCF-7 cells, but the effect of VDRCs on IGF-I receptor (IGF-IR) intracellular signaling has not been elucidated. We report here that the vitamin D analogue EB1089 interferes with the IGF-IR signaling pathway by attenuating IGF-I-induced tyrosine phosphorylation of IRS-1, and to a lesser extent, IRS-2. It does not affect protein levels of IRS-1, IRS-2 or IGF-IR. However, EB1089 does not inhibit tyrosine phosphorylation of IRS-1 induced by des(1-3) IGF-I, an IGF-I analogue with greatly reduced affinity for IGFBPs. Furthermore, we demonstrate that an antisense IGFBP-5 oligodeoxynucleotide attenuates EB1089-induced inhibition of IGF-I-stimulated tyrosine phosphorylation of IRS-1 and EB1089-induced IGFBP-5 accumulation. These data strongly suggest that IGFBP-5 plays a functional role in the interfering action of EB1089 with the IGF-IR signal transduction pathway.

Introduction

The biologically active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$], apart from its classical role as a regulator of calcium homeostasis (1), is known to regulate cell growth and differentiation and may be an essential determinant of progression toward terminal differentiation of epithelial cells (2-6). The vitamin D receptor (VDR) has been detected in kidney, bone, intestine, (reviewed in ref. 6), and in many neoplastic cell lines, including MCF-7 breast cancer cells (3,4,7). $1,25(OH)_2D_3$ has been shown to have antiproliferative effects (8,9), and to induce apoptosis in breast cancer cells (10). However, the clinical application of $1,25(OH)_2D_3$ as an antineoplastic agent is severely limited by the hypercalcemia associated with high dose administration. More recently, synthetic vitamin D analogues with potent antineoplastic but reduced calcemic activity have been described (11-15). One compound, EB1089, has been shown to inhibit the growth of breast cancer cells *in vitro* and *in vivo* (11).

Insulin-like growth factors I and II (IGF-I and IGF-II) are potent mitogens and antiapoptotic agents for many neoplastic cell types, as well as their untransformed precursors (16). IGF bioactivity is either enhanced or inhibited by specific insulin-like growth factor binding proteins (IGFBPs), 7 of which have been described to date (17,18). The inhibitory effects of IGFBPs have been attributed to competitive scavenging of IGF peptides away from the type-1 IGF receptor (IGF-IR) or direct (IGF independent) mechanisms (17). The IGF-IR belongs to the tyrosine kinase receptor family (19). Both IGF-I and IGF-II act as ligands for the IGF-IR, although the IGF-IR has higher affinity for IGF-I than IGF-II.

Signal transduction through the IGF-IR activates intracellular signaling cascades that lead to both mitogenic and antiapoptotic effects (16). The activation of the IGF-IR appears to play a key role in the regulation of breast cancer cell growth (20). Its activation by ligand binding causes rapid tyrosine phosphorylation of IRS-1, -2 and SHC, and intracytoplasmic assembly of a complex consisting of a variety of proteins that are responsible for stimulating diverse downstream signal transduction pathways, as reviewed in (21,22). IRS-1 is a docking protein that, upon phosphorylation by the IGF-IR, interacts with various effector proteins through SH2-type interactions. It is found at the crossroads of several signaling pathways. In addition to IRS-1 binding and activation of PI-3 kinase and SYP phosphatase, its binding to GRB-2/SOS complexes stimulates the p21 *ras*/MAP pathway (23). IRS-1 is required for signaling by the growth hormone receptor and γ-interferon (24), and has been found to interact with the JAK family of

transducing molecules (22,25), certain integrins (26), and G protein-coupled receptors (27). IRS-1 is therefore central to signaling in different pathways and may play a broader role than previously anticipated both in cell proliferation and in gene expression.

As the molecular mechanisms underlying growth inhibition induced by vitamin D-related compounds (VDRCs) are incompletely described, we investigated the possibility that these compounds interfere with the activity of IGFs. Our previous results indicate that $1,25(OH)_2D_3$ and EB1089 stimulate autocrine production of IGFBP-5 by MCF-7 cells, thereby indirectly suppressing their proliferation (28). The VDRCs attenuated the growth-promoting activity of IGF-I on MCF-7 cells; however, these compounds did not inhibit the growth-promoting activity of long R3 IGF-I, an IGF-I analogue with greatly reduced affinity for IGFBPs. These data provide evidence for an important role for IGFBPs in the antiproliferative effect mediated by VDRCs. The interaction of VDRCs with the IGF-IR signaling pathway has not been characterized. Here we explored this aspect of EB1089 action in MCF-7 cells by determining if EB1089 interferes with IRS-1 phosphorylation by the IGF-IR, and if this effect is mediated by IGFBPs.

Materials and methods

Cell culture. MCF-7 human breast cancer cells were obtained from American Type Culture Collection (Rockville, MD) and maintained as monolayer cultures in Alpha Modified Eagle Medium (α-MEM; Life Technologies, Inc., Gaithersburg, MD) that was supplemented with 5 μg/ml bovine insulin (Sigma Chemical Co., St. Louis, MO) and 10% fetal calf serum (FCS; Life Technologies, Inc.) in a humidified incubator at 37°C and 5% CO_2.

Cell lysates. Confluent stock cultures of cells were treated with trypsin and plated in α-MEM supplemented with 5% FCS at $4x10^6$ cells per 100-mm tissue culture dish (Becton Dickinson, Lincoln Park, NJ). After 48 h, the cells were washed three times with serum-, estrogen- and phenol red-free (SEPF) α-MEM, and then incubated in this media for 48 h in the presence or absence of 10^{-7} M EB1089 (kindly provided by Dr Lise Binderup, LEO Pharmaceuticals, Ballerup, Denmark) as indicated in the figure legends. In some experiments, sense and antisense IGFBP-5 oligodeoxy-nucleotides (Sheldon Biotechnology Center, Montreal, Quebec) were added at a concentration of 5 μg/ml, and described previously (29). Cells were stimulated with $1.4x10^{-9}$ M IGF-I (Celtrix Pharmaceuticals, Santa Clara, CA) or des(1-3) IGF-I (GroPep, Adelaide, Australia) for 5 min wherever indicated, washed twice with PBS, and lysed in 10 mM $Na(PO_4)_2$, 100 mM NaCl, 0.5% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 5 mM EDTA containing the protease inhibitor cocktail tablet Complete™ (Boehringer Mannheim, Germany) for 10 min at 4°C. Cell lysates were centrifuged at 3,000 rpm for 10 min at 4°C and used for Western blotting or immuno-precipitation.

Western blot analysis. Equal amounts of protein (50-100 μg) were resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions and then transferred to nitrocellulose membranes. The membranes were blocked for 1 h, incubated for 1 h with an anti-phosphotyrosine monoclonal antibody PY20 (Transduction Laboratories, Lexington, KY), anti-IRS-1 or -2 polyclonal antibody (Upstate Biotechnology Inc., Lake Placid, NY), or anti-IGF-IRß polyclonal antibody (Santa Cruz Biotechnology Inc., Santa Cruz, CA) wherever indicated in the figure legends, washed, and then incubated for 1 h with horseradish peroxidase-conjugated secondary antibody. Antigen-antibody reactions were visualized by means of enhanced chemilumine-scence (Amersham, Oakville, Ontario, Canada).

Immunoprecipitation. 200 μg of cell lysate was immuno-precipitated with 2 μg anti-IRS-1 or -2 overnight at 4°C. 40 μl of a protein A-Sepharose slurry (Pharmacia) was added to the immunocomplex for 2 h at 4°C. Immunoprecipitated proteins were washed twice with PBS, electrophoresed by SDS-PAGE, and analyzed by Western blotting as described above.

Western ligand blotting. Cells were treated with EB1089 in the presence or absence of sense and anti-sense IGFBP-5 oligodeoxynucleotides for 48 h as described above. Proteins in 10x-concentrated cell-conditioned media were separated by SDS-PAGE under non-reducing conditions and electro-blotted onto nitrocellulose membranes. The membranes were blocked, labeled with [^{125}I]-IGF-I, and exposed to X-ray film (X-Omat AR; Kodak, Rochester, NY) as previously described (30).

Results

In order to investigate the initial signaling events in IGF-IR activation by its ligand, quiescent MCF-7 cells were stimulated with $1.4x10^{-9}$ M IGF-I and total cell lysates were prepared. Proteins were separated by SDS-PAGE, transferred to nitrocellulose filters, and immunoblotted with anti-phospho-tyrosine antibody (PY20). Fig. 1A demonstrates the tyrosine phosphorylation profile of proteins in a total cell lysate, where treatment with IGF-I for 1 min results in a predominant tyrosyl-phosphorylated species migrating as a broad band at ~180 kDa. This species can be detected up to 24 h post-stimulation with IGF-I (data not shown). As we have previously shown that the vitamin D analogue EB1089 attenuated the growth-promoting activity of IGF-I (28), we wished to determine whether EB1089 interfered with early events in the IGF-IR signal transduction pathway by antagonizing IGF-I induced tyrosine phosphorylation of the ~180 kDa species. Fig. 1B demonstrates that stimulation with IGF-I for 5 min increased tyrosine phosphorylation 9-fold over control levels. While EB1089 had no effect on the basal level of tyrosine phosphorylation, it reduced IGF-I induced tyrosine phosphorylation of the ~180 kDa species by 80% (as estimated by densitometric scanning of the band).

The ~180 kDa band approximated the size of IRS-1 and/or IRS-2. Although IRS-2 is slightly larger (~190 kDa) and differs immunologically from IRS-1, it is difficult to distinguish the two by migration on SDS-PAGE. In an attempt to determine the identity of the protein(s), total cell lysates were immunoprecipitated with anti-IRS-1 and anti-

WB: PY20

kDa

← 220 →
← 97 →
← 66 →

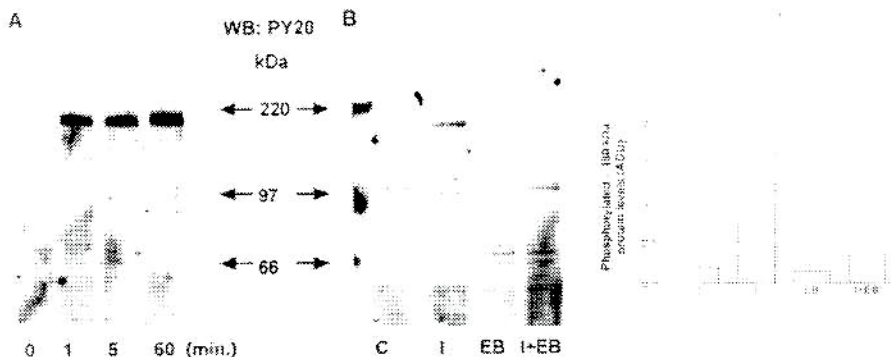0   1   5   60 (min.)   C   I   EB   I+EB

Figure 1. Tyrosine phosphorylation in response to IGF-I and effect of EB1089 preincubation. A, Cells were incubated for 48 h in SEPF medium and stimulated with 1.4×10^{-9} M IGF-I for indicated times prior to cell lysis. Total cell lysates were separated by SDS-PAGE and immunoblotted with PY20 as described in Materials and methods. Migration positions of molecular size markers are indicated on the right. B. Cells were incubated for 48 h in SEPF medium in the absence (C) or presence (EB, I + EB) of 10^{-7} M EB1089 and stimulated for 5 min with 1.4×10^{-9} M IGF-I (I, I + EB) prior to cell lysis. Total cell lysates were separated by SDS-PAGE and immunoblotted with PY20 in a representative experiment as described above. Molecular size markers are indicated on the left. The band corresponding to the ~180 kDa species was quantified densitometrically and plotted. WB, Western blot.

A   →   IP : α-IRS-2   WB: PY20

B   →   IP : α-IRS-1   WB: PY20

C   →   IP : α-IRS-1   WB: α-IRS-1

D   →   WB : α-IRS-1

E   →   WB : α-IRS-2

F   →   WB : α-IGF-IRß
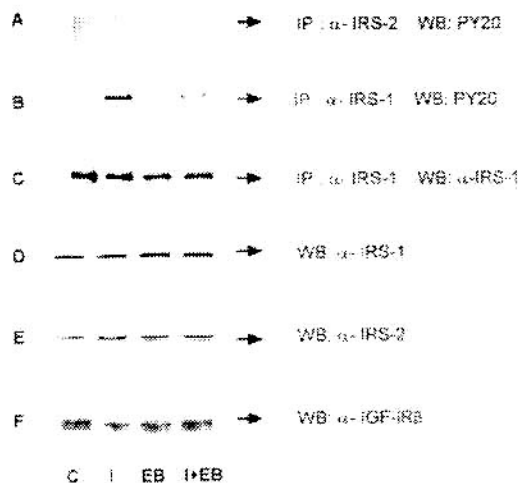
C   I   EB   I+EB

Figure 2. Effect of EB1089 on IGF-I-induced phosphorylation of IRS-1 and IRS-2 and on IRS-1, IRS-2, and IGF-IR protein levels. A, Cells were incubated in the absence or presence of EB1089 and stimulated with IGF-I as described in Fig. 1. Total cell lysates were immunoprecipitated with α-IRS-2, followed by immunoblotting with PY20 as described in Materials and methods. B, Total cell lysates were immunoprecipitated with α-IRS-1, followed by immunoblotting with PY20. C, The α-IRS-1 immunoprecipitated blot in (B) was stripped and reprobed with α-IRS-1. D, E, and F, Total cell lysates were immunoblotted with α-IRS-1, α-IRS-2 and α-IGF-IRß, respectively. IP, immunoprecipitate; WB, Western blot.

IRS-2, followed by Western blotting with PY20 (Fig. 2). While IGF-I weakly stimulated IRS-2 tyrosine phosphorylation (which was inhibited by EB1089, Fig. 2A), the major tyrosyl-phosphorylated protein induced by IGF-I and attenuated by EB1089 is IRS-1 (Fig. 2B). To control for equal immunoprecipitation of IRS-1, lysates were immuno-precipitated with anti-IRS-1 and immunoblotted with anti-IRS-1 (Fig. 2C). Treatment with IGF-I, EB1089 or both did not alter IRS-1, IRS-2 or IGF-IR protein levels (Fig. 2D, E and F).

In order to determine whether IGFBPs play a functional role in the interference of EB1089 with the IGF-IR signaling pathway, we induced tyrosine phosphorylation of IRS-1 with des(1-3) IGF-I, an IGF-I analogue that exhibits greatly reduced affinity for IGFBPs but similar affinity for IGF-I receptors (31). Consistent with its greater biological potency than IGF-I *in vitro* and *in vivo* (32-35), densitometric scanning demonstrated that des(1-3) IGF-I stimulated tyrosine phosphorylation of IRS-1 30-fold over control levels, as compared to IGF-I, where a 15-fold increase in tyrosyl-phosphorylated IRS-1 levels was observed (Fig. 3). More importantly, the IGF-I-induced tyrosine phosphorylation of IRS-1 was reduced by 65% in the presence of 10^{-7} M EB1089. In contrast, EB1089 had no inhibitory effect on tyrosyl-phosphorylated IRS-1 levels stimulated by des(1-3) IGF-I (Fig. 3). IRS-1 protein levels were not affected by stimulation with des(1-3) IGF-I or IGF-I (Fig. 3).

To determine if IGFBP-5 specifically contributes to the blocking action of EB1089 on IGF-IR signaling, we used an IGFBP-5 antisense oligodeoxynucleotide to examine the effect of EB1089-induced IGFBP-5 accumulation on IGF-I-stimulated tyrosine phosphorylation of IRS-1 (Fig. 4). Densitometric scanning of ligand blots revealed a 15-fold increase over control levels of IGFBP-5 accumulation in MCF-7 conditioned media in the presence of 10^{-7} M EB1089 [Fig. 4A and (28)]. Western blotting experiments confirmed the induction of IGFBP-5 accumulation in the presence of EB1089 [(1724) and data not shown]. While 5 μg/ml IGFBP-5 antisense oligodeoxynucleotide antagonized EB1089-induced IGFBP-5 accumulation, an equal concentration of the sense oligodeoxynucleotide did not (Fig. 4A). Similarly, EB1089 inhibited IGF-I stimulated tyrosine phosphorylation of IRS-1, which was attenuated 2-fold by 5 μg/ml IGFBP-5
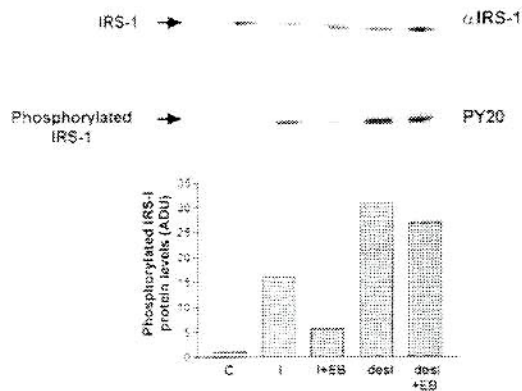
Figure 3. Effect of EB1089 on IGF-I- and des(1-3) IGF-I-induced tyrosine phosphorylation of IRS-1. Cells were incubated for 48 h in SEPF medium in the absence (C) or presence (EB, I + EB, desI + EB) of 10^{-7} M EB1089 and stimulated for 5 min with 1.4×10^{-9} M IGF-I (I, I + EB) or des(1-3) IGF-I (desI, desI + EB) prior to cell lysis. Total cell lysates were immunoblotted with PY20 (lower blot) and the densitometric quantification is shown below. The blot was stripped and reprobed with α-IRS-1 (upper blot). Representative data are shown. WB, Western blot.

Effects of EB1089 on the IGF signal transduction pathway have not previously been described. Our data provide evidence for two novel aspects of EB1089 action: i) the anti-proliferative effect of EB1089 on MCF-7 cells is associated with interference of EB1089 with IRS-1 phosphorylation by the IGF-IR and ii) the interfering action of EB1089 on IRS-1 phosphorylation is mediated at least in part by IGFBPs in these cells. Our experiments focused on IGF-I-induced IRS-1 tyrosine phosphorylation by the IGF-IR, its major cellular substrate. No other IGF-responsive tyrosine phosphorylated proteins were evident under our conditions, or as reported by Kleinman et al in this cell line (36). Karas et al demonstrated that due to the low IGF-IR number in Ishikawa cells (17,000/cell), sensitivity of the tyrosine phosphorylation assay in whole cells was not sufficient to detect IGF-I-induced receptor autophosphorylation (37). 28,000 IGF-IRs per MCF-7 cell (37) could similarly explain our failure to detect IGF-I-induced IGF-IR autophosphorylation in this assay.

The demonstration that the antiproliferative actions of p53 (38), retinoids (39), antiestrogens (40), TGFβ (41), vitamin D analogues (28) and TNF-α (42) all entail increased IGFBP expression, implies that modulation of IGF bio-activity is a common mechanism for the growth inhibitory activities of these antineoplastic agents in various cell types. In addition to EB1089, p53 and the antiestrogens tamoxifen and ICI 182780 decrease IGF-I-induced tyrosine phos-phorylation of IRS-1 (43,44) (data not shown). The involvement of IGFBPs in these inhibitory processes remains to be seen. Additionally, IGF-IR protein levels and MAP kinase activity are not modulated by EB1089 (Fig. 2 and data

antisense oligodeoxynucleotide (Fig. 4B). In contrast, an equal concentration of the sense oligodeoxynucleotide had no substantial effect. Treatment with sense or antisense oligo-deoxynucleotides did not affect IRS-1 protein levels (data not shown).
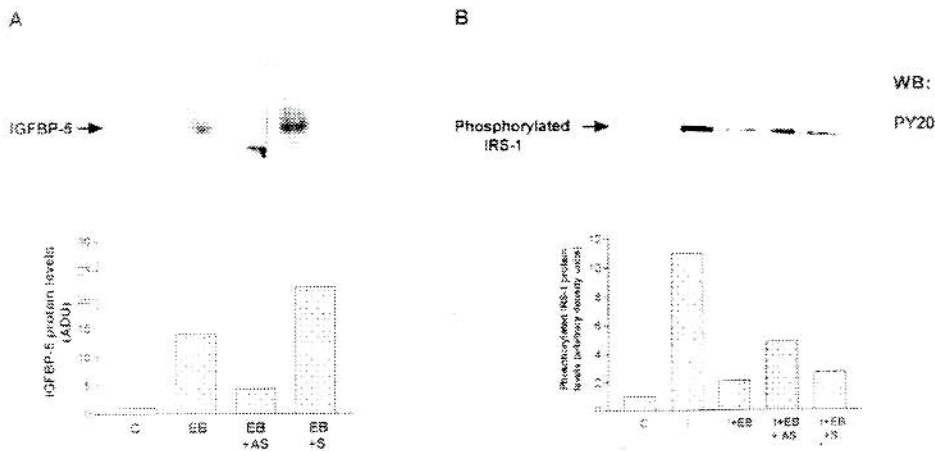

Figure 4. Effect of IGFBP-5 antisense oligodeoxynucleotide on EB1089-induced IGFBP-5 accumulation and EB1089-induced inhibition of IGF-I-stimulated phosphorylation of IRS-1. A. Cells were incubated for 48 h in SEPF medium with 5 μg/ml of antisense (AS) or sense (S) oligodeoxy-nucleotide in the presence of 10^{-7} M EB1089. Conditioned media were collected and concentrated, and Western ligand blot analysis was performed as described in Materials and methods. The densitometric quantification is shown below. B, Cells were incubated with antisense or sense oligodeoxy-nucleotides in the presence of EB1089 as described in (A) and stimulated for 5 min with 1.4×10^{-9} M IGF-I prior to cell lysis. Total cell lysates were immunoblotted with PY20 and the densitometric quantification is shown below. Representative data are shown. WB, Western blot.

not shown) or tamoxifen (44), suggesting a convergence in effector pathways for the antiproliferative actions of vitamin D-related compounds and antiestrogens. Interestingly, TNF-α does not attenuate IGF-I-induced tyrosine phosphorylation of IRS-1 (data not shown). Interleukin 1-α and -ß (which increase IGFBP-3 accumulation in MCF-7 conditioned media, data not shown), have been shown to inhibit phosphotransferase activity of IGF-IR [as measured by ^{32}P incorporation into the synthetic substrate polyGlu:Tyr, (45)], yet do not antagonize IGF-I-induced tyrosine phosphorylation of IRS-1 under our conditions (data not shown). Therefore, while modulation of IGF action may be the common mechanism for the growth inhibitory activities of the above mentioned antiproliferative agents, the inhibition of IGF-I-induced IRS-1 phosphorylation is not a universal feature. Inhibition up- or downstream of IRS-1 in the IGF-IR signaling pathway by these antiproliferative agents can not be excluded.

Our present study implicates IGFBP-5 in the inhibitory action of EB1089 on IGF-IR signal transduction, as demonstrated by experiments using des(1-3) IGF-I and IGFBP-5 antisense oligodeoxynucleotides. The affinity of soluble IGFBPs for des(1-3) IGF-I is reported to be 25-50-fold lower than that for IGF-I (46). The greater biological potency of des(1-3) IGF-I can be attributed to the reduced ability of IGFBP-5 (which is upregulated by EB1089) to effectively sequester des(1-3) IGF-I, explaining the lack of attenuation of des(1-3) IGF-I-induced phosphorylation of IRS-1 by EB1089. Furthermore, we demonstrate that EB1089-induced inhibition of IGF-I-stimulated IRS-1 phosphorylation is associated with increased IGFBP-5 accumulation, and that attenuation of this EB1089-induced inhibition by an IGFBP-5 antisense oligodeoxynucleotide is associated with a decrease in IGFBP-5 accumulation. The attenuation by an IGFBP-5 antisense oligodeoxynucleotide likely results from a reduction in soluble IGFBP-5 sequestering IGF-I and inhibiting its biological action by preventing receptor interaction. It is not necessary to postulate a role for a putative IGFBP-5 receptor (47-49) to account for our results, but an additional direct (IGF independent) growth inhibitory activity of IGFBP-5 cannot be excluded as a possible mechanism.

The IGF-IR plays an important role in the pathophysiology of breast tumors (20,50), and activation by its ligand promotes cell growth, transformation, and cell survival by directly inhibiting apoptosis (16,20). It is possible that breast carcinomas expressing IGF-IRs are responsive to measures that inhibit IGF bioactivity. Thus our findings may have relevance to the use of vitamin D analogues for treatment and/or prevention of breast cancer.

Acknowledgments

References

1. Reichel H, Koeffler HP and Norman AW: The role of the vitamin D endocrine system in health and disease. N Engl J Med 320: 980-991, 1989.

2. DeLuca HF: The vitamin D story: a collaborative effort of basic science and clinical medicine (review). FASEB J 2: 224-236, 1988.

3. Eisman JA, Martin TJ, Macintyre I, Frampton RJ, Moseley JM and Whitehead R: 1,25-Dihydroxyvitamin D3 receptor in a cultured human breast cancer cell line (MCF 7 cells). Biochem Biophys Res Commun 93: 9-15, 1980.

4. Colston K, Colston MJ, Fieldsteel AH and Feldman D: 1,25-dihydroxyvitamin D3 receptors in human epithelial cancer cell lines. Cancer Res 42: 856-859, 1982.

5. Colston KW, Berger U and Coombes RC: Possible role for vitamin D in controlling breast cancer cell proliferation. Lancet 1: 188-191, 1989.

6. Walters MR: Newly identified actions of the vitamin D endocrine system (review). Endocrinol Rev 13: 719-764, 1992.

7. Buras RR, Schumaker LM, Davoodi F, Brenner RV, Shabahang M, Nauta RJ and Evans SR: Vitamin D receptors in breast cancer cells. Breast Cancer Res Treat 31: 191-202, 1994.

8. DeLuca HF and Ostrem V: The relationship between the vitamin D system and cancer. Adv Exp Med Biol 206: 413-429, 1986.

9. Niendorf A, Arps H and Dietel M: Effect of 1,25-dihydroxyvitamin D3 on human cancer cells in vitro. J Steroid Biochem 27: 825-828, 1987.

10. Welsh J: Induction of apoptosis in breast cancer cells in response to vitamin D and antiestrogens. Biochem Cell Biol 72: 537-545, 1994.

11. Colston KW, Mackay AG, James SY, Binderup L, Chander S and Coombes RC: EB1089: a new vitamin D analogue that inhibits the growth of breast cancer cells in vivo and in vitro. Biochem Pharmacol 44: 2273-2280, 1992.

12. Zhou JY, Norman AW, Chen DL, Sun GW, Uskokovic M and Koeffler HP: 1,25-Dihydroxy-16-ene-23-yne-vitamin D3 prolongs survival time of leukemic mice. Proc Natl Acad Sci USA 87: 3929-3932, 1990.

13. Elstner E, Linker-Israeli M, Said J, Umiel T, de Vos S, Shintaku IP, Heber D, Binderup L, Uskokovic M and Koeffler HP: 20-epi-vitamin D3 analogues: a novel class of potent inhibitors of proliferation and inducers of differentiation of human breast cancer cell lines. Cancer Res 55: 2822-2830, 1995.

14. Abe J, Nakano T, Nishii Y, Matsumoto T, Ogata E and Ikeda K: A novel vitamin D3 analog, 22-oxa-1,25-dihydroxyvitamin D3, inhibits the growth of human breast cancer in vitro and in vivo without causing hypercalcemia. Endocrinology 129: 832-837, 1991.

15. Bikle DD: Clinical counterpoint: vitamin D: new actions, new analogs, new therapeutic potential (review). Endocrinol Rev 13: 765-784, 1992.

16. Baserga R: The insulin-like growth factor I receptor: a key to tumor growth? Cancer Res 55: 249-252, 1995.

17. Clemmons DR: Insulin-like growth factor binding proteins and their role in controlling IGF actions. Cytokine Growth Factor Rev 8: 45-62, 1997.

18. Oh Y, Nagalla S, Yamanaka Y, Kim HS, Wilson E and Rosenfeld RG: Synthesis and characterization of insulin-like growth factor binding protein (IGFBP)-7. J Biol Chem 271: 30322-30325, 1996.

19. Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachendran J and Fujita-Yamaguchi Y: Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 5: 2503-2512, 1986.

20. Lee AV and Yee D: Insulin-like growth factors and breast cancer. Biomed Pharmacother 49: 415-421, 1995.

21. LeRoith D, Werner H, Beitner-Johnson D and Roberts C: Molecular and cellular aspects of the insulin-like growth factor I receptor. Endocrinol Rev 16: 143-163, 1995.

22. White M and Kahn C: The insulin signalling system. J Biol Chem 269: 1-4, 1994.

23. 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.

24. Argetsinger L, Hsu G, Meyers M, Billestrup N, White M and Carter-Su C: Growth hormone, interferon-gamma, and leukemia inhibitory factor promoted tyrosine phosphorylation of insulin receptor substrate-1. J Biol Chem 270: 14685-14692, 1995.

25. Ihle J: Cytokine receptor signalling. Nature 377: 591-594, 1995.

26. Vuori K and Ruoslahti E: Association of insulin receptor substrate-1 with integrins. Science 266: 1576-1578, 1994.
27. Kowalski-Chauvel A, Pradayrol L, Vaysse N and Seva C: Gastrin stimulates tyrosine phosphorylation of insulin receptor substrate-1 and its association with Grb2 and the phosphatidylinositol 3-kinase. J Biol Chem 271: 26356-26361, 1996.
28. Rozen F, Yang X, Huynh HT and Pollak M: Antiproliferative action of vitamin-D-related compounds and insulin-like growth factor binding protein 5 accumulation. J Natl Cancer Inst 89: 652-656, 1997.
29. Huynh HT, Yang XF and Pollak M: A role for insulin-like growth factor binding protein 5 in the antiproliferative action of the antiestrogen ICI 182780. Cell Growth Differ 7: 1501-1506, 1996.
30. 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.
31. Francis GL, Ross M, Ballard FJ, Milner SJ, Senn C, McNeil KA, Wallace JC, King R and Wells JR: Novel recombinant fusion protein analogues of insulin-like growth factor (IGF)-I indicate the relative importance of IGF-binding protein and receptor binding for enhanced biological potency. J Mol Endocrinol 8: 213-223, 1992.
32. McGrath MF, Collier RJ, Clemmons DR, Busby WH, Sweeny CA and Krivi GG: The direct in vitro effect of insulin-like growth factors (IGFs) on normal bovine mammary cell proliferation and production of IGF binding proteins. Endocrinology 129: 671-678, 1991.
33. Russo VC and Werther GA: des(1-3) IGF-I potently enhances differentiated cell growth in olfactory bulb organ culture. Growth Factor 11: 301-311, 1994.
34. Gillespie C, Read LC, Bagley CJ and Ballard EJ: Enhanced potency of truncated insulin-like growth factor-I [des(1-3)IGF-I] relative to IGF-I in lit/lit mice. J Endocrinol 127: 401-405, 1990.
35. Tomas FM, Knowles SE, Owens PC, Chandler CS, Francis GL, Read LC and Ballard FJ: Insulin-like growth factor-I (IGF-I) and especially IGF-I variants are anabolic in dexamethasone-treated rats. Biochem J 282: 91-97, 1992.
36. Kleinman D, Karas M, Danilenko M, Arbeli A, Roberts C, LeRoith D, Levy J and Sharoni Y: Stimulation of endometrial cancer cell growth by tamoxifen is associated with increased insulin-like growth factor (IGF)-I induced tyrosine phosphorylation and reduction in IGF binding proteins. Endocrinology 137: 1089-1095, 1996.
37. Karas M, Danilenko M, Fishman D, LeRoith D, Levy J and Sharon Y: Membrane-associated insulin-like growth factor-binding protein-3 inhibits insulin-like growth factor-I-induced insulin-like growth factor-I receptor signaling in Ishikawa endometrial cancer cells. J Biol Chem 272: 16514-16520, 1997.
38. Buckbinder L, Talbott R, Velasco-Miguel S, Takenaka I, Faha B, Seizinger BR and Kley N: Induction of the growth inhibitor IGF-binding protein 3 by p53. Nature 377: 646-649, 1995.
39. Fontana JA, Burrows-Mezu A, Clemmons DR and LeRoith D: Retinoid modulation of insulin-like growth factor binding proteins and inhibition of breast carcinoma proliferation. Endocrinology 128: 1115-1122, 1991.
40. 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.
41. 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.
42. Rozen F, Zhang J and Pollak M: Antiproliferation action of tumor necrosis factor-alpha on MCF-7 breast cancer cells is associated with increased insulin-like growth factor binding protein-3 accumulation. Int J Oncol 13: 865-869, 1998.
43. Ohlsson C, Kley N, Werner H and LeRoith D: p53 regulates insulin-like growth factor-I (IGF-I) receptor expression and IGF-I-induced tyrosine phosphorylation in an osteosarcoma cell line: interaction between p53 and Sp1. Endocrinology 139: 1101-1107, 1998.
44. Guvakova MA and Surmacz E: Tamoxifen interferes with the insulin-like growth factor-I receptor (IGF-IR) signaling pathway in breast cancer cells. Cancer Res 57: 2606-2610, 1997.
45. Constantino A, Vinci C, Mineo R, Frasca F, Pandini G, Milazzo G, Vigneri R and Belfiore A: Interleukin-1 blocks insulin and insulin-like growth factor-stimulated growth in MCF-7 human breast cancer cells by inhibiting receptor tyrosine kinase activity. Endocrinology 137: 4100-4107, 1996.
46. Heding A, Gill R, Ogawa Y, De MP and Shymko RM: Bio-sensor measurement of the binding of insulin-like growth factors I and II and their analogues to the insulin-like growth factor-binding protein-3. J Biol Chem 271: 13948-13952, 1996.
47. Andress DL: Insulin-like growth factor-binding protein-5 (IGFBP-5) stimulates phosphorylation of the IGFBP-5 receptor. Am J Physiol 274: E744-E750, 1998.
48. Andress DL: Heparin modulates the binding of insulin-like growth factor (IGF) binding protein-5 to a membrane protein in osteoblastic cells. J Biol Chem 270: 28289-28296, 1995.
49. Mohan S, Nakao Y, Honda Y, Landale E, Leser U, Dony C, Lang K and Baylink DJ: 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.
50. Peyrat JP and Bonneterre J: Type I IGF receptor in human breast diseases. Breast Cancer Res Treat 22: 59-67, 1992.