EXPERIMENTAL STUDY

1,25-Dihydroxyvitamin D₃ stimulates the production of insulin-like growth factor-binding proteins-2, -3 and -4 in human bone marrow stromal cells

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Abstract

Background: 1,25-Dihydroxyvitamin D₃ (calcitriol) inhibits proliferation and stimulates differentiation of multiple cell types, including osteoblasts. Human (h) bone marrow stromal cells (MSCs) are a homogenous non-hematopoietic population of cells present in the bone marrow and exhibit a less differentiated osteoblastic phenotype. The IGF system, including IGFs-I, and -II and IGF binding proteins (IGFBPs), plays an important role in osteoblast cell proliferation and differentiation.

Objective: To examine the pattern of expression of the IGF system in hMSCs and its regulation by calcitriol.

Methods and results: hMSCs express mRNA of both IGFs-I, and -II and IGFBPs-1 to -6 as shown by RT-PCR and northern blot analysis. As assessed by western ligand blotting (WLB) and western immunoblot analysis, hMSCs secrete 38–42 kDa IGFBP-3, 24–28 kDa IGFBP-4 and a 33 kDa IGFBP-2. Calcitriol (dose range 10⁻¹⁰–10⁻⁷ mol/l) exerted no consistent dose-dependent effects on either IGF-I or IGF-II mRNA levels. In contrast, calcitriol treatment increased steady-state mRNA levels of IGFBPs-2, -3 and -4, but had no effect on IGFBP-5 or -6. Similarly, calcitriol increased the secretion of IGFBPs-2, -3 and -4 as determined by WLB. We found no detectable basal IGFBP-3 or IGFBP-4 protease activities in the absence or presence of calcitriol treatment.

Conclusions: Our results demonstrate that hMSCs expressed a distinct pattern of IGFs and IGFBPs that may be related to their stage of differentiation. The observed increase in production of IGFBPs-2, -3 and -4 by hMSCs upon treatment with calcitriol may be an important mechanism mediating the effects of calcitriol on MSC proliferation and differentiation.

European Journal of Endocrinology 144 549–557

Introduction

1,25-Dihydroxyvitamin D₃ (calcitriol) has an important role in the maintenance of skeletal integrity and osteoblast functions (1, 2). *In vitro*, calcitriol inhibits osteoblast cell proliferation and stimulates the production of several non-collagenous proteins, including osteocalcin, osteopontin and alkaline phosphatase, thereby promoting the phenotype of mature osteoblasts (3, 4). The anti-proliferative effect of calcitriol and transcriptional regulation of several target genes are mediated through interaction with the vitamin D receptor, known to be present in osteoblasts (5, 6).

Insulin-like growth factors (IGFs-I and -II) are important autocrine and paracrine regulators of proliferation and differentiation of various cell types, including osteoblasts (7, 8). The bioactivity of IGFs is

regulated through their interaction with a group of high-affinity binding proteins (IGF binding proteins, IGFBPs). At least six IGFBPs have been characterized and found to have a variety of biological effects that are both IGFBP and cell-type-specific (9). The activities of IGFBPs are regulated through changes in their rates of degradation by multiple specific and non-specific proteases and protease inhibitors (10, 11).

Several studies using cultured osteoblasts have demonstrated that different components of the IGF system are regulated by several calciotropic hormones, including calcitriol in addition to growth factors and cytokines known to be important for bone metabolism (12–16). It has also been suggested that the IGF system may mediate the effects of these agents on bone cells (13, 15, 17).

Human bone marrow stromal cells (hMSCs) are derived from mesenchymal stem cells in the bone marrow and can differentiate into a variety of cell types including osteoblasts, chondrocytes or adipocytes, under appropriate *in vitro* and *in vivo* conditions (18–22). The molecular signals and mechanisms necessary for their osteoblastic differentiation are not known in detail. Among other factors, calcitriol treatment seems to be an important inducer of hMSCs differentiation, as indicated by increasing production of osteoblast-specific proteins and bone matrix formation *in vitro* (21, 23, 24). In the current study, we characterized the gene expression and protein production of components of the IGF system in hMSCs and examined the regulation of this system by calcitriol in short-term cultures.

Materials and methods

Cell culture

Cultures of hMSCs were established as described previously (19). Briefly, bone marrow was aspirated from the posterior iliac spine of seven healthy volunteers (aged 20–33 years) after they had given informed consent and approval had been received from the Regional Ethics Committee. Low-density mononuclear cells were isolated by density gradient centrifugation in Ficoll-Hypaque (density 1.077 g/ml). Plating was performed in T-75 culture flasks in minimal essential medium containing 10% fetal calf serum and supplemented with glutamine, penicillin and streptomycin (complete medium) (Life Technology, Copenhagen, Denmark). Cells were incubated in a humidified atmosphere of 5% CO₂ at 37 °C. After 5 days, the non-adherent cells were removed and the cultures were fed with fresh complete medium. The medium was changed once a week until the cells became confluent, usually after 4–6 weeks. They were trypsinized and used in subsequent assays. All experiments were performed using cells from the first passage.

[³H]Thymidine incorporation assay

Mitogenic effects of calcitriol were studied by incorporation of [³H]thymidine tracer into cell DNA. hMSCs were plated at 20 × 10³ cells/well in 96-wells culture plates. Twenty-four hours after seeding, the medium was changed to serum-free medium containing 0.1% BSA (SFM) to slow down cell proliferation. After an additional 48 h, the cells were washed and were incubated for another 24 h with SFM in the presence of various concentrations of calcitriol or vehicle together with [³H]thymidine (90 kBq/well) (Amersham, Amersham, Bucks, UK). After the cells had been washed three times with PBS, scintillation liquid was added and the amount of radioactivity incorporated into the cells was measured using a scintillation counter.

RNA preparation

Total RNA for analysis of expression of IGFs and IGF-BPs was isolated using the TRIzol method (25). In short, hMSCs were grown for 2 h in SFM, followed by culturing in fresh SFM containing various concentrations of calcitriol or vehicle for the time indicated. Cells were washed with PBS and lysed in TRIzol solution (Sigma, Copenhagen, Denmark). The organic phase containing the protein was extracted with chloroform, and 2-propanol was added to the remaining aqueous phase for RNA precipitation. The precipitate was collected by centrifugation and the RNA pellet was dissolved in diethylpyrocarbonate-treated H₂O (DEPC H₂O), quantitated by its absorbance at 260 nm and stored at –80 °C.

Reverse transcriptase polymerase-chain reaction

cDNA was synthesized from 4 µg total RNA in a 20-µl reaction mixture containing 1 × reverse transcriptase buffer (5 × = 50 mmol/l MgCl₂, 250 mmol/l KCl, 250 mmol/l Tris-HCl (pH 8.3), 50 mmol/l dithiothreitol, 2.5 mmol/l spermidine), dCTP, dGTP, dATP and dTTP each at 2 mmol/l, 20 U RNase inhibitor, 8–10 U avian myeloblastosis virus (AMV) reverse transcriptase (all from Promega, Madison, WI, USA), 200 pmol random hexamer primer, and 50 pmol poly-dT₁₅ primer (Roche, Frankfurt, Germany). Reaction times were at least 4 h at 42 °C.

Aliquots (5%) of the total cDNA were amplified in each PCR in a 20-µl reaction mixture that contained 2–20 pmol 5' and 3' primer, 1 × PCR buffer (10 × = 500 mmol/l KCl, 100 mmol/l Tris-HCl (pH 9), 1% Triton X-100, dCTP, dGTP, dATP, and dTTP each at 0.2 mmol/l, 1.5 mmol/l MgCl₂, 0.2 µl [α-³²P]dATP (Amersham), 0.4 µl anti-Taq buffer, 0.1 µl anti-Taq (Perkin Elmer, Boston, MA, USA) and 0.5 U Taq polymerase (Promega). Each cDNA sample was run in triplicate for every PCR. Having established the linear range for each primer sets, we performed amplifications for 25, 30 and 35 cycles for GAPDH, IGF-II and IGF-I respectively. The same reaction profile was used for all the primer sets: after an initial denaturation at 94 °C for 2 min, cycles of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min were run and, finally, full-length products were obtained by ending the program with 8 min at 72 °C. PCR reaction products were analysed by agarose gel electrophoresis, visualized by ethidium bromide staining, and quantitated using gel-doc software.

Primer sequences and concentrations were as follows: GAPDH sense (3 pmol/µl): 5' ACCACAGTC-CATGCCATCAC 3'; GAPDH antisense (3 pmol/µl): 5' TTCACCACTGTTGCTGTA 3'; IGF-I sense (20 pmol/µl): 5' ATGCTCTTCAGTTCGTGTGT 3'; IGF-I antisense (20 pmol/µl): 5' AGCTGACTTGGCAGGCTTGT 3'; IGF-II

sense (2 pmol/ μ l): 5' CTGTGCTACCCCGCCAAAGT 3'; IGF-II antisense (2 pmol/ μ l): 5' ACGTTTGGCCTCCCTGAACG 3'.

Northern blot analysis

Fifteen micrograms of total RNA for each sample was heated in 1/6 vol loading buffer (0.25% bromophenol blue, 0.25% xylene cyanol, 30% glycerol, 1.2% SDS, 60 mmol/l NaAc, pH 6.8) for 10 min at 65 °C, electrophoresed on a 1% agarose formaldehyde gel, transferred to nylon membranes (Hybond N, Amersham) by capillary action using 10 $\times$ SSC (1.5 mol/l NaCl, 0.3 mol/l sodium citrate, pH 7) and subsequently baked at 80 °C for 2 h. Prehybridization and hybridization were performed in 15 ml hybridization buffer (0.5 mol/l Na₂HPO₄ pH 7.2, 7% SDS, 1.25 mmol/l EDTA pH 8) at 65 °C for 1 h and 12–16 h respectively. Labelling of cDNA probes was performed by random labelling using Klenow (New England Biolab, Copenhagen, Denmark) and [α -³²P]dATP (Amersham) and subsequent purification on Wizard mini-columns (Promega), as described by the manufacturer. Each hybridization contained approximately 5 $\times$ 10⁷ c.p.m./150 ng labelled cDNA. After hybridization, the membranes were washed for 1 h at 65 °C in wash solution (40 mmol/l Na₂HPO₄ pH 7.2, 1% SDS, 2 mmol/l EDTA pH 8) and then for 30 min at 65 °C in solution 2 (40 mmol/l Na₂HPO₄ pH 7.2, 0.1% SDS, 2 mmol/l EDTA pH 8). Finally, membranes were exposed to X-ray film (Kodak, Copenhagen, Denmark) at –80 °C or to a phosphor-imaging screen and the size of transcripts were estimated from relative migration rates of 18S and 28S rRNA. The bands of IGFBPs were quantitated using ImageQuant-software (Molecular Dynamics Inc., Santa Cruz, CA, USA) and their expression levels were corrected for GAPDH (a housekeeping gene).

Preparation of conditioned media

Conditioned media were collected from cells grown in 24-well plates, rinsed once with PBS and allowed to incubate 2 h in SFM before culturing with fresh SFM containing various concentrations of calcitriol or vehicle at the time indicated. Media were centrifuged at 4000 r.p.m. at 4 °C for 10 min, aliquoted, and stored at –80 °C and cells were trypsinized and counted with a Coulter counter.

Western ligand blot analysis

Seventy microlitres of the conditioned media samples were separated by SDS–PAGE and the separated proteins were electroblotted onto nitrocellulose filters. The filters were blocked with buffer (150 mmol/l NaCl, 10 mmol/l Tris–HCl, pH 7.4) plus 3% Nonidet P40 for 30 min, with blocking buffer plus 1% BSA for 120 min and with blocking buffer plus 0.1% Tween-20 at 4 °C.

Filters were incubated in buffer containing 1% BSA, 0.1% Tween-20 and 6000–7000 c.p.m./100 μ l ¹²⁵I-labeled IGF-I overnight at 4 °C. The filters were washed twice in buffer and 0.1% Tween-20 for 20 min and three times in buffer, and dried before being visualized by autoradiography. Band intensities were normalized to the number of cells.

Immunoblot blot analysis

Fifty microlitres of the conditioned media samples were separated by SDS–PAGE and the separated proteins were electroblotted onto nitrocellulose filters. The filters were blocked with buffer (150 mmol/l NaCl, 10 mmol/l Tris–HCl pH 7.4, 0.2% Tween-20) plus 5% BSA for 1 h at room temperature. Filters were washed three times for 10 min each time in buffer and incubated with a rabbit antibody against IGFBP-2 (Upstate Biotechnology, Boston, MA, USA) diluted 1:500 in buffer overnight at 4 °C. The filters were washed 3 $\times$ 10 min in buffer and incubated for 1 h at room temperature with horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (Dako, Copenhagen, Denmark) diluted 1:650 000 in buffer. Finally, the filters were washed 3 $\times$ 10 min and 3 $\times$ 20 min in buffer, before being visualized by SuperSignal Ultra (Pierce, Rockford, IL, USA).

Cell-free assay for IGFBP-3 protease activity

The IGFBP-3 degradation assay was performed as previously described (26), using a preparation of recombinant human (rh) ¹²⁵I-IGFBP-3 without enzyme-inhibiting additive (Diagnostic Systems Laboratories, Webster, TX, USA). ¹²⁵I-rhIGFBP-3 (approximately 30 000 c.p.m.) was incubated for 18 h at 37 °C with 50 μ l conditioned media and subjected to SDS–PAGE, as described above. Electrophoresed gels were fixed in a 7% acetic acid solution, dried and autoradiographed. The amount of proteolysis was calculated as a ratio of the intensity of fragmented ¹²⁵I-IGFBP-3 divided by the sum of all ¹²⁵I-IGFBP-3 (38–42, 30, and 16–18 kDa) in each lane.

Cell-free assays for IGFBP-4 protease activity

IGFBP-4 protease activity was monitored as described previously (27). Seventy microlitres conditioned media were incubated in a microfuge tube with 10 nmol/l IGF-I or an equivalent volume of SFM at 37 °C overnight. Proteolysis of IGFBP-4 was measured as the loss of 24 kDa IGFBP-4, by western ligand blotting.

Statistical analysis

For RT-PCR experiments, the concentrations of IGFs were corrected for variations between different samples by using a housekeeping gene (GAPDH) and presented

as a ratio of target gene/GAPDH (mean $\pm$ S.E.M.; $n = 3$). Differences between calcitriol- and vehicle-treated values were assessed by unpaired Student's *t*-test.

Results

In order to confirm that hMSCs used in these experiments are responsive to calcitriol, we examined the effects of calcitriol treatment (dose range 10^{-9} – 10^{-7} mol/l) on cell proliferation. Calcitriol inhibited hMSCs proliferation, in a dose dependent fashion, to $47 \pm 8\%$ of that in vehicle-treated controls at a concentration of 10^{-7} mol/l (data not shown).

Basal steady-state mRNA levels and production of components of the IGF system in hMSCs

Using RT-PCR, we found that hMSCs expressed both IGF-I and IGF-II. These results were confirmed using an RNase-protection assay with specific probes for IGFs-I and -II (data not shown). Although the absolute concentrations of IGFs-I and -II can not be compared using these assays, we have previously reported that IGF-II concentrations in osteoblast media were much greater than those of IGF-I (15, 28).

Steady-state mRNA levels of IGFBPs-1 to -6 were examined by northern blot analysis in primary cultures and in first-passage hMSCs. As shown in Fig. 1, 1.6 kb IGFBP-1, 1.4 kb IGFBP-2, 2.6 kb IGFBP-3, 2.2 kb IGFBP-4, 6.0 kb IGFBP-5 and 1.3 kb IGFBP-6 transcripts were all detectable by northern blot analysis. In WLB, hMSCs produced 38–42 kDa IGFBP-3, and 24–28 kDa IGFBP-4 and a weak band of 33 kDa IGFBP (Fig. 2a). The 33 kDa IGFBP was further characterized by immunoblot analysis and it was proved to be IGFBP-2, by recognition with an IGFBP-2 antibody, whereas no immunoreactive bands were observed using an IGFBP-5-specific antibody (Fig. 2b).

Effect of calcitriol on steady-state mRNA levels of IGFs and IGFBPs in hMSCs

In a quantitative non-competitive RT-PCR, calcitriol (dose range 10^{-10} – 10^{-7} mol/l) exerted no statistically significant dose-dependent effects on either IGF-I or IGF-II mRNA levels (Fig. 3). Similarly, time-course experiments (6–72 h) showed no significant effect of calcitriol (10^{-8} mol/l) on IGF-I or IGF-II expression (data not shown).

Calcitriol treatment (dose range 10^{-10} – 10^{-7} mol/l) increased steady-state mRNA expression of IGFBP-2 (2.1-fold), IGFBP-3 (1.8-fold) and IGFBP-4 (1.9-fold) after 48 h treatment (Fig. 4a). Furthermore, time-course experiments showed that the effects of calcitriol (10^{-8} mol/l) on steady-state mRNA levels of IGFBPs-3

Figure 1 Northern blot analysis of basal levels of IGFBPs-1 to -6 mRNAs in hMSCs, using 15 μ g total RNA from primary cultures and first-passage cells. Identification and sizes of hybridized bands are indicated in the figure.

and -4 were still observed after 72 h of continuous treatment (Fig. 4b).

Effect of calcitriol on IGFBP secretion in hMSCs

We also examined dose- (10^{-10} – 10^{-7} mol/l; 48 h) and time-dependent (10^{-8} mol/l; 6–72 h) effects of calcitriol on IGFBP production as revealed by WLB (Fig. 5 and Fig. 6). Similar to results of northern blot analysis, calcitriol increased protein secretion of IGFBP-2 (1.9 ± 0.3 -fold), IGFBP-3 (2.1 ± 0.5 -fold) and IGFBP-4 (2.8 ± 0.6 -fold) in a dose- (Fig. 5) and time-dependent manner (Fig. 6).

Effects of calcitriol on IGFBPs-3 and -4 protease activity

As the concentrations of IGFBPs in cell culture conditioned media are controlled not only by the production rate but also by their degradation rate, we tested for the presence of IGFBP protease activity in hMSCs cultures. We were not able to detect any significant levels of IGFBP-3 protease activity or IGF-dependent IGFBP-4 protease activity in media from hMSCs, whereas serum from a pregnant woman, used as a positive control, demonstrated clear degradation. Treatment with calcitriol did not change these results (results not shown).

Figure 2 (a) Western ligand blot of IGFBPs in conditioned media from hMSCs after 72 h culturing in SFM. Results from two different experiments are shown. [125 I]-IGF-I was used as ligand. The migrations of molecular size markers are indicated. (b) Immunoblot analysis of conditioned media using a rabbit anti-IGFBP-2 antibody. Identification of a 33 kDa protein is indicated.

Figure 3 Relative expression of RT-PCR analysis of dose-response effects of calcitriol (1,25D) on IGFs-I and -II mRNAs in hMSCs. Each cDNA sample was amplified in triplicate and normalized to the amount of GAPDH product detected in the same cDNA sample. The data (mean $\pm$ S.E.M.; $n = 3$) are presented as fold change relative (rel.) to control conditions (C).

Discussion

Previous studies on cultured osteoblasts have reported the presence of a cell-type- and species-specific expression pattern of IGFs and IGFBPs (29, 30). In our studies we have demonstrated that hMSCs, which represent a model for the less differentiated osteoblastic phenotype, express mRNA of both IGFs-I and -II and the six known IGFBPs. Also, hMSCs secrete into their conditioned media mainly IGFBPs-3 and -4 and to a lesser extent IGFBP-2. Using human trabecular osteoblasts representing the mature phenotype, Okazaki *et al.* (14) reported an expression pattern of components of the IGF system that is similar to that shown in our current study. Interestingly, hMSCs produced minute quantities of a 33 kDa protein that was immunologically identified as IGFBP-2, and mature osteoblasts produced a 31 kDa IGFBP-5. We also found no noticeable differences between the expression of IGFs or their binding

Figure 4 Northern blot analysis of the effects of calcitriol (1,25D) on IGFBP steady-state mRNA levels in hMSCs. Autoradiographs (representative of two independent experiments) showing hybridizing bands of the indicated transcripts (a) after 48 h stimulation with various concentrations of calcitriol (10^{-10} – 10^{-7} mol/l) and (b) after stimulation with 10^{-8} mol/l calcitriol for the time indicated.

Figure 5 Western ligand blot of IGFBPs in the conditioned medium from hMSCs after 48 h stimulation with various concentrations of calcitriol (1,25D; 10^{-10} – 10^{-7} mol/l). (a) Autoradiograph showing identification and sizes of hybridized bands. C, Control. (b) Relative amount of IGFBPs examined in (a), presented as (mean $\pm$ S.E.M.; $n = 3$) fold change after stimulation with calcitriol (●) from production under control conditions (■) (relative production = 1). [125 I]-IGF-I was used as ligand.

proteins between primary cultures and first-passage hMSCs, demonstrating the stability of expression of these genes in culture. Recently, an SV-40-immortalized human stromal cell line was shown to constitutively express and secrete IGFBPs-3, -4, -5 and -6 (31). The differences between this cell line and the cells used in our experiments may be due to cellular changes induced by the immortalized phenotype. Thus the present data suggest that human osteoblast differentiation is not associated with major qualitative changes in the expression pattern of the components of the IGF system. This contrasts with previous studies using murine osteoblasts showing complex changes in the pattern of IGFs and IGFBPs with differentiation (32, 33). However, hMSCs used in the current studies were obtained at confluency and from the first-passage cells, and at this stage most of the hMSCs expressed markers of osteoblastic phenotype (19, 21). We can therefore not exclude the possibility that the initial phases of human osteoblast differentiation may show a different pattern of expression of IGFs and IGFBPs compared with mature osteoblasts.

Our results showed that calcitriol did not affect steady-state mRNA levels of IGFs-I or -II. Similar to our findings, calcitriol did not regulate IGF-II gene expression or production in primary rat osteoblast cultures (34). In contrast to our findings is the report of Chenu *et al.* (18) that calcitriol increased IGF-I production in the conditioned media of human trabecular osteoblasts. However, the authors in that study used a commercial assay for measuring IGF-I that does not take into account changes in IGFBPs (28, 35). Calcitriol has also been reported to inhibit both expression and release of IGF-I in mouse bone cells (36, 37). These discrepancies may suggest the presence of species differences in the regulation of the IGF system (17).

Calcitriol treatment increased production of IGFBPs-2, -3 and -4 in hMSCs cells in a dose- and time-dependent fashion and the same results were obtained at the mRNA level. These changes were of modest magnitude (usually less than twofold change), but they were consistent in different experiments and were statistically significant. Our findings agree with those of several other studies that reported a calcitriol-induced increase in IGFBPs-3

Figure 6 Western ligand blot of IGFBPs in the conditioned medium from hMSCs after stimulation with 10^{-8} mol/l calcitriol (1,25D) for the time indicated. (a) Autoradiograph showing identification and sizes of hybridized bands. C, Control. (b) Relative amount of IGFBPs examined in (a), presented as secretion levels (mean $\pm$ S.E.M., $n = 3$) relative (Rel.) to control (0 h treatment). [125 I]-IGF-I was used as ligand.

and -4 mRNA expression and secretion in human bone-derived cells (12, 13, 36, 38). Our finding of a stimulatory effect of calcitriol on IGFBP-2 expression and secretion has not been reported previously.

The molecular mechanisms of calcitriol-mediated stimulation of IGFBPs are not completely understood. Our findings suggest that the changes in IGFBPs-3 and -4 are mediated through changes in gene transcription, as demonstrated by increased mRNA levels of these binding proteins. We examined the proteolytic activity of both IGFBP-3- and IGFBP-4-specific proteases in hMSC conditioned media. No evident degradation of IGFBP-3 or -4 protein was observed, and no protease activity could be induced by calcitriol treatment. Our results thus corroborate previous studies demonstrating the transcriptional regulation of calcitriol on IGFBPs-3 and -4 and the absence of effects on the protease system (13, 14). We found that the increase in mRNA and protein levels of IGFBPs required continuous exposure to calcitriol for more than 48 h, suggesting that these changes are secondary to effects on immediate responsive genes. As no consensus vitamin D response element has been found in the promoter regions of the IGFBPs, it is plausible that calcitriol effects are mediated through other signalling pathways such as cAMP or AP-1 transcription factors, which have been demonstrated to mediate some of the genomic effects on components of the IGF system (39, 40).

On the basis of known biological effects of IGFBPs, we suggest that the observed changes in IGFBPs may lead to inhibition of proliferation of hMSCs and induction of their differentiation. IGFBPs are known to exert complex effects on the biological effects of IGFs. Whereas IGFBP-3 has been shown to either potentiate (41, 42) or inhibit (43) IGF-mediated effects on osteoblasts, IGFBP-4 has consistently antagonized the IGF stimulatory effects (44, 45). IGFBP-2 has been shown to potentiate (46) or antagonize the IGF-mediated effects (47, 48) and its role in osteoblast biology is not known. IGFBPs can have direct effects on osteoblast differentiation (49, 50), or exert their effects through changes in local availability of IGFs in the bone microenvironment. It is possible that the physiological effects of calcitriol on bone can be mediated by differential regulation of IGFBPs and the local availability of IGFs in bone microenvironment, however, the fine details of this complex interaction remains to be determined.

Acknowledgements

This work was supported by grants from the Danish Centre for Molecular Gerontology, Danish Medical Research Council, the Novo Nordisk Foundation, Aage & Johanne Louis-Hansens Memorial Foundation, Director E. Danielsen & Hustrus Foundation, the Nordic Insulin Foundation, the Eva and Henry Fränkels

Memorial Foundation, and the Aarhus University-Novo Nordisk Center for Research in Growth and Regeneration. The authors thank Liselotte Steenkær, Lotte Sørensen and Karen Mathiasen for excellent technical assistance, Dr S Shimasaki (USA) for providing the cDNA probes for IGFBPs, and Dr Ryo Okazaki (Japan) for providing IGF probes for RNase protection assay. Drs Suresh I S Rattan and Brian F C Clark are thanked for helpful discussions.

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Received 5 October 2000

Accepted 18 January 2001