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*Research Communication*

# Parathyroid Hormone (PTH) Peptides Through the Regulation of Hyaluronan Metabolism Affect Osteosarcoma Cell Migration

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***Summary***

**Parathyroid hormone (PTH) strongly stimulates hyaluronan (HA) synthesis and secretion of both normal and carcinogenic cells of the osteoblastic lineage and improves skeletal microarchitecture. HA, a glycosaminoglycan component of the extracellular matrix (ECM), is capable of transmitting ECM-derived signals to regulate cellular function. In this study, we investigated whether the changes of HA metabolism induced by PTH (1–34) and PTH (7–84) peptides in moderately MG-63 and well-differentiated Saos 2 osteosarcoma cell lines, are correlated to their migration capabilities. Our results demonstrate that intermittent PTH (1–34) treatment significantly ($P \leq 0.01$) supported the migration of MG-63 cells, increased their HA-synthase-2 (HAS2) expression ($P \leq 0.001$), and enhanced their high-molecular size HA deposition in the pericellular matrix. Both increased endogenous HA production ($P \leq 0.01$) and treatment with exogenous high-molecular weight HA ($P \leq 0.05$) correlated to a significant increase of MG-63 cell migration capacity. Transfection with siHAS2 showed that PTH (1–34), mainly through HAS2, enhanced HA and regulated MG-63 cell motility. Interestingly, continuous PTH (1–34) treatment stimulated both Saos 2 cell HAS2 ($P \leq 0.001$) and HAS1 ($P \leq 0.001$) isoform expression inhibited their HYAL2 expression ($P \leq 0.001$) and modestly ($P \leq 0.05$) enhanced their migration. Therefore, the PTH (1–34) administration mode appears to distinctly modulate the migratory responses of the MG-63 moderately and Saos 2 well-differentiated osteosarcoma cell lines. Conclusively, the obtained data suggest that there is a regulatory effect of PTH (1–34), in an administration mode-dependent manner, on HA metabolism that is essential for osteosarcoma cell migration.** 
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**Keywords** hyaluronan (HA); parathyroid hormone (PTH); PTH peptides; osteosarcoma; cell migration; wound healing.

## INTRODUCTION

Parathyroid hormone (PTH) is an 84-amino acid polypeptide hormone produced by the parathyroid gland that regulates calcium homeostasis and bone remodeling. The anabolic effect of intermittent administration yielding a transient peak blood level and the catabolic effect of continuous administration of PTH on bone formation have been demonstrated in human and animal models (*1–3*). The effects of PTH in osteoblastic cells are mostly perpetrated through the activation of the specific G-protein–coupled receptor, PTH1R either through the formation of cyclic 30,50-adenosine monophosphate or alternatively through the activation of the mitogen-activated protein kinase (*4, 5*).

The aminoterminal peptide 1–34 (PTH 1–34) is a PTH analogue, which acts in an identical way as the full length protein (*4*). Large N-terminally truncated PTH fragments such as PTH (7–84) are also normally present in peripheral blood (*6*). However, PTH (7–84) is suggested to inhibit PTH signaling and to initiate adverse biological effects (*6, 7*).

Hyaluronan (HA) is a heteropolysaccharide with a molecular mass between $10^5$ and $10^7$ Da, localized primarily in the extracellular matrix (ECM) of cells (*8*). HAS1 and HA-synthase-2 (HAS2) HA synthase isoforms synthesize high-molecular weight HA, whereas HAS3 synthesizes shorter forms of HA (*9*). In somatic tissues HYAL1 and HYAL2 are the major hyaluronidases responsible for the degradation of HA (*10, 11*). Importantly, HA plays a role in a number of biological activities ranging from tissue hydration to cell migration in a manner dependent on its molecular mass (*12*). In addition, changes in HA metabolism have been proven to be of high importance in cancer cell

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**Table 1**
Sequence of primers for the genes of interest

| Primer name | Sequence |
|---|---|
| HYAL1_F | 5′ CCG GTG CTG CCC TAT GTC 3′ |
| HYAL1_R | 5′ AGG CTG TGC TCC AGC TCA TC 3′ |
| HYAL2_F | 5′ GGC GCA GCT GGT GTC ATC 3′ |
| HYAL2_R | 5′ CCG TGT CAG GTA ATC TTT GAG GTA CT3′ |
| CD44_F | 5′ GGT CCT ATA AGG ACA CCC CAA AT 3′ |
| CD44_R | 5′ AAT CAA AGC CAA GGC CAA GA 3′ |
| HAS2_F | 5′ GTG TTA TAC ATG TCG AGT TTA CTT CC 3′ |
| HAS2_R | 5′ GTC ATA TTG TTG TCC CTT CTT CCG C 3′ |
| HAS1_F | 5′ GGT GGG GAC GTG GGA TC 3′ |
| HAS1_R | 5′ ATG CAG GAT ACA CAG TGG AAG TAG 3′ |
| HAS3_F | 5′ GGT ACC ATC AGA AGT TCC TAG GCA GC 3′ |
| HAS3_R | 5′ GAG GAG AAT GTT CCA GAT GCG 3′ |
| PTH1R_F | 5′ CCT GTC CGG ACT ACA TTT ATG 3′ |
| PTH1R_R | 5′ GCC CAC GGT GTA AAT CAT GC 3′ |
| GAPDH_F | 5′ GGA AGG TGA AGG TCG GAG TCA 3′ |
| GAPDH_R | 5′ GTC ATT GAT GGC AAC AAT ATC CAC T 3′ |

function (*13*). Factors affecting HA metabolism, consequently, regulate the migration capacity of carcinoma cell lines (*14*). PTH has been reported to strongly stimulate the HA production of both normal (*15, 16*) and carcinogenic cells of the osteoblastic lineage (*17*). It has been previously demonstrated that the antisense inhibition of HAS2, *via* reduction of HA accumulation and cell-associated matrix formation (*18*), inhibits MG-63 osteosarcoma cell proliferation, motility, and invasiveness. In view of the fact that PTH-related pathways have been previously suggested to affect osteoblastic cell migration (*19*), we hypothesized that in osteosarcoma cell lines PTH peptides could modulate the expression of HA metabolism-related genes and, consequently, the migration capacity of these cells.

Our results demonstrate for the first time that the PTH peptides in an administration mode–dependent manner specifically regulate HAS isoform and hyaluronidase gene expressions to stimulate osteosarcoma cell HA production. Increased HA synthesis was correlated to the enhancement of osteosarcoma cell ability to migrate as demonstrated by short interfering RNA (siRNA) transfection experiments.

## EXPERIMENTAL PROCEDURES

### *Materials*

Recombinant human PTH (1–34) peptide and hyaluronidase (*Streptomyces hyalurolyticus*) were obtained from Sigma, whereas recombinant human PTH (7–84) peptide was obtained from Bachem (UK). The commercial name of the high-molecular weight HA preparation used is Healon (10 mg/mL, also containing sodium chloride 8.5 mg, disodium hydrogen phosphate dihydrate 0.28 mg, sodium dihydrogen phosphate hydrate 0.04 mg, and water for injection USP; Pharmacia AB Sweden). HA binding protein (HABP) was purchased from Seikagaku (Japan). The respective, secondary and primary antibodies (polyclonal goat anti-actin, sc-1616; polyclonal goat anti-HAS2, sc-34068) were purchased from SantaCruz. Cell culture reagents were obtained from GIBCO-Invitrogen.

### *Cell Culture*

In this study, Saos 2 and MG-63 human osteosarcoma cell lines of low and high metastatic capacities were utilized. Saos 2 are well-differentiated osteoblast-like cells, whereas MG-63 cells are moderately differentiated fibroblastoid-type cells (*20, 21*). Saos 2 and MG-63 cells were grown in DMEM (Biochrom KG) supplemented with 10% fetal bovine serum. Before stimulation with PTH peptides, the cells were cultured in serum free medium for 24 h at 37°C and 5% $CO_2$. The treatments with PTH (1–34) and PTH (7–84) peptides were performed in serum free medium either intermittently (during the first 6 h in each 24-h period) for two cycles or continuously for 48 h.

### *RNA Isolation and Real-Time PCR*

The TRIzol method (GibcoBRL) and the DyNAmo cDNA synthesis Kit (Finnzymes, Finland) were utilized for mRNA extraction and cDNA synthesis, respectively. Primers were designed to be mRNA specific (Table 1). QuantiTech SYBR Green master mix (Qiagen) was used for the real-time PCR reaction (20 $\mu$L reaction volume) and performed by an M×300P cycler. The quantity of each target was normalized against the quantity of GAPDH.

### Western Blot

Cells were harvested using RIPA solution. The samples were electrophoresed on 8% polyacrylamide Tris/Glycine gels and transferred to nitrocellulose membranes [10 mM CAPS (3-(Cyclohexylamino)-1-propanesulfonic acid; linear formula: $C_6H_{11}NH(CH_2)_3SO_3H$; C2632 by Sigma), pH 11, containing 10% methanol]. Membranes were blocked and incubated for 1 h at room temperature (RT) with primary antibody (1:200). The immune complexes were detected after incubation with the appropriate peroxidase-conjugated secondary antibody (1:2,000) with the SuperSignalWest Pico Chemiluminescent substrate (Pierce).

### Wound Healing Assay

The utilized "wound healing assay," an established method of assessing cancer cell motility was performed as previously described (*14, 22*). Briefly, MG-63 and Saos 2 cells were seeded in 24-well culture plates at a concentration of 60,000 and 80,000 cells per well, respectively. After serum starvation for 24 h, treatments were added (NPTH, 50 nM; hyaluronidase, 7 units/mL; HA, 50 $\mu$g/mL) either intermittently (during the first 6 h in each 24-h period, where the medium was changed to 0% DMEM) for two cycles or continuously for 48 h when the cell layer was wounded. Detached cells were removed by washing with serum free media. The wound closure was monitored at 0 and 24 h. The experiment was also performed with cells transfected with siRNA. After transfection for 48 h, the "wound healing assay" was performed as described above. The wound closure was monitored using a digital image processor connected to a microscope at six different positions across the wound. Cell motility was quantified by ImageJ 1.4.3.67 Launcher Symmetry Software.

### Transfection with siRNA

siRNA specific for HAS-2 (variant 97, 98, and 99) and Stealth™ RNAi negative control (siScr) were purchased from Invitrogen. Optimization of the transfection protocol conditions was performed. Finally, siRNA-99 (100 nM; Invitrogen) and Lipofectamine™2000 (1 $\mu$L; Invitrogen) were first diluted separately in 50 $\mu$L Opti-MEM© I Reduced Serum Medium (Invitrogen). Lipofectamine™2000 was mixed with 50 $\mu$L diluted siRNA for 20 min (RT) to allow siRNA-liposome complexes to form. Cells were counted, added on top of the lipofectamine and siRNA mix, and shaken gently. The transfection took place in a 6-h period when the medium was replaced with fresh containing antibiotics and the incubation period continued for 48 h. At these time point the cells were harvested and RNA was extracted or treatments were added. All transfection experiments were repeated at least three times and performed in triplicates.

### Immunofluorescence

Cells were plated on coverslips, placed in 24-well plates, and after a 24-h serum starvation, treatments were added. The cells were fixed with a 5% formaldehyde and 0.02 g/mL sucrose for 10 min at RT. Biotinylated HABP (1:10 dilution in PBS 1% bovine serum) was added for 1 h at RT. Coverslips not incubated with HABP were utilized as negative controls. Finally, the coverslips were washed and incubated for 1 h, in the dark at RT, with FITC-Streptavidin (Zymed). Hoescht dye was used for the nuclear staining. Coverslips were then mounted onto slides using ProLong gold antifade reagent (Molecular Probes) and visualized using a LEICA DM2500 microscope fitted with a DFC490 digital camera.

### Statistical Analysis

The statistical significance was evaluated by student's $t$ test using GraphPad Prism (version 4.0) software.

## RESULTS

### Effects of PTH Peptides on Osteosarcoma Cell Migration

MG-63 and Saos 2, human osteosarcoma cell lines of different differentiation status that express PTRH receptors (data not shown), were intermittently treated with PTH (1–34) and PTH 7–84 peptides that are normally found in peripheral blood (during the first 6 h in each 24-h period) for two cycles or continuously for 48 h, and their cell motility was determined utilizing a "wound healing" assay. Intermittent PTH (1–34) treatment of MG-63 cells significantly ($P < 0.01$) supported the migration of these cells (Figs. 1A and 1C). In contrast, Saos 2 cells under the same treatment conditions did not exhibit changes in their ability to migrate (Figs. 1B and 1D) whereas continuous treatment with PTH (1–34) during 48 h (Figs. 1A and 1C), moderately upregulated Saos 2 cells' migration ($P = 0.05$; Figs. 1B and 1D). PTH (7–84) did not affect osteosarcoma cell motility under any treatments that are utilized. Following the observed treatment data, further studies have been performed utilizing intermittent treatment for MG-63 cells and continuous treatment for Saos 2 cell lines.

### Effects of PTH Peptides on HAS Isoform Expression

Both cell lines with different basal HA production (*23*) were treated with the PTH (1–34) and PTH (7–84) peptides intermittently (during the first 6 h in each 24-h period) for two cycles or continuously for 48 h, and the expression of HAS1, 2, and 3 genes was evaluated at the beginning and at the end of each treatment type. Real-time PCR demonstrated that MG-63 cells express HAS2 and HAS3 products (Figs. 2A and 2B), whereas HAS2 and HAS1 transcripts were detected in various amounts in Saos 2 cells (Figs. 2C and 2D). Intermittent PTH (1–34) treatment of MG-63 cells resulted in a strong stimulation ($P \leq 0.001$) of their HAS2 expression (Fig. 2A) whereas, the same treatment with PTH (7–84) modestly inhibited HAS isoform expression ($P \leq 0.05$) (Fig. 2A). Interestingly, continuous treatment of MG-63 cells with both utilized peptides did not affect HAS isoform expressions. On the other hand, continuous treatment of Saos 2 cells with PTH (1–34) resulted in a significant stimulation of both their HAS1 ($P \leq 0.001$) and HAS2 ($P \leq 0.001$) expression (Figs. 2C and 2D). As HAS1 and HAS2 are known to produce

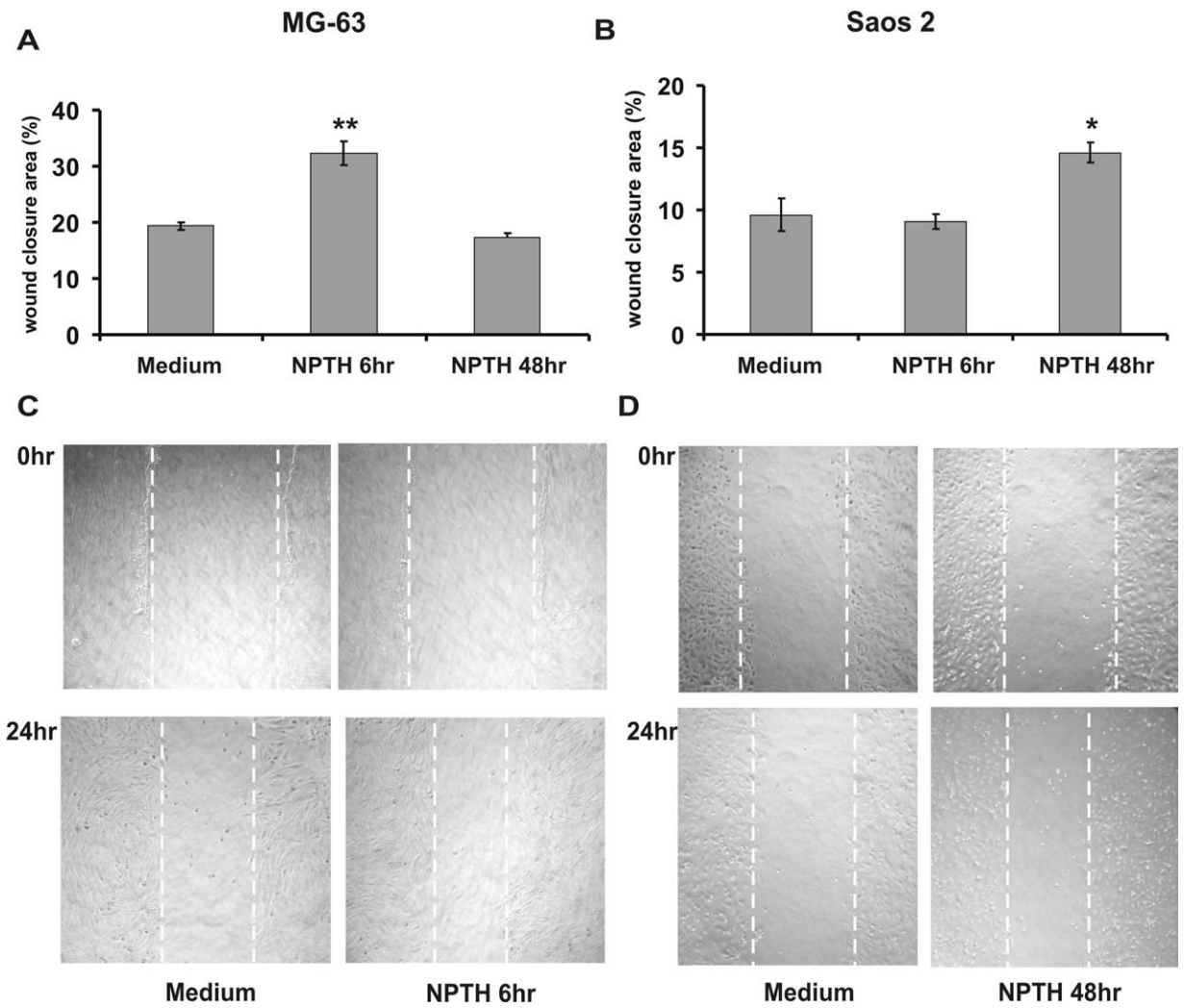


**Figure 1.** Effects of PTH peptides on osteosarcoma cell migration. MG-63 (A) and Saos 2 (B) human osteosarcoma cell lines were treated with PTH (1–34) (NPTH) (50 nM) intermittently (during the first 6 h in each 24-h period) for two cycles or continuously for 48 h at which point they were confluent. Cell layer was "scratched" with a 10 $\mu$L sterile pipette tip and the "wound" surface area was measured at the 0 and 24 h points. Cells grown in 0% serum free culture medium were utilized as control. The results were expressed as % of closure of wound surface area at 0-h time point (at 0-h time point, the wound surface area was 100%). (Means $\pm$ SEM plotted; $n = 3$). *Depicts statistical significance $P \leq 0.05$ compared with 0% control cells (unpaired $t$ test).

high-molecular size HA, our results collectively suggest that PTH (1–34) increases the high-molecular size HA content of osteosarcoma cells in a manner dependent on the treatment type.

### *Effects of PTH Peptides on HYAL Isoforms and CD44 Expression*

Real-time PCR demonstrated that HYAL2, but not HYAL1, isoform was expressed by both MG-63 and Saos 2 cells. After intermittent treatment, no effect of either PTH peptide was observed on HYAL2 expression (Figs. 3A and 3B). In contrast, both MG-63 and Saos 2 cells exhibited a statistically significant reduction in their HYAL2 mRNA expression ($P \leq 0.01$ and $P \leq 0.001$) after continuous treatment with PTH (7–84; Figs. 3A and 3B). On the other hand, a significant upregulation of HYAL2 expression ($P < 0.001$) was observed in Saos 2 cells as it was continuously treated with PTH (1–34; Fig. 3B). The continuous treatment with both PTH (1–34) and PTH (7–84) peptides did not affect CD44 expression in MG63 and Saos 2 cells (Fig. 3D), whereas a moderate inhibition in the levels of the MG63 cells' CD44 transcripts ($P < 0.05$) after intermittent PTH (7–84) was demonstrated.

### *Visualization of HA Matrix of Osteosarcoma Cells*

The ECM surrounding MG-63 has a marked higher staining intensity on intermittent addition of PTH (1–34), whereas only a

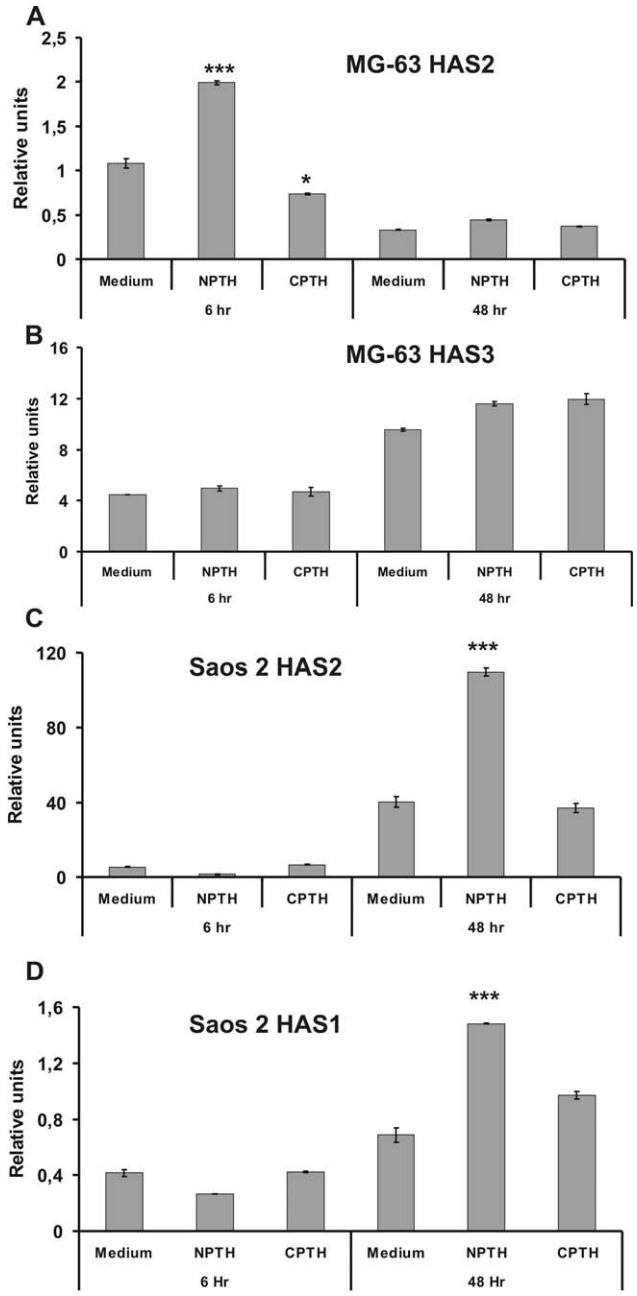


**Figure 2.** Effects of PTH peptides on HAS isoform expression. MG-63 and Saos 2 human osteosarcoma cells were treated with PTH (1–34) (NPTH) (10 nM) or PTH (7–34) (CPTH) (10 nM) intermittently (during the first 6 h in each 24-h period) for two cycles or continuously for 48 h, and their HAS isoform expression was determined by utilizing the real-time PCR. (A) MG-63 HAS2 isoform expression; (B) MG-63 HAS3 isoform expression; (C) Saos 2 HAS2 isoform expression; and (D) Saos 2 HAS1 isoform expression. The results represent the average of three separate experiments in triplicate. Means ± SEM plotted; statistical significance: $*P \leq 0.05$; $***P \leq 0.001$ compared to control.

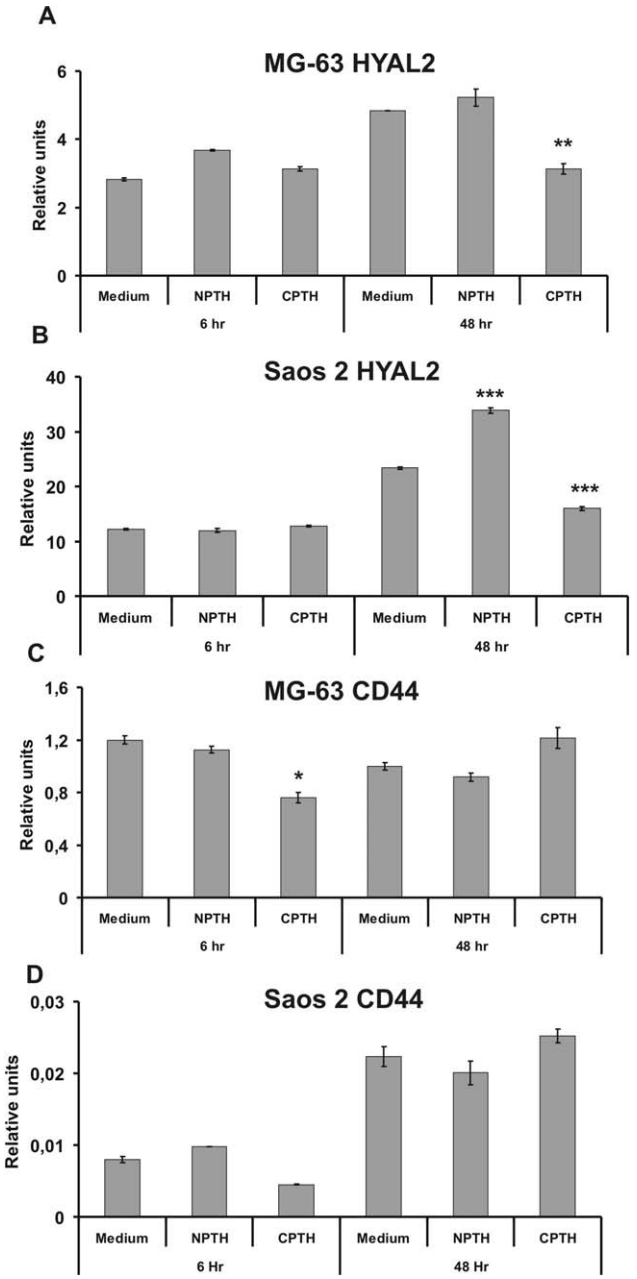


**Figure 3.** Effects of PTH peptides on HYAL isoforms and CD44 expression. MG-63 and Saos 2 human osteosarcoma cell lines were treated with PTH (1–34) (NPTH) (10 nM) or PTH (7–34) (CPTH) (10 nM) intermittently (during the first 6 h in each 24-h period) for two cycles or continuously for 48 h, and their HYAL 2 and CD44 expression were determined by utilizing the real-time PCR. (A) MG-63 HYAL2 isoform expression; (B) Saos 2 HYAL2 isoform expression; (C) MG-63 CD44 expression; and (D) Saos 2 CD44 expression. The results represent the average of three separate experiments in triplicate. Means ± SEM plotted; statistical significance: $*P \leq 0.05$; $**P \leq 0.01$; $***P \leq 0.001$ as compared to control.

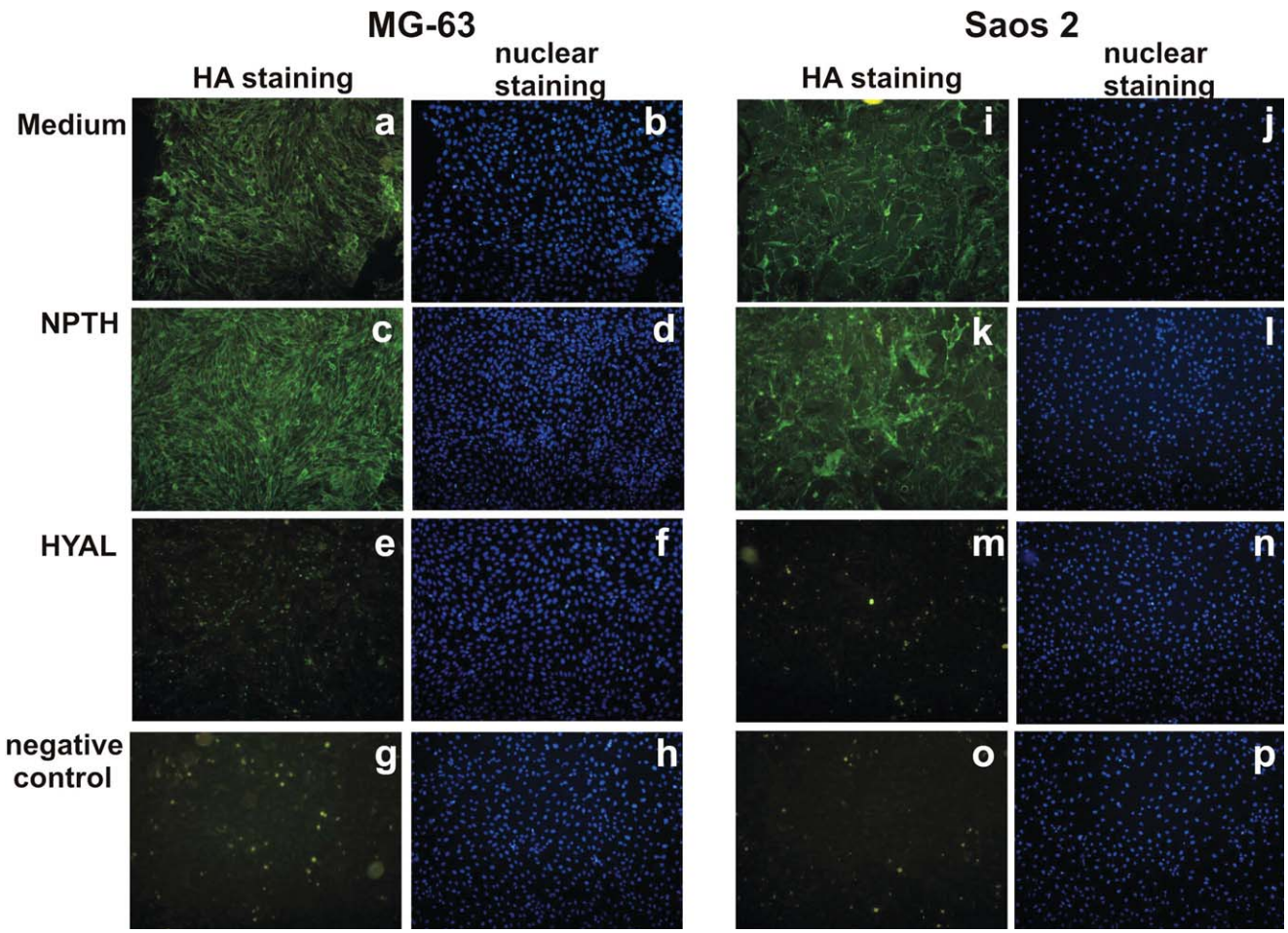


**Figure 4.** Visualization of HA matrix of osteosarcoma cells. MG-63 (a–h) and Saos 2 (i–p) cells were stained for HA using biotinylated HABP (hyaluronan binding protein) as well as the respective nuclear staining (using Hoechst), following a 48 h incubation period with (MG-63 a and b; Saos 2 i and j) 0% serum free culture medium, (MG-63 c and d; Saos 2 k and l) NPTH (50 nM) intermittently (during the first 6 h in each 24-h period) for two cycles for MG-63 cells or continuously for 48 h for Saos 2 cells, (MG-63 e and f; Saos 2 m and n) hyaluronidase (*Streptomyces hyalurolyticus*) (7 units/mL)—used as a negative control—and (MG-63 g and h; Saos 2 o and p) the second negative control where biotinylated HABP incubation was omitted. Pictures were taken using ×20 magnification.

minor increase in Saos 2 cell staining intensity on continuous addition of PTH (1–34), was discerned (Figs. 4a, c, i, and k). These findings correlate well with the data showing distinct modulation of HAS1 and 2 and HYAL2 isoform expression by PTH (1–34) in these cell lines (Fig. 2). In control experiments, treatment with exogenous hyaluronidase decreases the staining of the HA matrix, which was especially evident in the pericellular matrix of these cells (Figs. 4E and 4M). Our results demonstrate that PTH (1–34) specifically in a treatment-type–dependent manner modulates the HA content of the osteosarcoma cell pericellular matrix.

### *Effect of Exogenous HA on Osteosarcoma Cell Migration*

As HAS1 and HAS2 isoforms synthesize high-molecular size HA, we examined the effect of exogenous high-molecular weight HA (Healon; MW 3–4 $\times$ $10^6$ Da) and hyaluronidase treatment on osteosarcoma cell motility utilizing a "wound healing" assay. Exogenous HA significantly stimulated the migration of MG-63 cells ($P < 0.0354$) and moderately enchanced Saos 2 cell migration ($P < 0.05$; Figs. 5A and 5B). On the other hand, treatment with hyaluronidase significantly lowered the capability of MG-63 cells to migrate ($P < 0.001$; Fig. 5C).

### *Effect of HAS2 Inhibition by siRNA on Osteosarcoma Cell Migration*

To test our hypothesis, we performed downregulation of HAS2 expression utilizing the RNA interference (RNAi) method. Transfection of MG-63 and Saos 2 cells with siHAS2

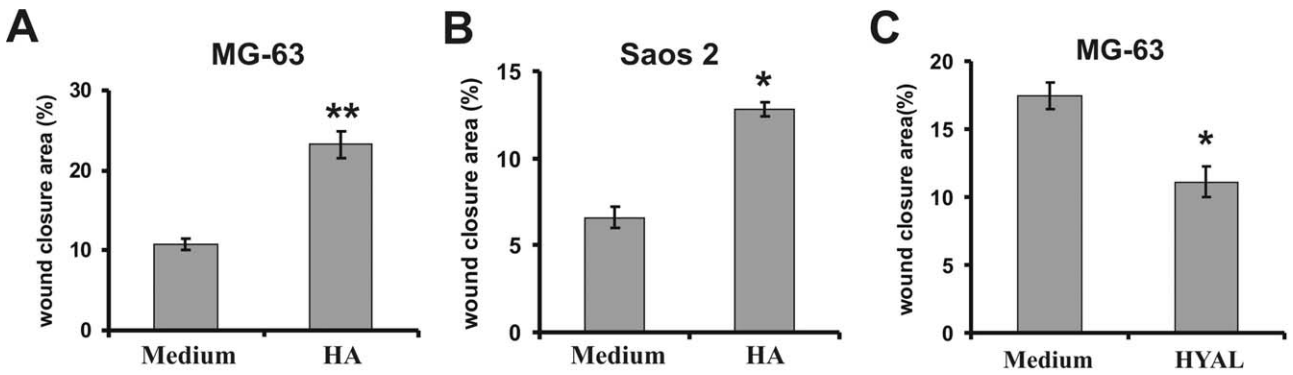


**Figure 5.** MG-63 cells were treated with (A) HA (50 $\mu$g/mL, Healon), and (C) hyaluronidase (*Streptomyces hyalurolyticus*; 7 units/mL), whereas Saos 2 cells were treated with (B) HA (50 $\mu$g/mL, Healon) up to the confluence (48 h). Cell layer was "scratched" with a 10 $\mu$L sterile pipette tip and the "wound" surface area was measured at 0 and 24 h points. Cells grown in 0% serum free culture medium were utilized as control. The results were expressed as % of closure of wound surface area at 0-h time point (at 0-h time point, the wound surface area was 100%). (Means $\pm$ SEM plotted; $n = 3$). *Depicts statistical significance: $P < 0.05$, **$P \leq 0.01$ compared to 0% control cells (unpaired $t$ test).

resulted in a significant decrease of their HAS2 mRNA expression (61 and 88%) and protein expression (66 and 74%), respectively (Figs. 6A and 6B).

Thus, MG-63 HAS2-defficient cells (siHAS2) had significantly decreased the ability to migrate ($P < 0.001$) as compared with both the control cells treated with scrambled siRNA (siScr) and untransfected cells (M) ($P \geq 0.001$; Fig. 7A). Furthermore, when MG-63 siHAS2 transfected cells were intermittently treated with PTH (1–34), a strong reduction of their migration capacity was observed as compared to transfected with nonspecific siScr cells treated with PTH (1–34) ($P < 0.001$; Fig. 7A). Saos 2 cell motility was negatively affected with downregulation of their HAS2 expression (Fig. 7B) and continuous treatment with PTH (1–34) was not able to restore their basal level migration (Fig. 7B).

These results point to a key role of PTH (1–34) on osteosarcoma cell motility and suggest that PTH (1–34) by regulating HAS2 expression levels may modulate the migration capabilities of osteosarcoma cells.

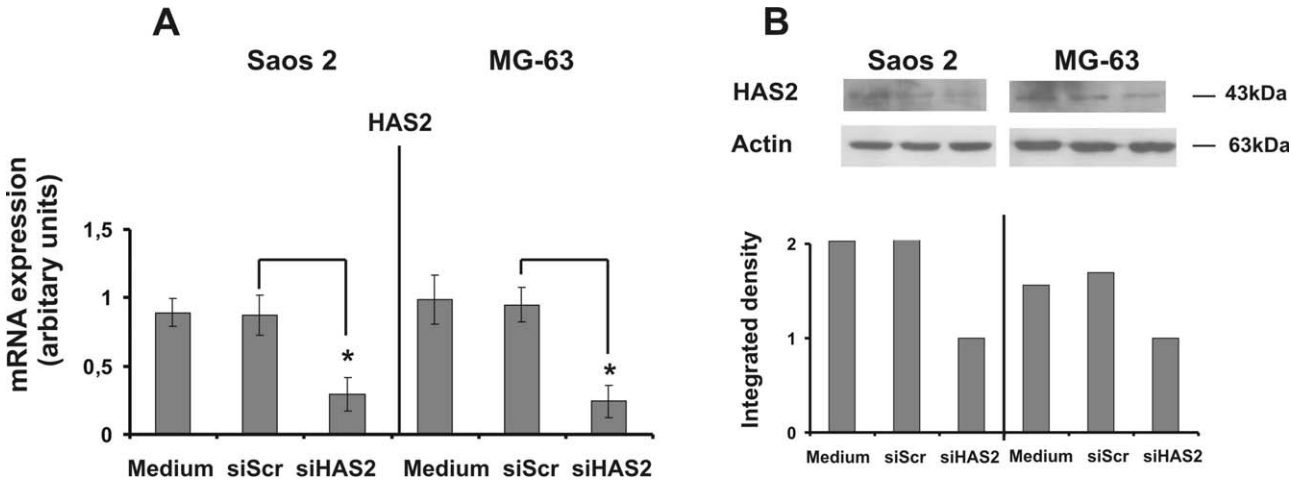


**Figure 6.** Transfection of MG-63 and Saos 2 cells with HAS2 siRNA. MG-63, and Saos 2 cells were transfected with HAS2 short interfering RNA (siHAS2) that are utilizing scramble interfering RNA (siScr) as control. (A) Inhibition of HAS2 mRNA expression was verified by real-time PCR as compared with siScr control, 48 h after transfection. (Means $\pm$ SEM plotted; $n = 3$). *Depicts statistical significance: $P \leq 0.05$ compared with siScr control cells (unpaired $t$ test). (B) Inhibition of HAS2 protein expression was verified by western blot analysis. Densitometric analysis of specific HAS2 protein bands as compared with actin showed agreement with real-time PCR.

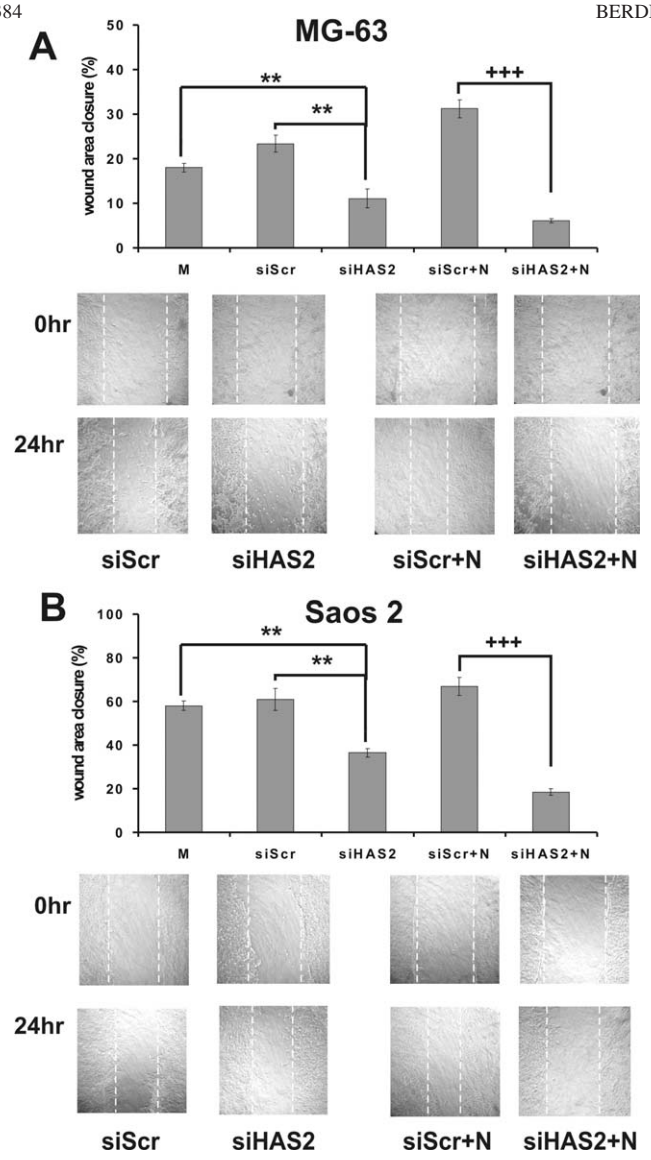


**Figure 7.** Effect of HAS2 inhibition by siRNA on osteosarcoma cell migration. (A) The migration capacity of MG-63 siHAS2 transfected cells (siHAS2) was compared with untransfected cells (M) and transfected cells with nonspecific scrambled siRNA (siScr). Furthermore transfected cells were treated intermittently for two 24-h cycles with PTH (1–34) (siHAS2 + N) utilizing scrambled interfering RNA incubated with PTH as control (siScr + N). B: The migration capacity of Saos 2 siHAS2 transfected cells (siHAS2) was compared with untransfected cells (M) and transfected cells with nonspecific scrambled siRNA (siScr). In contrast to MG-63, Saos 2 siHAS2 transfected cells were treated continuously for 48 h with PTH (1–34) (siHAS2 + N) utilizing scrambled interfering RNA (siScr + N) as a control. The cell layer was "scratched" with a 10 $\mu$L sterile pipette tip and the "wound" surface area was measured at the 0 and 24 h points. The results were expressed as % of closure of wound surface area at 0-h time point (at 0-h time point, the wound surface area was 100%). (Means $\pm$ SEM plotted; $n = 3$). $^{**}P \leq 0.01$ compared to control cells; $^{***}P \leq 0.01$ compared to control cells (unpaired $t$ test).

## DISCUSSION

Contacts between the cells of the osteoblast lineage and surrounding ECM play a key role in the mechanisms that dictate cellular behavior (*24*). PTH, a powerful osteotropic agent, was established to have net anabolic effects on bone (*2*). In this study, we demonstrated for the first time that PTH (1–34)-induced changes in HA production modulate the migration capacity of osteosarcoma cells in a manner dependent on treatment and cell differentiation type.

Previously, PTH (1–84) has been reported to strongly stimulate HA production of both normal (*15, 16*) and carcinogenic cells (*17*) of the osteoblastic lineage. Likewise, it has been reported that PTH (1–34) stimulates HA synthesis in calvarial explants, periosteal rat osteoblasts, and osteoblast-like rat cell lines (*16, 17*) whereas, the effects of PTH (7–84) on osteoblastic cell HA metabolism have not been addressed so far. It is worthwhile to note that these previous reports had established a correlation between the effects of PTH (1–34) on osteoblastic lineage cell HA metabolism and differentiation status (*16, 17*). In the present study, the effects of PTH (1–34) on HA metabolism were strongly dependent on the administration mode and the differentiation of the osteosarcoma cells, in accordance with previous findings (*16, 17*). Thus, intermittent treatment with PTH (1–34) increased the synthesis of HA and its' deposition in the pericellular matrix of MG-63 cells by stimulating their HAS2 expression. This increased content of endogenous HA correlated to a significant increase in MG-63 cell migration capacity. Treatment of these cells with exogenous high-molecular weight HA showed a similar increase in cell motility. Exogenous HA was recently reported to stimulate osteosarcoma cell migration in support of our data (*18*). In the present study and in agreement with previous (*18*), downregulation of HAS2 expression by siRNA inhibited MG-63 cell ability to migrate. The addition of PTH (1–34) to HAS2-defficient osteosarcoma cells did not stimulate their migration, suggesting that HAS2-synthesized HA participates in the PTH (1–34) effect. Interestingly, an *in vivo* study Lossdorfer et al. had demonstrated that the intermittent treatment with PTH (1–34) was correlated to an influx of osteoblast-like cells into the resorption lacunae (*19*). These recent data implied that PTH (1–34) through an unidentified mechanism(s) can influence osteoblastic lineage cell migration (*19*). Our results demonstrate that intermittent PTH (1–34) treatment stimulates osteosarcoma cell migration through a HA-dependent mechanism. Thus, these findings collectively propose that PTH (1–34) in addition to its well-established anabolic effect on bone may also regulate the capability of osteoblastic lineage cells to migrate. Furthermore, this regulatory effect of PTH (1–34) appears at least partly, to be correlated to HA metabolism. The effect was administration-type dependent as continuous administration of PTH (1–34) did not significantly affect neither HA metabolism nor MG-63 cell migration.

The well-differentiated nonmetastatic Saos 2 cells (*20*) showed a distinctly different response to intermittent and con-

tinuous treatment with PTH (1–34), which correlates well to earlier findings demonstrating that PTH effects depend on the *in vivo* location and differentiation status of the osteoblastic lineage cells (*16, 25*). Furthermore, PTH involvement in osteoblast differentiation is implicated (*26*). The intermittent administration mode did not affect Saos 2 cells, HAS isoforms, and HYAL expression and had no effect on their migration capacity, whereas the continuous treatment with PTH (1–34) significantly upregulated their HAS 1, 2 isoforms, and HYAL expression and was correlated to their modestly enhanced migration.

The last years have witnessed remarkable progress in understanding PTH physiology and in identifying various biological activities exhibited by different-sized PTH peptides. Thus, the accumulation of PTH (7–84) in renal failure may lead to PTH resistance by internalizing and downregulating PTH receptors without their activation, having an inhibiting net effect on PTH signaling (*27*). In this study PTH (7–84) treatment of both cell lines frequently exerts antagonistic effects on the regulation of genes related to HA metabolism as compared with PTH (1–34). These actions could be due to a non-PTH (1–34)–like effect on the PTH1R, since PTH (7–84) can bind to this receptor, inhibit the action of the N-terminally intact agonists, and induce internalization in some cells (*27*). Alternatively, signaling of the N-terminal truncated peptides through a novel putative PTH receptor with high affinity binding site for carboxyl terminal PTH peptides has been proposed (*7*).

In summary, our results demonstrate for the first time that PTH (1–34) and PTH (7–84) peptides discretely modulate the expression of genes involved in osteosarcoma HA metabolism and that this regulation was dependent on the administration mode and cell differentiation type. Importantly, the shown modulation of HA metabolism by PTH peptides was correlated to the migration capacity of osteosarcoma cells.

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