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Original

PTHrP-stimulated osteocytes stimulated prevent the differentiation of osteoclasts through the modulation of the cytokines CXCL5 and IL-6

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Abstract

Osteocytes respond to mechanical forces by controlling the function of osteoblasts and osteoclasts. Mechanical stimulation decreases osteocyte apoptosis and promotes bone formation. However, the lack of mechanical load induces osteocytes to favor osteoclastic migration and differentiation, ultimately resulting in bone mass loss. The primary cilium has been described as an important mechanoreceptor in bone cells. PTH1R, the type 1 receptor of parathyroid hormone (PTH), modulates the effects on osteoblasts, osteoclasts, and osteocytes upon activation by PTH or parathyroid hormone-related protein (PTHrP) in osteoblastic cells. Recently, it has been described that mechanical stimulation in osteocytes inhibits osteoclast recruitment and differentiation through a mechanism dependent on PTH1R and the primary cilium. Mechanical stimulation in osteocytes induces the translocation of PTH1R to the primary cilium in MLO-Y4 osteocytes. In this work, we propose to study whether PTHrP reproduces the effects observed with mechanical stimulation regarding the relocation of the receptor to the primary cilium and whether it is also capable of inhibiting osteoclast differentiation through the regulation of cytokines CXCL5 and IL-6. Our results show that stimulation with PTHrP (1-37) triggers a significant mobilization of PTH1R along with the primary cilium in MLO-Y4 osteocytic cells. Additionally, it is observed that PTHrP inhibits osteoclast differentiation through cytokines CXCL5 and IL-6.

Keywords:
Osteocytes. Osteoclasts. PTHrP. CXCL5. IL-6.

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INTRODUCTION

The primary cilium is a unique and immobile appendicular organelle found in numerous cell types, including bone cells, where it functions similarly to an antenna, receiving chemical and mechanical signals (1). Despite being present in almost all human cells, its presence is not constant, as it assembles and resorbs in a cell cycle-dependent process: it forms during the quiescent phase and disappears before entering mitosis (1,2).

Mechanical load is one of the most relevant modulators for the formation and maintenance of bone mass and architecture (3). The presence of the primary cilium in osteocytes is essential for mechanical function (4-6). Besides its role as a mechanoreceptor, the primary cilium accumulates a large number of receptors, including the type 1 receptor for parathyroid hormone (PTH) and the parathyroid hormone-related protein (PTHrP), PTH1R (7-9).

PTH1R is expressed in osteoblasts and osteocytes in which it plays major roles in the regulation of bone metabolism (10). PTHrP is a cytokine expressed in numerous tissues. In bone tissue, it exerts local functions mainly through the PTH1R receptor, although other effects independent of this receptor are known (11-14). PTH1R, in addition to being activated upon binding to its agonists, PTH and PTHrP, is sensitive to mechanical stimuli. It has been reported that mechanical stimulation can directly activate PTH1R in the absence of a ligand, indicating the importance of the PTH receptor as a mechanosensor in osteocytes and osteoblasts (15,16). Therefore, our group has demonstrated that PTH1R is a fundamental component in the process of mechanical signal transduction in MLO-Y4 osteocytic cells (16,17). In these cells, PTH1R activation in the absence of a ligand occurs immediately after mechanical stimulation with fluid flow, thanks to an increase in intracellular calcium influx (18). Moreover, fluid flow induces an increase in PTH1R on the plasma membrane of MLO-Y4 cells (19).

Recently, we have demonstrated that mechanical stimulation by fluid flow in MLO-Y4 cells induces the translocation of PTH1R to the primary cilium (20). Under these conditions, osteocytes inhibit the migration and differentiation of osteoclastic precursors through the alteration of the secretion of the cytokines CXCL5 and IL-6 (21). Although the presence of the PTH1R receptor in the primary cilium is also relevant for the actions of PTHrP (22), it is unknown whether PTHrP (1-37) induces changes in the translocation of the receptor to the primary cilium similarly to those exerted by mechanical stimulation.

In the present work, we aim to study the effects of stimulation with PTHrP (1-37) in the MLO-Y4 osteocyte line on the mobility of the PTH1R receptor in the plasma membrane, analyzing the possible colocalization of the receptor in the primary cilium, as well as the involvement of cytokines CXCL5 and IL-6, produced by osteocytes after stimulation with PTHrP, in the differentiation of osteoclastic precursors.

MATERIALS AND METHODS

CELL CULTURE

Mouse MLO-Y4 osteocytic cells (generously donated by Dr. Lynda Bonewald) were cultured in α-MEM supplemented with 2.5 % fetal calf serum (FCS), 2.5 % fetal bovine serum (FBS), penicillin (100 units/mL), and streptomycin (100 µg/mL) in a humidified incubator with 5 % CO_2 at 37 °C. Cells were seeded at a density of 25,000 cells/cm² in conventional culture plates or on glass coverslips (FlexCell International Corp., Hillsborough, NC, United States) coated with a type I collagen matrix (Sigma Aldrich, St. Louis, MO, United States). The cells were maintained in culture until they were almost fully confluent. The next day, the culture medium was replaced with α-MEM without phenol red containing 1 % FBS, and the cells were kept under this condition for 24 hours. Subsequently, the cells were stimulated with the exogenous peptide PTHrP (1-37) (Bachem, Bubendorf, Switzerland) at a concentration of 100 nM for 10 minutes. The unstimulated cells served as static controls (SC). Afterwards, the cells then incubated for 18 hours with α-MEM without phenol red, without FBS or FCS. After this time, conditioned media (CM) was collected. Additionally, blood was obtained from the Blood Transfusion Center of the Community of Madrid. Peripheral blood mononuclear cells (PBMCs) for generating human osteoclasts in vitro were isolated from the buffy coat. These cells were cultured in α-MEM supplemented with 10 % FCS, 100 units/mL of penicillin, and 100 µg/mL of streptomycin, in a humidified atmosphere with 5 % CO_2 at 37 °C. Human monocytes were grown until they reached 90 % confluence.

INHIBITION OF THE PRIMARY CILIUM, PTH1R, GLI1, cAMP, AND PHOSPHOLIPASE C

The formation of the primary cilium and the activity of PTH1R were inhibited by treating the cells for 1 hour with 1 mM aqueous chloral hydrate or 100 nM PTHrP (7-34), respectively. The Gli transcription factor was inhibited using 10 µM Gli-1-Antagonist 61 (GANT61; Santa Cruz Biotechnology) for 1 hour as appropriate. cAMP and phospholipase C were inhibited for 1 hour with 100 µM of the adenylate cyclase inhibitor SQ22536 or with 1 µM of the phospholipase C inhibitor U73122, respectively.

CELL TRANSFECTION

MLO-Y4 cells were transiently transfected with a plasmid carrying complementary DNA (cDNA) encoding human PTH1R fused with the green fluorescent protein (GFP) reporter gene (*^{GFP}PTH1R*) (generously donated by Dr. Peter Friedman) using Lipofectamine 3000 (Life Technologies) for 4 hours at 37 °C, following the manufacturer's guidelines.

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Cells were fixed using 4 % paraformaldehyde (PFA) in PBS, pH 7.4, for 10 minutes and subsequently permeabilized with 0.5 % Triton X-100 in PBS, pH 7.4 for 5 minutes. To block nonspecific binding, the cells were incubated for 1 hour with 10 % bovine serum albumin (BSA) supplemented with 5 % goat serum. Afterwards, they were incubated with the monoclonal antibody produced in mouse anti-α-acetylated tubulin (Sigma Aldrich), diluted 1:1000 in 1X BSA (100 mL of 10 % BSA diluted in 900 mL of PBS), overnight at 4 °C with agitation. The next day, several washes were performed with 1X PBS, and the secondary anti-mouse immunoglobulin G (IgG) antibody conjugated with Alexa Fluor 546 (Invitrogen, Waltham, MA, United States), diluted 1/1000 in 1X BSA, was added for 1 hour at room temperature. Subsequently, several washes were performed with 1X PBS, and the mounting was performed with FluorSafe reagent (Calbiochem, La Jolla, CA, USA). The samples were analyzed with the Leica DMI8 confocal microscope, evaluating the colocalization between the primary cilium and the PTH1R receptor in cells transfected with the *^{GFP}PTH1R* plasmid, while considering whether the colocalization occurred only at the base or along the entire primary cilium. The length of the primary cilium was also analyzed using ImageJ software.

GENERATION OF OSTEOCLASTS FOR OSTEOCLASTOGENESIS EVALUATION

Human monocytes obtained from the isolation of the buffy coat were used for the differentiation assay into osteoclasts. Once they reached 90 % confluence, the culture medium was removed, washed twice with 1X PBS, and trypsinized (in 0.25 % v/v trypsin and 1 mM ethylenediaminetetraacetic acid [EDTA]) for 30 minutes, using a cell scraper. For the differentiation assay, 20,000 cells/well were seeded in a 96-well plate with α-MEM culture medium supplemented with 10 % FCS, 100 units/mL of penicillin, and 100 µg/mL of streptomycin plus 20 ng/mL of M-CSF and 20 ng/mL of RANKL (ProSpec, Ness-Ziona, Israel). The controls were cultured in the same medium but without RANKL. After three days, the culture medium was replaced with fresh medium without phenol red, containing M-CSF and RANKL, as appropriate, and additionally, 20 % of the various CM from MLO-Y4 cells was added. Controls received 20 % α-MEM medium without FCS. Additionally, neutralizing antibodies were added; 2 µg/mL of anti-CXCL5 and 1 µg/mL of anti-IL-6. Human monocytes were in contact with the CM from MLO-Y4 cells for 3 days. After this time, the culture medium was removed and washed with 1X PBS to eliminate non-adherent cells. Cells were fixed with 4 % PFA for 10 minutes, permeabilized with 100 % methanol for 20 minutes, and stained with hematoxylin for 5 minutes. The differentiation of human monocytes into osteoclasts was determined by the morphology of the obtained cells, observing the formation of giant cells with 3 or more nuclei. Images were obtained using the Leica DMI1 microscope, evaluating the number of osteoclastic cells with three or more nuclei using ImageJ software.

STATISTICAL ANALYSIS

Data are expressed as means ± standard deviations (SD). Statistical analysis was performed using GraphPad Prism (GraphPad software). Differences between conditions were evaluated using non-parametric analysis of variance (Kruskal-Wallis) followed by the Mann-Whitney test. A $p < 0.05$ was considered significant.

RESULTS

LOCALIZATION OF THE PTH1R IN THE PRIMARY CILIUM OF PTHrP-STIMULATED OSTEOCYTES (1-37)

First, we aimed to assess how treatment with PTHrP affects the mobility of PTH1R towards the primary cilium in MLO-Y4 osteocytic cells. Confocal microscopy revealed that PTH1R colocalized with the primary cilium in MLO-Y4 cells. Under static conditions, 52 % of ciliated osteocytic cells showed some colocalization between PTH1R and the primary cilium. Of these, only 33 % of the cells showed colocalization along the entire primary cilium, while 19 % showed colocalization only at the base of the cilium. However, after stimulation with PTHrP, we observed a significant increase in the presence of PTH1R along the entire primary cilium (79 % of ciliated osteocytic cells showed colocalization between PTH1R and the primary cilium; of this percentage, 50 % of the ciliated cells showed localization of PTH1R along the entire length of the cilium, while 29 % showed colocalization only at the base of the cilium) (Fig. 1 A and B). Pretreatment with CH inhibited

the formation of the primary cilium as well as the mobilization of PTH1R towards it, while treatment with the PTHrP (7-34) antagonist did not significantly affect the mobilization of PTH1R (Fig. 1 A and B). Under static conditions and inhibition with PTHrP (7-34), 58 % of ciliated osteocytic cells showed some colocalization between PTH1R and the primary cilium. Of these, only 36 % of the cells showed colocalization along the entire primary cilium, while 22 % showed colocalization only at the base of the cilium. After treatment with PTHrP, 40 % of ciliated osteocytic cells showed colocalization between PTH1R and the primary cilium. Of this percentage, 23 % of the ciliated cells showed localization of PTH1R along the entire length of the cilium, while 17 % showed colocalization only at the base of the cilium (Fig. 1 A and B).

We also wanted to determine if the length of the cilium experienced any changes when stimulated with PTHrP (1-37) compared to static conditions. In both cases, the primary cilium had a similar length (Fig. 1C). This effect was inhibited by pretreatment with CH but was not affected by the PTH1R antagonist, PTHrP (7-34) (Fig. 1C).

These results show that stimulation with PTHrP triggers a significant mobilization of the PTH1R receptor to the primary cilium in MLO-Y4 osteocytic cells.

INVOLVEMENT OF CXCL5 AND IL-6 IN OSTEOCLAST DIFFERENTIATION

The regulation of the secretion of CXCL5 and IL-6 cytokines in MLO-Y4 osteocytes through mechanical stimulation has been shown to play a role in the recruitment and differentiation of osteoclastic precursors (21). This regulation associated with mechanotransduction is mediated by the primary cilium and the activation of PTH1R (21). Based on this background and the results obtained regarding the translocation of PTH1R to the cilium (Fig. 1), we wanted to verify whether the cytokines CXCL5 and IL-6 also mediate the actions performed by PTHrP in the communication between osteocytes and osteoclasts using specific neutralizing antibodies (Figs. 2 and 3). The results showed that the conditioned media (CM) from osteocytes under static conditions caused an increase in osteoclast differentiation; an effect that was inhibited by pretreatment of the osteocyte CM with the CXCL5 neutralizing antibody or the IL-6 neutralizing antibody. Additionally, the CM from osteocytes treated with PTHrP (1-37) decreased differentiation towards osteoclasts with or without neutralization of CXCL5 or IL-6 (Figs. 2 and 3). The neutralization of CXCL5 did not cause any effect on osteoclast differentiation when the primary cilium or PTH1R in osteocytes was inhibited with CH or PTHrP (7-34), respectively (Fig. 2 A and B). Similarly, the neutralization of CXCL5 also did not cause any ef-

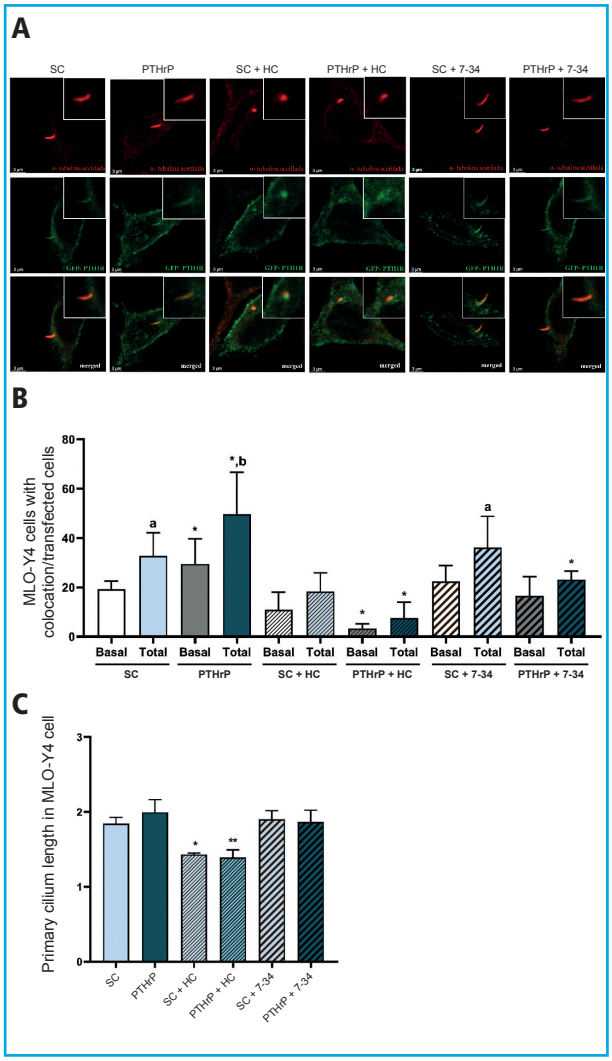

Figure 1. PTH1R colocalizes with the primary cilium in PTHrP-stimulated MLO-Y4 cells (1-37). MLO-Y4 cells were transfected with 1 µg of the $^{GFP}PTH1R$ plasmid using Lipofectamine 3000 for 4 h at 37 °C. Subsequently, the cells were serum-starved for 6 h, treated with 1 mM chloral hydrate or 100 nM PTHrP (7-34) for 1 h, and then stimulated with 100 nM PTHrP (7-34) for 1 h. Afterward, they were stimulated with 100 nM PTHrP (1-37) for 10 min. To evaluate the colocalization of PTH1R with the primary cilium, the cells were fixed, permeabilized, blocked, and incubated overnight at 4 °C with the mouse anti-α-acetylated tubulin antibody. The cells were then incubated for 1 h with the Alexa Fluor 546-conjugated anti-mouse IgG secondary antibody. Representative images of each condition are shown (A). The percentage of cells with PTH1R colocalization at the base (basal) and along the entire primary cilium (total) was analyzed under each condition in MLO-Y4 cells transfected with the $^{GFP}PTH1R$ plasmid (B). The length of the primary cilium in MLO-Y4 cells was evaluated using ImageJ software (C). The results are the mean ± SD of triplicates. $*p < 0.05$ vs. corresponding control; $**p < 0.01$ vs. corresponding control; $^{a}p < 0.05$ vs. corresponding basal condition; $^{b}p < 0.001$ vs. corresponding basal condition (SC, static control; PTHrP, parathyroid hormone-related protein; HC, chloral hydrate; 7-34: PTHrP (7-34); $^{GFP}PTH1R$, green fluorescent protein-PTH1R; PTH1R, type 1 PTH receptor; IgG, immunoglobulin G; SD, standard deviation).

fect on osteoclastogenesis when the GLI, PKA, and PKC pathways were inhibited with GANT61, SQ22536, and U73122, respectively (Fig. 3 A and B). On the contrary, the anti-IL-6 antibody not only reversed osteoclastogenesis under static conditions but also in the presence of CH or PTHrP (7-34) (Fig. 2 A and B). Similarly, inhibition of the GLI, PKA, and PKC pathways also reversed osteoclastogenesis (Fig. 3 A and B).

These findings indicate that both a functional primary cilium and the PTH1R receptor in osteocytes are necessary for proper communication with osteoclasts and suggest that stimulation with PTHrP in osteocytes inhibits osteoclast differentiation through CXCL5, while the activation of PTH1R and the primary cilium in osteocytes regulate osteoclasts through IL-6.

DISCUSSION

The primary cilium is a well-known mechanosensor present in osteocytes among other cells. However, its functions in bone cells do not seem to be limited solely to promoting mechanotransduction. In this work,

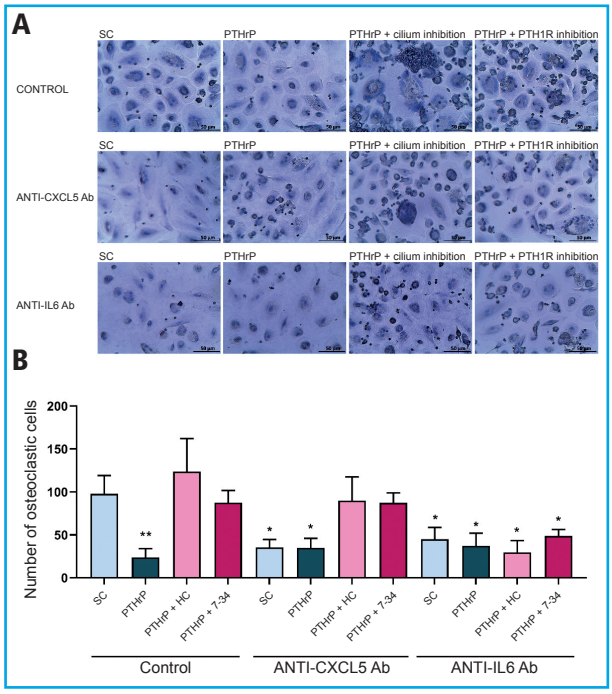

Figure 2. Stimulation with PTHrP (1-37) inhibits osteoclast differentiation through a mechanism dependent on CXCL5 and IL-6. MLO-Y4 osteocytic cells were serum-starved for 24 h and then treated with 1 mM chloral hydrate or 100 nM PTHrP (7-34) for 1 h. The cells were then stimulated with 100 nM PTHrP (1-37) for 10 minutes. CM was collected after 18 h, and 2 µg/mL anti-mCXCL5 neutralizing antibody or 1 µg/mL anti-mIL-6 neutralizing antibody was added. To evaluate the differentiation of monocytes into osteoclasts, human monocytes were treated with 20 ng/mL M-CSF and 20 ng/mL RANKL plus the corresponding CM from MLO-Y4 cells at 20 % with the corresponding neutralizing antibody. The cells were then fixed, permeabilized, and stained with hematoxylin. Representative images of each condition are shown (A). The percentage of cells with three or more nuclei was evaluated using ImageJ software (B). The results represent the mean ± SD of 2 experiments, each in triplicate for each experimental condition. *$p < 0.05$ vs. SC or vs. corresponding inhibition of the cilium or PTH1R; **$p < 0.01$ vs. SC (CM, conditioned medium; M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor kappa-B ligand; CXCL5, C-X-C motif chemokine 5; IL-6, interleukin-6; Ab, antibody; SC, static control; PTHrP, parathyroid hormone-related protein; HC, chloral hydrate; 7-34: PTHrP (7-34); SD, standard deviation).

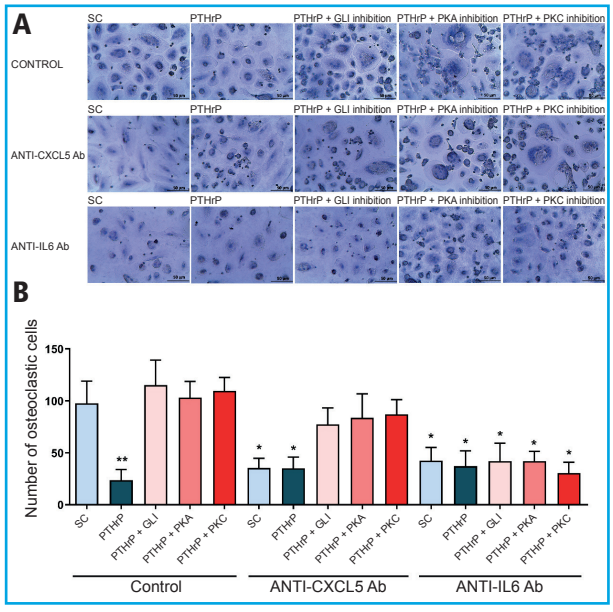

Figure 3. Stimulation with PTHrP (1-37) inhibits osteoclast differentiation through a mechanism dependent on CXCL5 and IL-6. MLO-Y4 osteocytic cells were serum-starved for 24 h and then treated with 10 µM GANT61, 100 µM adenylate cyclase inhibitor SQ22536, or 1 µM phospholipase C inhibitor U73122 for 1 h. The cells were then stimulated with 100 nM PTHrP (1-37) for 10 minutes. CM was collected after 18 h, and 2 µg/mL anti-mCXCL5 neutralizing antibody or 1 µg/mL anti-mIL-6 neutralizing antibody was added. To evaluate the differentiation of monocytes into osteoclasts, human monocytes were treated with 20 ng/mL M-CSF and 20 ng/mL RANKL plus the corresponding CM from MLO-Y4 cells at 20 % with the corresponding neutralizing antibody. The cells were then fixed, permeabilized, and stained with hematoxylin. Representative images of each condition are shown (A). The percentage of cells with three or more nuclei was evaluated using ImageJ software (B). The results represent the mean ± SD of 2 experiments, each in triplicate for each experimental condition. *$p < 0.05$ vs. SC or vs. corresponding inhibition of GLI, PKA, or PKC; **$p < 0.01$ vs. SC (CM, conditioned medium; M-CSF, macrophage colony-stimulating factor; RANKL, receptor activator of nuclear factor kappa-B ligand; CXCL5, C-X-C motif chemokine 5; IL-6, interleukin-6; Ab, antibody; SC, static control; PTHrP, parathyroid hormone-related protein; GANT61, GLI1-antagonist 61; GLI, glioma-associated oncogene family zinc finger 1 transcription factor; PKA, protein kinase A; PKC, protein kinase C; SD, standard deviation).

our results indicate that the primary cilium exposes the type 1 PTH and PTHrP receptor, PTH1R, along its entire length after stimulation with the PTHrP (1-37) peptide. Additionally, we observed that the cytokines CXCL5 and IL-6 appear to regulate the effects exerted by PTHrP on the communication between osteocytes and osteoclasts, specifically affecting the differentiation of the latter.

PTH1R is expressed in osteoblasts and osteocytes and is key to tissue formation and the maintenance of bone homeostasis (23). PTHrP signaling through PTH1R in osteocytes promotes the inhibition of sclerostin synthesis, leading to increased bone formation, but also activates bone remodeling through the regulation of RANKL (24). PTH1R can be activated by its two agonists, PTH secreted by the parathyroid glands, or PTHrP, secreted by a variety of tissues, such as bone, where it acts locally (10). Several studies indicate that this receptor can act as a mechanoreceptor, being directly activated by mechanical stimuli in the absence of its agonists (25). It has also been described that mechanical load, such as physical exercise, and PTH/PTHrP peptides can synergistically enhance each other's actions (13,26). In fact, physical activity such as running or swimming induces the transient secretion of PTH (27), and mechanical stimulation increases the production of PTHrP in osteocytes, which could induce the activation of PTH1R (18,28).

The primary cilium also acts as a mechanoreceptor in bone cells, receiving mechanical signals. The primary cilium presents numerous receptors and channels capable of being activated by different stimuli. In this work, our results show that after stimulation with the PTHrP (1-37) peptide, there is a migration of PTH1R towards the primary cilium, increasing the presence of the receptor along the entire ciliary projection (Fig. 1). A very similar effect to that described here is observed when these same MLO-Y4 cells are stimulated with mechanical fluid flow (21). Additionally, another study has shown that mechanical stimulation promotes the transport of PTH1R to the primary cilium, increasing PTH signaling in nucleus pulposus cells of the intervertebral disc (9).

Neutralization experiments point to CXCL5 as a cytokine regulated by osteocytes that, when secreted, controls the differentiation of osteoclasts. CXCL5 is a chemokine involved in leukocyte recruitment (29,30). This chemokine binds to the CXC1 receptor and the CXC2 receptor, both expressed in osteoclast precursors (31,32). Additionally, it has been described that CXCL5 modulates the expression of CXC1 and may have a functional role in increasing the expression levels of RANKL in human bone marrow stromal/preosteoblastic cells (33). Sundaram et al. showed that CXCL5 could have a functional role in increasing RANKL expression levels associated with Paget's disease of bone in humans, which presents very localized areas of bone turnover with increased osteoclast activity (33).

Regarding IL-6, its role as an inducer of osteocyte-mediated osteoclastogenesis through the activity of JAK2 and RANKL is well known (34). A study has demonstrated that the increase in IL-6 secretion by apoptotic osteocytic cells promotes the recruitment of osteoclastic precursors. This is because IL-6 secretion promotes endothelial ICAM-1 expression and osteoclastic precursor adhesion (35). The study conducted by Kazuhiro showed that the combination of TNF and IL-6 can induce bone resorption in osteoclast-like cells (36). In fact, IL-6 plays an important role as a regulator during osteoclastogenesis, bone resorption, and regeneration (35). Similarly to this study, osteoclast formation was enhanced after the secretion of both IL-6 and the soluble IL-6 receptor (37). Consistently, our results (Fig. 2 and 3) show that the IL-6 neutralizing antibody decreased cell differentiation when the cilium and PTH1R receptor were inhibited. The same thing occurs when the Hedgehog, adenylate cyclase, and phospholipase C pathways are inhibited. However, the CXCL5 neutralizing antibody had no effect in this regard.

Previous data from our research group show that the silencing of PTH1R is associated with an increase in monocyte migration and osteoclastogenesis, as well as an increase in IL-6 secretion by osteocytes, despite the cells being stimulated by FF. Since IL-6 neutralization under these conditions decreases both migration and differentiation of osteoclasts, these data suggest that the secretion of high levels of IL-6 may overcome the low FF-dependent secretion of CXCL5 and maintain monocyte migration and osteoclastogenesis (20). Similarly, this would occur when stimulation with PTHrP is produced in the process of osteoclastogenesis (Fig. 2).

Moreover, we demonstrated that the inhibition of the primary cilium was also associated with an increase in osteoclastic function, even under conditions of stimulation with PTHrP (1-37), and IL-6 neutralization reversed this effect. Collectively, these data suggest that primary cilium inhibition could induce high IL-6 secretion that overcomes the low CXCL5 secretion, as it occurs when the PTH1R receptor is inhibited (Fig. 4). However, it is also possible that the primary cilium —under conditions of stimulation with PTHrP (1-37)— modulates other alternative cytokines involved in osteoclastic communication. Even so, IL-6 neutralization was sufficient to prevent the differentiation of osteoclastic precursors.

Our findings support that a functional primary cilium and PTH1R are necessary in osteocytes to regulate the secretome of these cells and their communication with osteoclasts. Thus, PTHrP-simulated osteocytes (1-37) inhibit osteoclast differentiation by decreasing CXCL5 secretion, while PTH1R activation and the primary cilium in osteocytes regulate osteoclasts through the modulation of IL-6 secretion.

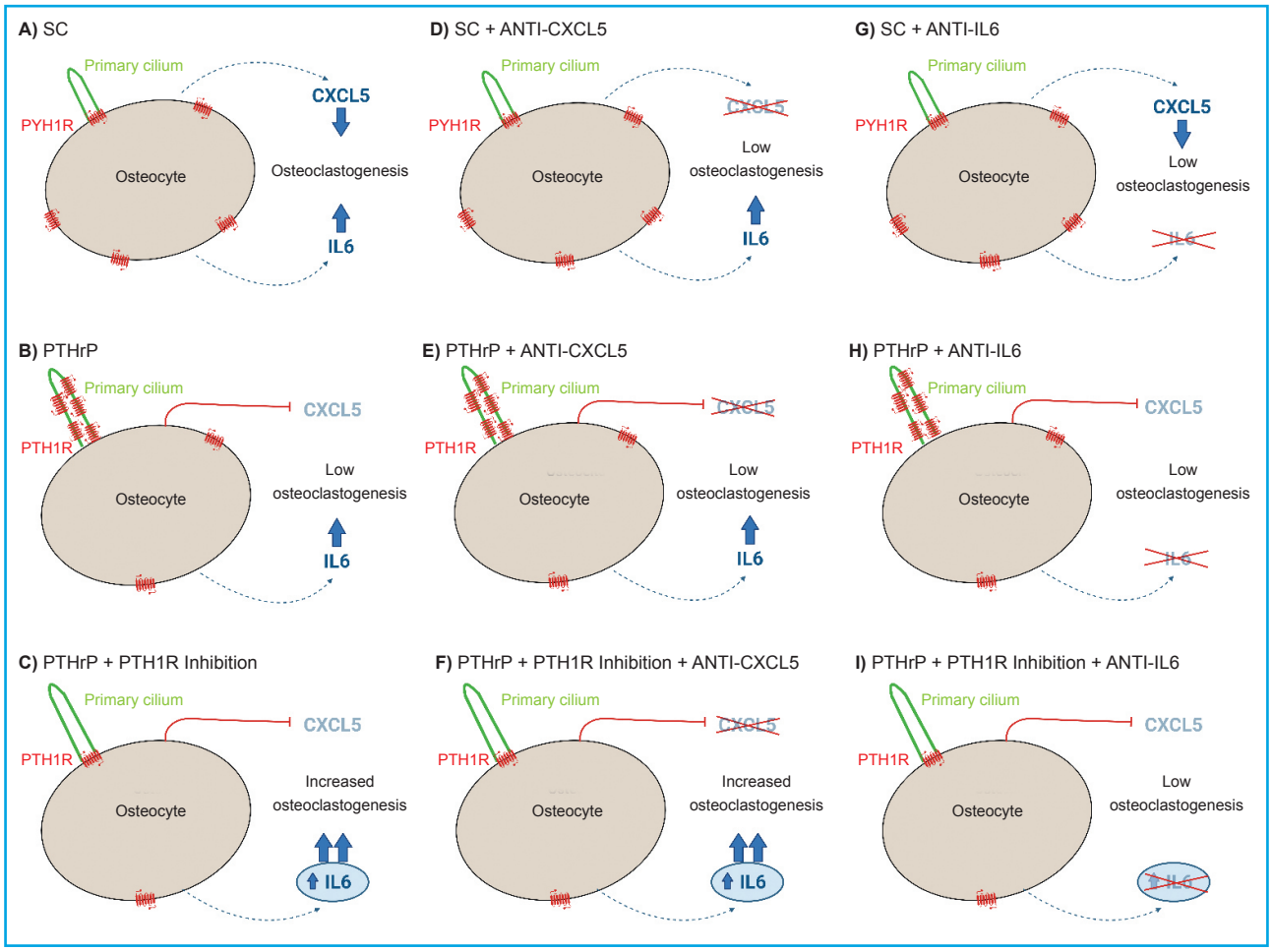

Figure 4. Proposed mechanism for the regulation of osteoclast differentiation by the primary cilium and PTH1R in osteocytes. Both a functional primary cilium and PTH1R in osteocytes are necessary for proper communication with osteoclasts. Stimulation with PTHrP (1-37) inhibits osteoclast differentiation through CXCL5, while PTH1R activation regulates osteoclasts through IL-6 (SC, static control; PTHrP, parathyroid hormone-related protein; CXCL5, C-X-C motif chemokine 5; IL-6, interleukin-6; PTH1R, type I parathyroid hormone receptor).

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