Importance of melastatin-like transient receptor potential 7 and magnesium in the stimulation of osteoblast proliferation and migration by platelet-derived growth factor

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Abed E, Moreau R. Importance of melastatin-like transient receptor potential 7 and magnesium in the stimulation of osteoblast proliferation and migration by platelet-derived growth factor. *Am J Physiol Cell Physiol* 297: C360–C368, 2009. First published May 27, 2009; doi:10.1152/ajpcell.00614.2008.—Bone is a dynamic tissue that is continuously being remodeled throughout life. Specialized cells called osteoclasts transiently break down old bone (resorption process) at multiple sites as other cells known as osteoblasts are replacing it with new tissue (bone formation). Usually, both resorption and formation processes are in balance and thereby maintain skeletal strength and integrity. This equilibrium is assured by the coordination of proliferation, migration, differentiation, and secretory functions of the osteoblasts, which are essential for adequate formation and resorption processes. Disturbances of this equilibrium may lead to decreased bone mass (osteoporosis), increased bone fragility, and susceptibility to fractures. Epidemiological studies have linked insufficient dietary magnesium (Mg$^{2+}$) intake in humans with low bone mass and osteoporosis. Here, we investigated the roles of Mg$^{2+}$ and melastatin-like transient receptor potential 7 (TRPM7), known as Mg$^{2+}$ channels, in human osteoblast cell proliferation and migration induced by platelet-derived growth factor (PDGF), which has been involved in the bone remodeling process. PDGF promoted an influx of Mg$^{2+}$, enhanced cell migration, and stimulated the gene expression of TRPM7 channels in human osteoblast MG-63 cells. The stimulation of osteoblast proliferation and migration by PDGF was significantly reduced under culture conditions of low extracellular Mg$^{2+}$ concentrations. Silencing TRPM7 expression in osteoblasts by specific small interfering RNA prevented the induction by PDGF of Mg$^{2+}$ influx, proliferation, and migration. Our results indicate that extracellular Mg$^{2+}$ and TRPM7 are important for PDGF-induced proliferation and migration of human osteoblasts. Thus Mg$^{2+}$ deficiency, a common condition among the general population, may be associated with altered osteoblast functions leading to inadequate bone formation and the development of osteoporosis.

TRPM7 channels; osteoblasts; adhesion

The bone is a dynamic tissue that is continuously being remodeled following two coordinated processes. The osteoclasts are constantly breaking down old bone (known as the resorption process) as the osteoblasts are replacing it with new tissue (termed the bone formation process). Osteoblasts ensure bone formation and tissue mineralization through the secretion of bone matrix components (type I collagen and noncollagenous proteins) and also provide factors essential for the differentiation of osteoclasts. By regulating osteoclast differentiation, the osteoblasts play a central role not only in bone formation, but also in the regulation of bone resorption (21). Therefore, the maintenance of bone remodeling equilibrium relies on the coordination of proliferation, migration, differentiation, secretion of matrix proteins, and apoptosis of osteoblastic cells. Disturbances of any of these processes that shift the balance of equilibrium toward bone resorption may cause loss of bone mass with a consequent increase in bone fragility and susceptibility to fractures (osteoporosis).

Osteoblasts arise from osteoprogenitor cells located in the bone marrow (2). Osteoprogenitors are induced to differentiate under the influence of growth factors, in particular, the bone morphogenetic proteins (BMPs). Aside from the BMPs, other growth factors including fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and transforming growth factor beta (TGF-β) promote the division of osteoprogenitors and increase osteogenesis. It has been known that PDGF promotes proliferation and migration in a variety of cell types including osteoblastic cells (23). This growth factor is one of the biological mediators of bone formation (5, 17, 29). Studies have shown that PDGF enhances DNA and collagen synthesis in rat osteoblast cultures (5) and increases bone matrix deposition in cultured calvaria (29). In an in vivo study, PDGF was also found to enhance bone formation (41).

Calcium (Ca$^{2+}$) influx is implicated in numerous cellular functions such as proliferation, differentiation, secretion, and apoptosis (3). In bone cells of the osteoblast lineage, calcium channels play fundamental roles in cellular responses to external stimuli including both mechanical forces and hormonal signals (11, 18).

Magnesium (Mg$^{2+}$) is the most abundant intracellular divalent cation in living cells. Virtually every biological process requires Mg$^{2+}$ as an essential cofactor for hundreds of enzymes and for the regulation of various transporters and ion channels (35). Furthermore, Mg$^{2+}$ is an important modulator of intracellular free Ca$^{2+}$ concentration and intracellular pH, which are major determinants of cell contraction, secretion, motility, and proliferation (10, 28, 46).

Epidemiologic studies have demonstrated a positive correlation between Mg$^{2+}$ intake and bone density, thereby linking insufficient dietary Mg$^{2+}$ intake to osteoporosis (for a review, see Ref. 31). By histomorphometry analysis, a reduction in osteoblast number was observed in Mg$^{2+}$-deficient rats and mice (33). In other studies, serum and bone alkaline phosphatase, osteocalcin, and bone osteocalcin mRNA were reduced, suggesting a decrease in osteoblastic functions (4, 9).

The “membrane magnesium mitosis” model of cell proliferation control suggests that, on mitogenic stimulus, cells are
able to increase their intracellular magnesium content, likely by activating Mg$^{2+}$ influx, to levels optimum for the initiation of protein synthesis. Therefore, influx of both extracellular Ca$^{2+}$ and Mg$^{2+}$ for proper intracellular ion homeostasis is likely solicited for cell proliferation. Melastatin-like transient receptor potential (TRPM) is a recently emerging subfamily of the transient receptor potential family, a diverse group of voltage-independent Ca$^{2+}$-permeable cation channels expressed in mammalian cells (7, 16, 25, 26) that encompasses eight distinct members, designated TRPM1–8. TRPM7 combines structural elements of both an ion channel and a protein kinase (for recent reviews, see Refs. 12 and 15). TRPM7 channels have been associated with cell proliferation and survival (27). TRPM7-deficient DT40 cells can be rescued from their cell growth defect by supplementary extracellular Mg$^{2+}$ homeostasis. Among TRPM members, TRPM2, TRPM6, and TRPM7 uniquely possess an enzyme domain in their long COOH termini, the latter two exhibiting spontaneously activated divalent cation (Ca$^{2+}$, Mg$^{2+}$, and other trace metals) entry, regulated by cytosolic Mg$^{2+}$ and ATP levels. Mutations in the TRPM6 gene have been shown in patients suffering from a hereditary form of hypomagnesemia caused by impaired Mg$^{2+}$ resorption (6). Life-long dietary Mg$^{2+}$ supplementation of these patients is sufficient to rescue the phenotype of affected human beings. Our previous results have indicated that TRPM7 channels are involved in the intracellular Mg$^{2+}$ homeostasis of osteoblastic cells as well as in basal cell proliferation (1).

To better understand the link between insufficient dietary Mg$^{2+}$ intake in humans with low bone mass and osteoporosis, the current study aimed to investigate the importance of Mg$^{2+}$ and TRPM7 channel in the stimulation of osteoblast proliferation and migration by the bone remodeling regulating factor PDGF.

MATERIALS AND METHODS

Cell culture. Human osteoblast-like MG-63 cells from the American Type Culture Collection (Rockville, MD) were grown in a 1:1 mixture of phenol-free DMEM/Ham’s F12 medium (DMEM/F12; Sigma, Oakville, Ontario, Canada) supplemented with 10% fetal bovine serum (FBS; Cansera, Etobicoke, Ontario, Canada), l-glutamine (Invitrogen, Burlington, Ontario, Canada), and penicillin/streptomycin (Invitrogen). Cells were cultured in 5% CO$_2$ at 37°C and were harvested weekly with Trypsin-EDTA solution (Invitrogen).

Cell proliferation assays. For proliferation experiments, cells were seeded in 96-well plates (Sarstedt, Montreal, Quebec, Canada) at 2,500 cells/cm$^2$. After 4 days of culture in supplemented media, cells were incubated in calcium (Ca$^{2+}$)- and Mg$^{2+}$-free DMEM/F12 (Sigma) supplemented with different concentrations of Ca$^{2+}$ and Mg$^{2+}$ without serum in the absence or the presence of 25 ng/ml of PDGF-BB (Sigma) for 48 h. Cell proliferation was determined by microtiter tetrazolium [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrasodium bromide (MTT)] reduction assays. Briefly, 1 h before the end of treatment the medium was replaced with DMEM/F12 containing 0.5 mg/ml MTT (Sigma). At the end of the incubation, media were aspirated and formazan crystals generated by the cellular reduction activity were dissolved in dimethylsulfoxide. Absorbance was measured at 575 nm, and data are expressed as the ratio of absorbance of treated cells versus initial MTT absorbance corresponding to the level of MTT reduced to formazan crystals by cells, the initial day of treatment.

Cell migration assays. To investigate the effects of Ca$^{2+}$, Mg$^{2+}$, and PDGF and the involvement of TRPM7 on MG-63 cell migration, a wound scratch assay was performed. Briefly, the cells were grown to confluent monolayers on 35-mm diameter dishes (Sarstedt). Monolayers were wounded by scratching the surface as uniformly as possible with a pipette tip, and the cells were then incubated for 18 h under different conditions. This initial wounding and the movement of the cells in the scratched area were photographically monitored using an Axiovert Zeiss 200 microscope with a ×10 [0.25 numerical aperture (NA)] objective linked to a Coolsnap ES charge-coupled device camera. The 18-h time interval has been chosen because it is shorter than MG-63 doubling time in these conditions. Four different fields from each sample were analyzed using the ImageJ software for quantitative estimations of the number of cells that have migrated to the wounded area. The values are expressed as the relative cell migration compared with control condition.

Adhesion assay. Cell adhesion was measured using a trypan blue exclusion assay (36). Briefly, cells were seeded in 12-well plates for 4 days and treated with different conditions for 16 h. Then cells were incubated with 1 ml of 0.05% trypsin-EDTA for 4 min to promote cell detachment. The enzymatic activity was stopped by the addition of 1 ml of culture medium containing 10% FBS, and detached cells were collected. The number of detached cells under limited trypsination was then manually counted using a hemocytometer. The remaining adherent cells was collected by complete trypsination and counted by hemocytometer. The percentage of adhesion was expressed as a ratio of detached versus total (detached and remaining) cells.

Cell morphology. MG-63 cells were cultured in four-well Lab-Tek (Nalge Nunc, Naperville, IL) dishes for 5 days in supplemented medium. Cells were then incubated for 18 h under different conditions. At the end of treatment, cells were fixed in 3.7% formaldehyde/phosphate-buffered saline (PBS) for 10 min, permeabilized with 0.1% Triton X-100/PBS for 3 min, and blocked in 3% bovine serum albumin-PBS. Cells were incubated with 6 μM Alexa Fluor 555-conjugated phallolidin (1:200; Invitrogen) with an equivalent volume of 20% Pluronic F127 (Invitrogen) for 45 min at room temperature in the dark to detect filamentous actin (F-actin). Cells were washed three times with PBS between each step. The cells were examined with a laser-scanning confocal (Bio-Rad) microscope with an Apochromatic ×40 NA 1.0 objective lens. Fluorescence was excited by an argon laser at 555 nm, and emission was collected with a 565-nm filter.

Reverse-transcription polymerase chain reaction. Total RNA from cells was extracted using TRIzol (Invitrogen) according to the manufacturer’s instructions. Reverse transcription (RT) reactions were carried out with Omniscript RT kit (Qiagen, Mississauga, Ontario, Canada) using hexamers. The PCR amplifications were conducted with Taq PCR core kit (Qiagen) using specific primer sets for human TRPM7 (sense: 5’-TGCACCTATACTAGGAAACGTTTTCG-3’; antisense: 5’-CATGATAAAAAGGCATATAACCTGCG-3’). Each primer was designed in distinct exons to ensure specific transcript amplifications. Briefly, amplification products were carried out for 40 cycles according to incubation of 1 min at 94°C, 30 s at 58°C, and 1 min at 72°C. Amplification products were resolved in 2% agarose gel with ethidium bromide revelation.

Measurements of intracellular calcium and magnesium levels. MG-63 cells were cultured in four-well Lab-Tek dishes for 5 days in supplemented media. Cells were then transfected to HEPS-buffered saline solution (HBSS; in mM: 121 NaCl, 5.4 KCl, 25 HEPS, 1.8 CaCl$_2$, and 6.0 NaHCO$_3$ at pH 7.3) or Ca$^{2+}$ and Mg$^{2+}$-free HBSS solution (HBSS without Ca$^{2+}$ and/or without Mg$^{2+}$) and loaded with 2 μM Fluor-3 AM or Magnesium Green (Invitrogen) with an equivalent volume of 20% Pluronic F127 for 45 min at room temperature in the dark. Thereafter, cells were washed with corresponding HBSS, and the loaded dye was allowed to deesterify for 45 min at room temperature in the dark. Following transfer to a Ca$^{2+}$-free and Mg$^{2+}$-free HBSS, additions of Ca$^{2+}$ or Mg$^{2+}$ were made in an open
chamber configuration at room temperature. The cells were examined with a laser-scanning confocal (Bis-Rad) microscope (Nikon TE300) with an Apochromatic ×40 NA 1.0 objective lens. Fluorescence was excited by an argon laser at 488 nm, and emission was collected with a 515-nm filter. Data were analyzed with Laser Sharp 2.1T. Time Course 1.0 software for 7 to 8 fields per experiments (between 10 and 20 cells per field). All experiments were performed for at least four individual experiments.

Interference with small interfering RNA. Small interfering RNAs (siRNAs) directed against human TRPM7 (si-TRPM7) and a nontargeting control (si-mock) were obtained from Qiagen. Transfection of the siRNAs was performed using HiPerFect reagent following the manufacturer’s instructions. Quantifications of transcripts were performed by RT-PCR 2 days posttransfection to evaluate the TRPM7 expression level as described previously (1). To investigate the role of TRPM7 in osteoblast proliferation, cells seeded in 96-well plates were transfected for 24 h and were thereafter incubated in appropriate conditions for 48 h, with the addition of MTT for the last 1 h of incubation. For the determination of the importance of TRPM7 in migration, adhesion, and morphology, cells seeded in 12-well plates were transfected for 48 h and were thereafter incubated in appropriate conditions for 18 h and studied as mentioned above. To study the involvement of TRPM7 in magnesium influx, cells seeded in four-well Lab-Tek dishes were transfected for 48 h and studied as mentioned above.

Statistical analysis. A nonparametric Mann-Whitney test was used to compare two independent groups of sampled data, or a two-way ANOVA with Bonferroni post test was used to test the effectiveness of two independent variables using GraphPad Prism 4 software. A level of $P < 0.05$ was considered significant.

RESULTS

The physiological concentration of Mg$^{2+}$ is between 0.8 to 1.0 mM, and concentrations ranging between 0.6 and 0.7 mM are frequent in apparently healthy Western people, with lowest levels of 0.4 to 0.5 mM in critically ill subjects (43). In experimental Mg$^{2+}$ deficiency, plasma Mg$^{2+}$ level of 0.1 mM has been observed in animal models (32). Mg$^{2+}$ depletion has been shown to influence endothelial cell functions in a dose- and time-dependent manner (22). Such alterations were associated with modulations of gene expression related to cell adaptation. On the basis of these findings, we used experimental conditions of severe Mg$^{2+}$ depletion for short periods of time to principally document the roles of intracellular Mg$^{2+}$ and TRPM7 activity on morphology, adhesion, and cell migration and exclude cell adaptation to low Mg$^{2+}$ concentration condition as reported by Maier et al. (22).

Influence of reduced extracellular magnesium and calcium on the osteoblast proliferation induced by PDGF. Influx of both extracellular Ca$^{2+}$ and Mg$^{2+}$ for proper intracellular ion homeostasis is likely solicited for cell proliferation. We determined the importance of extracellular Ca$^{2+}$ and Mg$^{2+}$ in the basal proliferation of osteoblastic cells and induced by PDGF. MTT assays were performed on MG-63 cells maintained for 48 h in 1 mM Ca$^{2+}$ and Mg$^{2+}$-free media supplemented with various concentrations of Mg$^{2+}$ ions (Fig. 1A) or in 0.8 mM Mg$^{2+}$ and Ca$^{2+}$-free media supplemented with various concentrations of Ca$^{2+}$ ions (Fig. 1B). As shown in Fig. 1A, concentrations of Mg$^{2+}$ below 0.8 mM gradually reduced basal cell proliferation compared with control condition. Moreover, the stimulation of cell proliferation by PDGF was significantly abolished by concentrations of Mg$^{2+}$ below 0.01 mM. As shown in Fig. 1B, no difference of cell proliferation was observed for concentration of Ca$^{2+}$ below 1 mM in the absence or presence of PDGF. Similar reduction of cell proliferation under extracellular low Mg$^{2+}$ conditions was obtained with two other human osteoblast-like cells, namely SaOs and U2OS (data not shown).

Influence of reduced extracellular magnesium and calcium on the osteoblast migration induced by PDGF. A previous study showed that PDGF induces human osteoblast cell migration (13). We therefore sought to determine the involvement of Mg$^{2+}$ or Ca$^{2+}$ in basal or induced cell migration by PDGF. As shown in Fig. 1C, treatment with low Mg$^{2+}$ (0.1 mM) concentrations reduced the basal migration of MG-63 cells, while cell migration was not affected in low Ca$^{2+}$ (0.1 mM) medium. In addition, we observed that the PDGF treatment stimulated MG-63 cell migration, which was reduced by decreasing Mg$^{2+}$ concentration in the media. Again, low Ca$^{2+}$ (0.1 mM) concentration had no effect on the migration of MG-63 induced by PDGF. These results indicate the importance of Mg$^{2+}$ in the stimulation of MG-63 migration by PDGF.

Importance of calcium and magnesium in cell morphology and adhesion. Migration phenomena implicate changes in the organization of cytoskeleton elements which correlate with changes in cell morphology and adhesion (38). Therefore, we investigated the role of extracellular Ca$^{2+}$ and Mg$^{2+}$ in osteoblast morphology and adhesion in the absence or presence of PDGF. As shown in Fig. 2A, the morphology of cells after 18 h of treatment in the low Ca$^{2+}$ (0.1 mM) condition medium was similar to the control (CTL; 1 mM Ca$^{2+}$ and 0.8 mM Mg$^{2+}$) condition; but when treated in the low Mg$^{2+}$ (0.1 mM) condition, cell morphology was different compared with the CTL condition and cells showed fewer projections (panels at right show higher magnification). Similar results were obtained for cells treated with PDGF. We undertook to correlate this difference with a reorganization of actin filaments of cytoskeleton. Indeed, in Fig. 2B, phalloidin staining for F-actin of cells incubated for 18 h in the low Ca$^{2+}$ (0.1 mM) condition was similar to the control condition; but when cells were treated in low Mg$^{2+}$ (0.1 mM) conditions, phalloidin staining showed less and more diffuse actin staining. PDGF stimulation revealed more actin-rich and fingerlike projections (arrows) extending from the membrane compared with control condition. Similar effects on F-actin were observed in low Mg$^{2+}$ (0.1 mM) or low Ca$^{2+}$ (0.1 mM) conditions in the presence of PDGF (data not shown). Furthermore, as shown in Fig. 2C, incubation of cells for 18 h under low Mg$^{2+}$ condition reduced both basal cell adhesion and cell adhesion induced by PDGF compared with the control condition, whereas reduction of extracellular Ca$^{2+}$ had no effect on basal adhesion and on cell adhesion induced by PDGF.

Effect of PDGF on the influx of calcium and magnesium. Our results indicate that extracellular magnesium is important for PDGF-induced human osteoblast cell proliferation and migration. To investigate the importance of Mg$^{2+}$- and Ca$^{2+}$ influx in the stimulation of cell proliferation and migration by PDGF, we performed intracellular Mg$^{2+}$ and Ca$^{2+}$ measurements with Magnesium Green and Fluo-3, respectively. As shown in Fig. 3A, the addition of PDGF to cells following prior incubation in 1 mM Ca$^{2+}$ and Mg$^{2+}$-free medium had no effect on the intracellular Mg$^{2+}$ level of MG-63 cells in the absence of extracellular Mg$^{2+}$. On the other hand, prior incubation of
cells in 1 mM Ca\(^{2+}\) and Mg\(^{2+}\)-free medium resulted in the observation of a Mg\(^{2+}\) influx on addition of PDGF to the incubation medium in the presence of extracellular 0.8 mM Mg\(^{2+}\) (Fig. 3B), suggesting that PDGF activated plasma membrane Mg\(^{2+}\) channels. Under preincubation conditions of the Fig. 3A, the addition of PDGF to cells in 0.8 mM Mg\(^{2+}\), Ca\(^{2+}\)-free media resulted in a mobilization of Ca\(^{2+}\) from intracellular stores (Fig. 3C). To note, although mobilization of Ca\(^{2+}\) was induced by PDGF, fluorescence of Magnesium Green was not modified as shown in Fig. 3A, indicating that the increase of Magnesium Green fluorescence was specific to Mg\(^{2+}\) influx.

Effect of PDGF on the expression of TRPM7 by osteoblast MG-63. Our results indicate that PDGF promotes the activation of plasma membrane channels that ensure Mg\(^{2+}\) influx. Our previous studies have shown that TRPM7 channels ensure...
Therefore, we speculated that TRPM7 channels may be important in the effects of PDGF on osteoblastic cells. First, we investigated the effect of PDGF on the expression levels of TRPM7 channels. As shown in Fig. 4, the expression of TRPM7 increased by approximately twofold after a 4-h treatment with PDGF compared with control condition where cells had received fresh medium. This upregulation of TRPM7 expression was sustained for a 24 h-period. On the other hand, no difference was noticed for the expression of TRPM6 (data not shown). Our results suggest that PDGF upregulates TRPM7 to ensure long-term Mg$^{2+}$ homeostasis in osteoblastic cells, which promotes proliferation and migration in osteoblastic cells.

**Effect of PDGF on cell proliferation, migration, adhesion, and Mg$^{2+}$ influx under condition of reduced TRPM7 expression.** To determine the importance of TRPM7 channels in the proliferation of osteoblastic cells, MTT assays were performed with cells transfected with specific siRNA against TRPM7 or nontargeted siRNA. Figure 5A shows that the basal and PDGF-induced proliferations of MG-63 cells were inhibited by 60–75% under conditions where TRPM7 expression was reduced with specific siRNAs against TRPM7 (right). Moreover, cell migration (Fig. 5B) and adhesion (Fig. 6B) induced by PDGF were prevented when cells were transfected with siRNA against TRPM7 compared with mock condition without PDGF. We also observed on reduction of TRPM7 expression that the morphology of cells was different from CTL and mock con-
ditions with less projection and the shape of cells was similar to the low Mg\(^{2+}\)/H\(_{11001}\) condition (Fig. 6A). This difference was correlated to a reorganization of actin filaments. Fluorescence intensity of Alexa Fluor 555-conjugated phalloidin in cells silenced for the expression of TRPM7 was reduced and more diffused compared with CTL and mock conditions where actin-rich and fingerlike projections (arrows, Fig. 6D) were easily distinguishable. Furthermore, the reduction of TRPM7 expression prevented Mg\(^{2+}\)/H\(_{11001}\) influx induced by PDGF (Fig. 6C) even when high concentrations of Mg\(^{2+}\) (10 mM) were added in the incubation medium (data not shown), which suggest the absence of compensation pathway for Mg\(^{2+}\) influx.

**DISCUSSION**

Because a reduction in osteoblast number and osteoporosis have been reported under insufficient dietary Mg\(^{2+}\) intake (33) and given that, during bone remodeling, osteoblast proliferation and migration are stimulated in response to growth factors such as PDGF (24), we investigated the importance of Mg\(^{2+}\) and of TRPM7, a channel known for its involvement in the homeostasis of intracellular Mg\(^{2+}\) (1), in the stimulation of osteoblast proliferation and migration by PDGF. Our study indicates that appropriate extracellular Mg\(^{2+}\) levels are essential to the stimulation of osteoblast proliferation and migration by PDGF. Moreover, TRPM7 channels appear a key player in the Mg\(^{2+}\) influx induced by PDGF. Therefore, we provide mechanistic evidence for the alterations of osteoblast functions associated with insufficient dietary Mg\(^{2+}\) intakes, which lead to the development of osteoporosis.

It has been shown that PDGF can promote proliferation and migration in a variety of cell types including osteoblastic cells (23). In accordance, we observed that PDGF stimulates proliferation and migration of human osteoblast-like MG-63 cells. Of interest, we observed a significant reduction of osteoblast proliferation and migration induced by PDGF under culture conditions with TRPM7 expression and Mg\(^{2+}\)/H\(_{11001}\) conditions.
conditions of low extracellular Mg\(^{2+}\) (0.1 mM), while conditions with low extracellular Ca\(^{2+}\) (0.1 mM) concentrations were without effect. These results highlight the important role of Mg\(^{2+}\) in osteoblast functions. The observation that the stimulation of osteoblast proliferation by PDGF was reduced under low extracellular Mg\(^{2+}\) conditions agrees with our previous report about the importance of this ion for osteoblast proliferation (1). Of interest in the current study is that Mg\(^{2+}\) influences the migration of osteoblastic cells because low extracellular concentrations reduced both basal and induced cell migration. Migration phenomena implicate changes in the organization of cytoskeleton elements, which is associated with modifications in cellular morphology and adhesion (38). So we further investigated the effects of low concentrations of extracellular Mg\(^{2+}\) (0.1 mM) or Ca\(^{2+}\) (0.1 mM) on cell morphology, adhesion, and F-actin organization. We observed that basal cell adhesion and cell adhesion induced by PDGF were reduced by low extracellular Mg\(^{2+}\) (0.1 mM), whereas the reduction of extracellular Ca\(^{2+}\) (0.1 mM) had no effect on basal cell adhesion or on cell adhesion induced by PDGF. A change of cell morphology (fewer projections) and reorganization of actin filaments were also observed under low Mg\(^{2+}\) (0.1 mM) conditions; similar effects were observed for low Mg\(^{2+}\) (0.1 mM) conditions in the presence of PDGF while under low Ca\(^{2+}\) (0.1 mM) condition, cells have similar shape compared with control conditions even when they were treated with PDGF. Therefore our results highlight the importance of Mg\(^{2+}\) in maintaining the structural integrity of osteoblastic cells, a necessary parameter for numerous cellular functions (30). Overall, these results indicate that Mg\(^{2+}\) is required for the effects of PDGF on proliferation, adhesion, and migration of osteoblastic cells. Extracellular Mg\(^{2+}\) has been associated with integrin-mediated cell adhesion of tumorigenic osteoblastic cells to type I collagen while this ion was without influence on cell adhesion to laminin-1 and fibronectin (44). However, it is not yet clear whether the importance of Mg\(^{2+}\) in cell adhesion relies on intracellular, extracellular or both levels. We speculated that the effects of PDGF on cell proliferation, morphology, adhesion, and migration rely on adequate intracellular Mg\(^{2+}\) homeostasis. So we hypothesized that PDGF may promote Mg\(^{2+}\) influx to induce cell proliferation and migration in human osteoblasts. Indeed, Mg\(^{2+}\) influx were observed on the addition of PDGF to the incubation medium, indicating that plasma membrane channels were activated. Our previous results have indicated that the TRPM7 channel is involved in the intracellular Mg homeostasis of osteoblastic cells (1). In accordance, PDGF may activate TRPM7 channels to assure Mg\(^{2+}\) influx. Indeed, the silencing of TRPM7 expression prevented the Mg\(^{2+}\) influx induced by PDGF while siRNA treatments had no effect on the depletion of intracellular Ca\(^{2+}\) stores induced by PDGF. Moreover, we showed that the expression of TRPM7 is upregulated by treatment with PDGF. The enhanced expression of TRPM7 was specific since the expression of TRPM6, a close homologue of TRPM7, was not yet clear whether the importance of Mg\(^{2+}\) in cell adhesion relies on intracellular, extracellular or both levels. We speculated that the effects of PDGF on cell proliferation, morphology, adhesion, and migration rely on adequate intracellular Mg\(^{2+}\) homeostasis. So we hypothesized that PDGF may promote Mg\(^{2+}\) influx to induce cell proliferation and migration in human osteoblasts. Indeed, Mg\(^{2+}\) influx were observed on the addition of PDGF to the incubation medium, indicating that plasma membrane channels were activated. Our previous results have indicated that the TRPM7 channel is involved in the intracellular Mg homeostasis of osteoblastic cells (1). In accordance, PDGF may activate TRPM7 channels to assure Mg\(^{2+}\) influx. Indeed, the silencing of TRPM7 expression prevented the Mg\(^{2+}\) influx induced by PDGF while siRNA treatments had no effect on the depletion of intracellular Ca\(^{2+}\) stores induced by PDGF. Moreover, we showed that the expression of TRPM7 is upregulated by treatment with PDGF. The enhanced expression of TRPM7 was specific since the expression of TRPM6, a close homologue of TRPM7, was...
not modified by PDGF. This growth factor may promote upregulation of TRPM7 expression to ensure long-term Mg\(^{2+}\) homeostasis in osteoblastic cells. Of interest, silencing TRPM7 expression, in addition to preventing PDGF-induced Mg\(^{2+}\) influx, inhibited the stimulation of cell proliferation and migration by PDGF. A change of cell morphology (fewer projections) was also observed when TRPM7 expression was reduced by specific siRNA, which correlates with our previous observations under low extracellular Mg\(^{2+}\) (0.1 mM) conditions. Therefore, our results suggest that TRPM7-mediated Mg\(^{2+}\) influx is important for PDGF-induced cell proliferation and migration in human osteoblasts. Such Mg\(^{2+}\) influx will likely ensure the adequate intracellular ion levels essential for numerous cellular functions triggered by PDGF. To note, no Mg\(^{2+}\) influx was seen in TRPM7-deficient cells stimulated by PDGF even when high 10 mM Mg\(^{2+}\) concentrations were added to the incubation medium, which suggests the absence of compensation pathway for Mg\(^{2+}\) influx. Our previous published results also indicate that no other Mg\(^{2+}\) entry pathway can supply for TRPM7 silencing in the osteoblasts (1). In accordance, studies have reported both rescue (19, 27, 37) and no effect (14, 45) by Mg\(^{2+}\) supplementation in TRPM7-deficient cells, which has been stated to depend on cell types. More specifically, overexpression of other Mg\(^{2+}\) entry pathways, such as the Mg\(^{2+}\) transporter SLC41A2, has been shown to compensate for the loss of TRPM7 channel and maintain Mg\(^{2+}\) at sufficient levels for the cells to proliferate in a manner which directly correlates with their level of expression (34).

In a molecular point of view, it has been reported that TRPM7 channels regulate cell adhesion through m-calpain (protease that control cell adhesion through focal adhesion disassembly) by mediating the local influx of calcium into peripheral adhesion complexes (39). The cellular adhesion and motility were enhanced when the expression levels of TRPM7 were reduced. In contrast, Clark et al. (8) have demonstrated in rat basophil leukemia cells that the COOH-terminal α-kinase activity of TRPM7 channels regulates myosin II A filament stability and localization by phosphorylating a short stretch of amino acids within the α-helical tail of the myosin II A heavy chain. They observed that low overexpression of TRPM7 induces cell spreading and increases cell adhesion. Sugden and Nihei (40) have suggested that both Ca\(^{2+}\) and Mg\(^{2+}\) can bind tightly to myosin, although only the binding of Mg\(^{2+}\) shows a direct influence on the enzymatic activity. On the other hand, Mg\(^{2+}\) is an important modulator of the intracellular Ca\(^{2+}\) levels, the latter being associated with cell contraction, proliferation, migration, and secretion (42). Therefore, changes in intracellular Mg\(^{2+}\) levels could lead to significant effects on Ca\(^{2+}\)-dependent signaling pathways that regulate cellular functions.

Magnesium deficiency is common among the general population because its intake has decreased over the years in a significant proportion of the population, especially in the Western world. Many important human pathologies such as hypertension, heart failure, several nervous system complaints, muscle diseases, and atherosclerosis have been associated with a decrease in Mg\(^{2+}\) availability (20). In addition, coexisting disorders that impair intestinal Mg\(^{2+}\) absorption and/or are associated with renal Mg\(^{2+}\) loss, such as malabsorption syndromes, alcoholism, diabetes mellitus, and drugs (e.g., diuretics), would place an individual at even greater risk. Physiological concentration of Mg\(^{2+}\) is 0.8 to 1.0 mM and concentrations ranging between 0.6 and 0.7 mM are frequent in apparently healthy Western people, with lowest levels of 0.4 to 0.5 mM in critically ill subjects (43). Of interest, epidemiologic studies provide a link associating insufficient dietary Mg\(^{2+}\) intake in humans with low bone mass and osteoporosis (for a review, see Ref. 31). Moreover, genetic hypomagnesaemia with renal Mg\(^{2+}\) wasting leads to low bone mass (31). Experimental Mg\(^{2+}\) deficiency in animal models, where plasma Mg\(^{2+}\) levels range from 0.1 to 0.4 mM, has resulted in impaired bone growth, osteopenia, and increased skeletal fragility with a decrease in both osteoblast number and osteoclast activity associated with a form of aplastic bone disease (32, 33). Progressive alterations of the endothelial cell functions, in accordance with a pro-atherogenic environment leading to cardiovascular disease, have been reported following 1 to 6 days of cell culture in low (0.1 and 0.5 mM) Mg\(^{2+}\) concentration conditions (22). Such alterations were associated with modulations of gene expression related to cell adaptation. Therefore, Mg\(^{2+}\) depletion influences cell functions in a dose- and time-dependent manner. Our experimental protocol corresponds to severe Mg\(^{2+}\) depletion conditions for short period of time that may not directly apply to human disease, which is more likely associated with moderate Mg\(^{2+}\) deficiency for a long period of time, as years. However, such experimental strategy has the benefit to exclude potential cell adaptation to low Mg\(^{2+}\) concentration condition as reported by Maier et al. (22) and allow investigating the roles of intracellular Mg\(^{2+}\) and TRPM7 activity on cell morphology, adhesion, proliferation, and migration. Our current results indicate that Mg\(^{2+}\) influx and TRPM7 are important for PDGF-induced human osteoblast proliferation and migration. Such reduction in osteoblast cell proliferation would lead to inadequate bone formation and poor regulation of resorption, resulting in the development of osteoporosis.

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GRANTS

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