Journal of Cellular Biochemistry 92:745–754 (2004)

# Differential Activation of ERK1,2 MAP Kinase Signaling Pathway in Mesenchymal Stem Cell From Control and Osteoporotic Postmenopausal Women

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**Abstract** Osteoblasts, the cells responsible for bone formation, derive from mesenchymal stem cells (MSCs) in bone marrow. To acquire a new cell phenotype, uncommitted MSCs must undergo several proliferation and differentiation changes. Although, it is known that extracellular signal-regulated protein kinases (ERKs) mitogen-activated protein (MAP) kinase pathway signaling is involved in the proliferation and differentiation processes, the role of ERKs in osteogenic differentiation it is controversial, at present. In addition, the function that ERK could play in MSCs derived from osteoporotic patients it is not well documented. In this study, we analyze whether previously observed differences in the dynamic response of MSCs from normal and osteoporotic postmenopausal women can be explained by changes in the activation of this signal transduction pathway. Levels of ERK phosphorylation and their correlation with osteogenic differentiation were evaluated in cultures of MSCs derived from osteoporotic postmenopausal women and ''healthy'' controls. The results show that, under basal conditions, MSCs derived from osteoporotic donors show a level of ERK phosphorylation 2.5 times higher than MSCs derived from control donors. The addition of the osteogenic stimulus only slightly increases the p-ERK level in cells derived from osteoporotic donors, and is higher in cells derived from control women. Important differences in the ability of PD98059 to inhibit phosphorylation of ERK in both types of cells were also observed, as well as the effect that this inhibition produced on calcium deposition. We conclude that the MAP kinase pathway signaling is differentially activated in MSCs derived from osteoporotic postmenopausal women. The high p-ERK levels in MSC derived from osteoporotic donors could determine the unresponsiveness of these cells to the osteogenic differentiation stimulus. J. Cell. Biochem. 92: 745–754, 2004. 

**Key words:** osteogenesis; osteoporosis; mesenchymal stem cells; mitogen-activated protein kinase; extracellular signal-regulated protein kinase; p-ERK

Bone formation is a complex process involving the proliferation of pluripotent progenitor cells, their differentiation into osteogenic progenitor cells, migration to the bone surface, and differentiation into osteoblasts. This results in the secretion of abundant extracellular bone matrix proteins and their eventual calcification [Long et al., 1995; Lecanda et al., 1997]. Osteoblasts, the cells responsible for bone formation, derive from mesenchymal stem cells (MSCs) present in the bone marrow. MSCs are multipotent, capable of differentiating into bone, cartilage, adipose, and other connective tissues [Caplan, 1991; Haynesworth et al., 1992; Dennis et al., 1999; Pittenger et al., 1999]. Under normal conditions, bone formation and resorption are processes finely regulated to maintain a constant bone mass. Altered dynamic responses of bone cells progenitors may be responsible for specific bone diseases [Gimble et al., 1996; Nuttall et al., 1998; Rodríguez et al., 1999, 2000].

To acquire a new cell phenotype, uncommitted MSCs must undergo proliferation and differentiation, processes which are fundamental biological in the cell life cycle. In this regard, it is well documented that mitogen-activated protein (MAP) kinases are involved in the proliferation and differentiation of MSCs derived from healthy donors [Jaiswal et al., 2000]. It is also known that growth factors, hormones such as estrogens and parathyroid hormone, which have a profound effect on bone

Grant sponsor: FONDECYT; Grant number: 1020728.

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Received 18 December 2003; Accepted 5 March 2004

DOI 10.1002/jcb.20119

development and remodeling, transiently activate extracellular signal-regulated protein kinase (ERK)1,2.

Evidence has been presented that MAP kinase pathway signaling is involved in the differentiation of MSCs into osteogenic and adipogenic lineages. However, results in the literature are still controversial [Jaiswal et al., 2000; Xiao et al., 2000, 2002; Gallea et al., 2001; Higuchi et al., 2002; Suzuki et al., 2002; Hu et al., 2003]. Some authors have shown that enhanced phosphorylation of the ERK1,2 stimulates differentiation of MSCs to the osteogenic lineage, while inhibition of this phosphorylation pathway leads to adipogenic differentiation of the cells [Jaiswal et al., 2000]. Other authors have demonstrated that continuous inhibition of ERK1,2 phosphorylation stimulates the osteogenic differentiation of MSCs [Higuchi et al., 2002].

Osteoporosis is an age-related disease characterized by decreased skeletal mass caused by an imbalance between bone resorption and formation [Manolagas and Jilka, 1995]. Research efforts to elucidate the origin and cause of osteoporosis could help develop new therapeutic approaches. Most studies have focused on the demonstration that enhanced osteoclastic activity is a key factor related to the bone mass decrease [Horowitz, 1993; Manolagas and Jilka, 1995]. Altered bone formation as a pathogenic factor has also been explored [Gimble et al., 1996; Nuttall et al., 1998; Bianco and Robey, 1999]. In this regard, we reported previously that MSCs derived from osteoporotic donors exhibit important functional differences compared to MSCs obtained from control donors: cell growth, proliferative responses, type I collagen synthesis, and osteogenic and adipogenic differentiation are significantly affected [Rodríguez et al., 1999, 2000].

In this study, we analyze whether the activation status of the ERK1,2 signal transduction mechanism is different in MSCs from osteoporotic donors compared with MSCs derived from control donors. Differences in the activation of this pathway may explain, at least in part, the changes observed previously in the dynamic response of MSCs derived from normal and osteoporotic postmenopausal women [Rodríguez et al., 1999, 2000]. Findings from this study may contribute to better understand some of the cellular and molecular mechanisms related to the pathogenesis of osteoporosis.

## MATERIALS AND METHODS

### Subjects

Control and osteoporotic postmenopausal women (65–75 years old), who were patients from the Bone Trauma Section, Hospital Sótero del Río, in Santiago, Chile, were selected as bone marrow donors. "Healthy" women, without bone disease, constituted the control group. Control and osteoporotic donors were not under any therapies, which may affect the cells of the osteoblastic lineage. After informed consent, bone marrow was obtained by iliac crest aspiration during surgical procedures [Rodríguez et al., 1999]. It is important to note that, although the marrow samples were derived from independent human donors, significant heterogeneity was not detected with respect to the functional characteristics analyzed in this study, for example, in the extent of the phosphorylation of ERK1,2 (p-ERK1,2), alkaline phosphatase activity, or calcium deposition.

Donors were considered osteoporotic when their bone mineral density was $<2.5$ standard deviations (SD) below the mean for young adults, and in addition had suffered hip fracture. The bone mineral density for control donors ranged between $>-1.0$ SD and $<2.5$ SD [Raisz, 1997]. Bone mineral density was measured using dual-energy X-ray absorptiometry (DXA).

### Cell Preparation and Culture Methods

MSCs were isolated from bone marrow as previously described [Jaiswal et al., 1997]. Briefly, 10 ml of bone marrow aspirate was added to 20 ml of Dulbecco's minimal essential (DME) medium (Sigma, St. Louis, MO) containing 10% fetal bovine serum (FBS) (basal medium), centrifuged to pellet cells and the fat layer was discarded. Cells were suspended in culture medium and fractionated on a 70% Percoll (Sigma) density gradient. The MSCs-enriched low-density fraction was collected, rinsed with culture medium, and plated at $1–2 \times 10^7$ nucleated cells/100 mm dish (Nunc, Naperville, IL). Cells were cultured at 37°C in a humidified atmosphere of 5% $CO_2$. After 4 days in culture, non-adherent cells were removed and fresh culture medium was added. The culture medium was replaced by fresh medium twice weekly. When culture dishes became near confluent, cells were detached by a mild treatment with trypsin (0.25%, 5 min, 37°C), and

replated at a 1/3 density for continued passaging. The experiments described here were performed after the fourth cell passage.

### Osteogenic Differentiation

MSCs from control and osteoporotic donors were maintained in osteogenic culture medium (basal medium supplemented with 100 nM dexamethasone, 10 mM β-glycerophosphate, and 50 μg/ml ascorbic acid added daily (OS)) for up to 10 days [Rodríguez et al., 1999]. The ability of MSCs to differentiate into the osteoblastic lineage in vitro was evaluated measuring an early marker, such as alkaline phosphatase activity, and a late marker, calcium phosphate deposition on the cell layer [Hu et al., 2003]. At selected times (0, 3, and 7 days of culture), the culture medium was removed and alkaline phosphatase activity was measured as previously described [Martínez et al., 1996]. After 10 days in culture, the calcium phosphate crystals deposited on the cell layer were solubilized in 0.5 N HCl, and the amount of calcium recovered was measured by atomic absorption spectroscopy (423 nm) [Rodríguez et al., 2002].

### Western Blot and Activation of ERK1,2

Cells ($1 \times 10^5$ cells) were seeded in 6-wells plates (Nunc) and cultured under basal and osteogenic conditions. At selected times (3 and 7 days), cells were lysed and 12 μg of protein were separated on a 10% sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) under reducing conditions [Laemmli, 1970]. Afterwards, gels were blotted onto polyvinylidenedifluoride (PDVF) membrane (BioRad, Hercules, CA) and ERK1,2 activation was determinated as previously described [Santibañez et al., 2002] using antibodies recognizing the phosphorylated and non-phosphorylated forms of ERK1,2 (Santa Cruz Biotech, Santa Cruz, CA). Peroxidase-conjugated goat anti-rabbit or peroxidase-conjugated goat anti-mouse secondary antibodies (Rockland, Gilbertsville, PA) were used. Immunoreactivity was determined using the ECL chemiluminescence reaction (Amersham Pharmacia Biotech, Piscataway, NJ). For relative levels of ERK1,2 and p-ERK1,2, blots were scanned (Snapscan 1212 AGFA scanner) and the Kodak Digital 1D image MDS 120 software was used for densitometric blot analysis.

To analyze the role of ERK1,2 activity, PD98059 (Calbiochem, La Jolla, CA) (25 μM), a specific MEK1,2 (MAP kinase/ERK kinase) inhibitor, was added to cell cultures at indicated times.

## RESULTS

### Phosphorylation Level of ERK1,2

The p-ERK1,2 was measured in MSCs derived from control (hC-MSCs) and osteoporotic (hO-MSCs) donors, cultured in basal and osteogenic culture medium.

Under basal conditions, the phosphorylation level of ERK1,2 measured in hO-MSCs was always higher than the level observed in hC-MSCs. Figure 1A shows ERK1,2 and p-ERK1,2-separated by SDS–PAGE and analyzed by Western blot from three independent control and three osteoporotic samples. The densitometric analysis of the different bands allowed to determine the p-ERK1,2/ERK1,2 ratio. As

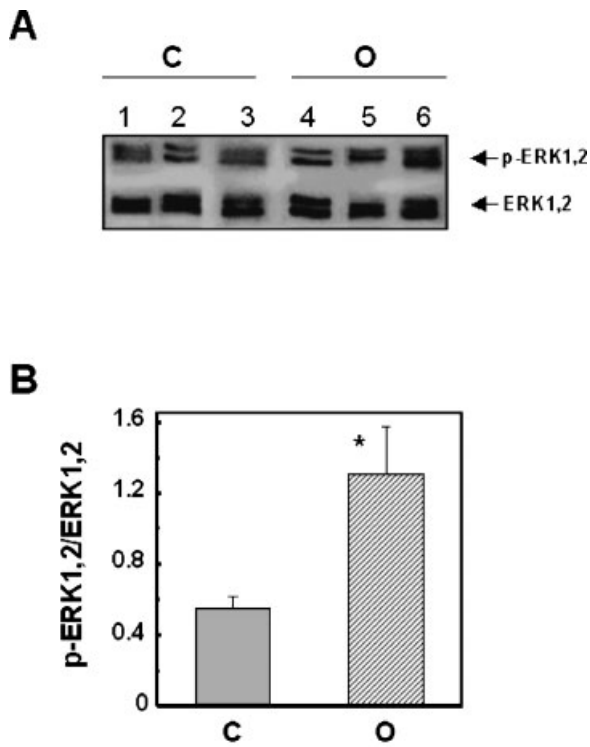


**Fig. 1.** Phosphorylation of extracellular signal-regulated protein kinase (ERK). Mesenchymal stem cells (MSCs) derived from control (3) and osteoporotic (3) donors were cultured in basal conditions. **Panel A**: ERK1,2 and the phosphorylated form of ERK1,2 (p-ERK1,2) were separated by sodium dodecylsulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and analyzed by Western blot. **B**: The ratio of p-ERK/ERK was determined from the control (3) and osteoporotic (3) samples analyzed in panel A. The results were expressed as mean ± standard deviation (SD). (*$P < 0.05$.)

shown in Figure 1B, the p-ERK1,2/ERK1,2 ratio determined in hO-MSCs was 2.5 times higher that the ratio determined in hC-MSCs ($1.34 \pm 0.23$ and $0.57 \pm 0.04$, respectively).

Under osteogenic (OS) conditions, the p-ERK1,2 level depends on whether MSCs were derived from control or osteoporotic donors. After 3 days in OS medium, the p-ERK1,2/ERK1,2 ratio of hC-MSCs was 2.5 times higher than the ratio in cells cultured under basal conditions. After the same time interval in culture, hO-MSCs did not exhibit significant changes in p-ERK1,2/ERK1,2 ratio (1.1 times) compared with the same cells cultured in basal conditions. After 7 days, the p-ERK1,2/ERK1,2 ratio in hC-MSCs decreased compared to the ratio at day 3 of culture, and it was only 1.6 times higher in OS than in basal conditions. Conversely, in OS conditions the p-ERK1,2/ERK1,2 ratio in hO-MSCs was a half of the ratio determined in basal conditions (Fig. 2A,B). The p-ERK1,2/ERK1,2 ratio in basal conditions did not show differences between day 3 and 7 of culture. These results show that not only the basal level of p-ERK1,2 was different in hO-MSCs compared to hC-MSCs, but also that the ability to increase the extent of ERK1,2 phosphorylation as response to the osteogenic stimuli was different.

### Inhibition of ERK1,2 Phosphorylation Level

We also investigated whether PD98059, a specific MEK1,2 inhibitor, produces a similar effect on MSCs derived from both types of

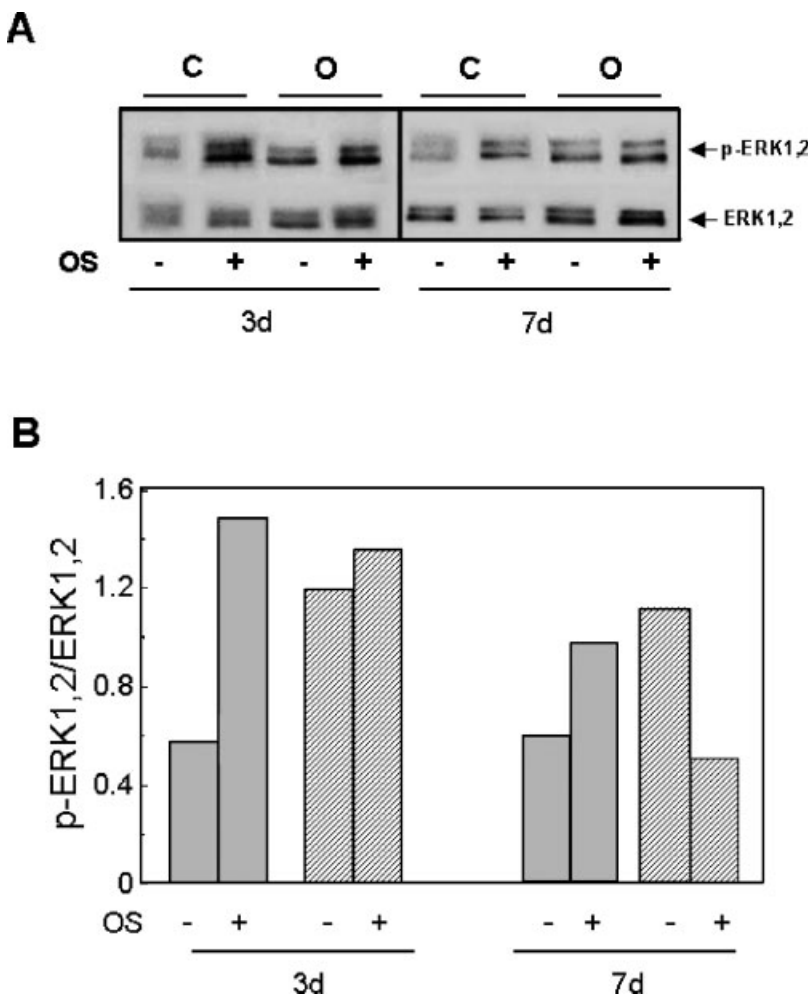


**Fig. 2.** Phosphorylation of ERK in basal and osteogenic conditions hC-MSCs (gray bars) and hO-MSCs (dashed bars) were cultured during 3 and 7 days in basal and osteogenic conditions. **Panel A**: ERK1,2 and p-ERK1,2 were separated by SDS–PAGE and analyzed by Western blot. **B**: The ratio p-ERK/ERK was determined from the results presented in panel A. Figure shows the results of a representative experiment from six experiments performed with different samples (control = 3 and osteoporotic = 3).

donors. In hC-MSCs the p-ERK1,2 was inhibited by PD98059 in the culture medium. However, hO-MSCs were insensitive to inhibition by PD98059 (Fig. 3A,B). In control cells cultured under osteogenic conditions, the maximal inhibition was reached as early as 3 h of treatment, and its extent was 65% compared with the p-ERK1,2 in the absence of the inhibitor. After this stage, the level of phosphorylation recovered slightly reaching 50% compared with the phosphorylation level in the absence of the inhibitor. hO-MSCs cultured in the same conditions, were insensitive to the presence of the inhibitor PD98059. After 1 h of treatment with the inhibitor, the pERK1,2 level remains constant, and after 3 h of treatment, time at which cells derived from control donors reached the maximum inhibition value, hO-MSCs not only

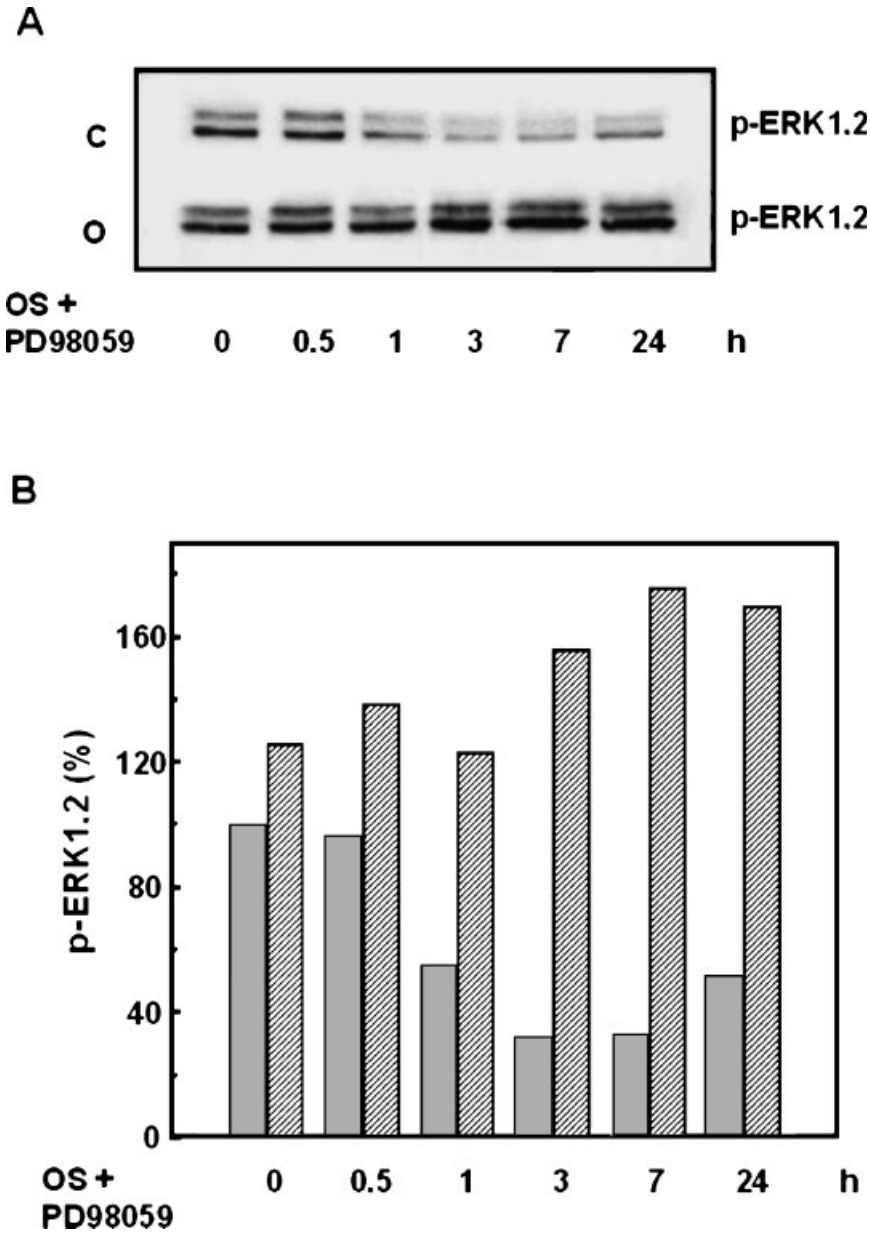


**Fig. 3.** Inhibition of ERK phosphorylation by PD98059. hC-MSCs and hO-MSCs were cultured in basal and osteogenic conditions in the presence or not of 25 μM PD98059. Figure shows the levels of p-ERK1,2 in osteogenic conditions. The p-ERK1,2 level at different times in culture is expressed as a percentage respect to the level of p-ERK at zero time. Gray bars represent hC-MSCs and dashed bars represent hO-MSCs. Results were obtained from six different donors: three controls and three osteoporotics. Figure shows the results of a representative experiment from six experiments performed with different samples (control = 3 and osteoporotic = 3).

did not exhibit an inhibition but showed a slightly increase in the ERK1,2 phosphorylation as compared with phosphorylation in the absence of inhibitor. At longer time intervals in culture, 7 and 24 h, the level of ERK1,2 phosphorylation by hO-MSCs continued to increase slightly (Fig. 3B). After 7 days of culture in the presence of PD98059, hC-MSCs showed a similar level of pERK1,2 that observed after 24 h of treatment and hO-MSCs did not exhibit inhibition in the ERK1,2 phosphorylation (data not shown). The same inhibition pattern was observed in cells derived from control and osteoporotic donors cultured under basal conditions (data not shown).

### Effect of PD98059 on Osteogenic Differentiation

We analyzed the ability of hC-MSCs and hO-MSCs to differentiate into the osteoblastic lineage in the absence or in the presence of PD98059. Alkaline phosphatase activity and the extent of the calcium deposition were determined as biochemical markers of osteogenic differentiation.

As reported previously [Rodríguez et al., 1999], hC-MSCs and hO-MSCs expressed alkaline phosphatase activity when cultured in osteogenic medium, but the enzymatic activity was 3.5–4 times higher in hC-MSCs than in hO-MSCs at day 7 of culture (Fig. 4).

To determine the existence of temporal changes in the expression of the alkaline phosphatase activity in the presence on PD98059, the enzyme activity was measured at selected times (0, 1, 3, 5, 7, and 10 days). The profile of alkaline phosphatase expression by MSCs derived from control and osteoporotic donors cultured in osteogenic medium was affected differently by the presence of PD98059. Thus, in hC-MSCs cultured in osteogenic medium, the inhibitor induced a shift in the expression of the enzyme towards earlier stages of culture (day 3) compared with the expression in the absence of the inhibitor (Fig. 4), increasing importantly the enzymatic activity at this time. Thus, at day 3 of culture, the activity of alkaline phosphatase in cells cultured in the presence of PD89059 was 3–4 times higher than the activity in hC-MSCs cultured in the absence of inhibitor. On day 7 of culture, alkaline phosphatase activity reached its maximum value, which is similar in cells cultured in the presence or in the absence of PD89059. Subsequently, the enzymatic activity of cells treated and untreated with the inhibitor decreased. In other hand, the expression of

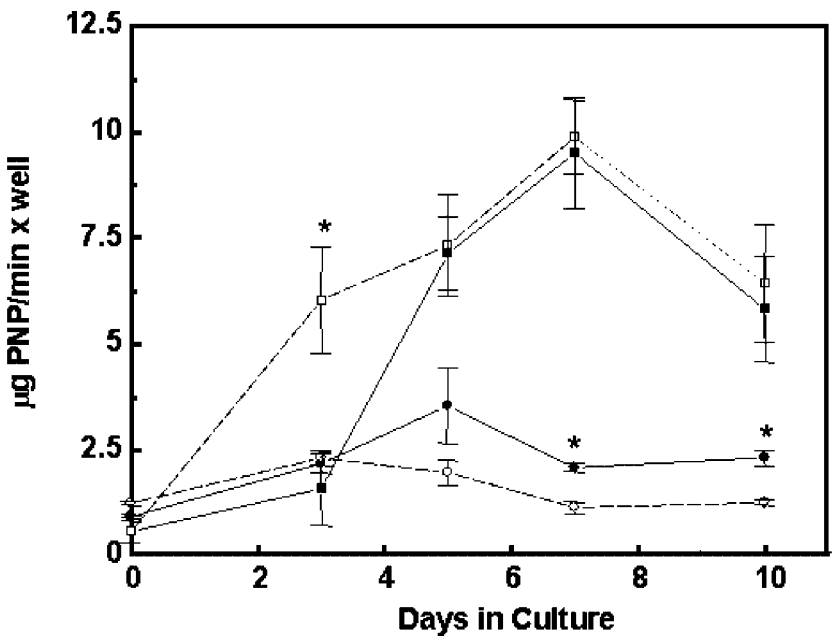


**Fig. 4.** Alkaline phosphatase activity was determined in hC-MSCs (square) and hO-MSCs (circle) cultured in osteogenic conditions in the absence (filled) or presence (open) of 25 µM PD98059. Results were obtained from six different donors: three controls and three osteoporotics. Each experiment was performed in triplicate and results were expressed as mean ± SD of micrograms of *p*-nitrophenol (PNP) produced/min × well. (*$P < 0.05$, as compared with values in the absence of inhibitor.)

alkaline phosphatase activity in hO-MSCs cultured in osteogenic medium remained close to the basal level during all the period tested. The presence of PD98059 in the osteogenic medium did not induce changes in the expression of the alkaline phosphatase activity by hO-MSCs during the first 3 days of culture (Fig. 4). After this, the presence of PD98059 produced a slight inhibition of the enzymatic activity.

Both, hC-MSCs and hO-MSCs cultured in osteogenic medium were able to deposit calcium phosphate. As we reported previously [Rodríguez et al., 1999], calcium deposition by hC-MSCs was 2–3 times higher than the amounts deposited by hO-MSCs (data not shown). The addition of the inhibitor PD98059 to the osteogenic differentiation medium also affected in a different manner the ability of hC-MSCs and hO-MSCs to stimulate calcium deposit. The presence of the inhibitor in the culture medium increased the ability of hC-MSCs to deposit calcium by 3.0 times as compared with cells cultured in osteogenic medium in the absence of the inhibitor. However, the presence of PD98059 in the osteogenic medium had a lesser effect on the calcium deposition by hO-MSCs as compared with the effect produced on hC-MSCs. As shown in Figure 5, the calcium deposited by hO-MSCs in the presence of the inhibitor showed only a discrete increase (1.5 times) compared with control cells (Fig. 5). Under basal conditions, calcium deposition was not affected in both types of cells by PD98059 (data not shown).

## DISCUSSION

Activation of the MAP kinase-signaling cascade has been associated with many different cellular signaling events. Thus, some reports implicated this pathway in the differentiation of MSCs to the osteoblastic lineage [Jaiswal et al., 2000; Higuchi et al., 2002], and others in cell proliferation and survival [Lai et al., 2001; Hu et al., 2003].

In this regard, several reports have suggested a role for the involvement of ERK-MAP kinase during differentiation of MSCs to osteoblasts [Jaiswal et al., 2000; Xiao et al., 2000, 2002; Gallea et al., 2001; Higuchi et al., 2002; Suzuki et al., 2002], however, this role still remains controversial. In this study, we analyze whether the phosphorylation status of ERK1,2 is different in MSCs derived from control and osteoporotic postmenopausal women.

We have previously reported that hO-MSCs exhibit important differences in some functional characteristics as compared with hC-

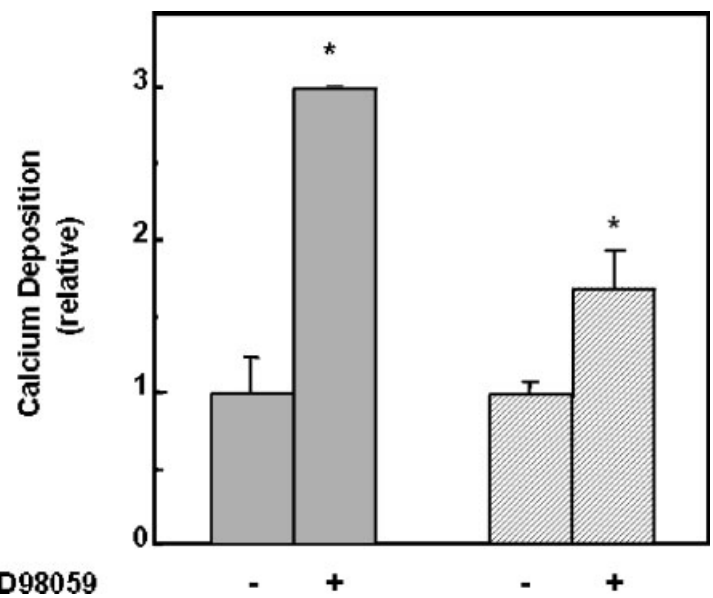


**Fig. 5.** Calcium deposition was determined in hC-MSCs (gray bars) and hO-MSCs (dashed bars) after 10 days of culture in osteogenic conditions, in the absence or presence of 25 μM PD98059. Results were obtained from six different donors: three controls and three osteoporotics. Calcium deposition was expressed in a relative manner respect to calcium deposited in osteogenic conditions in the absence of inhibitor. Each experiments was performed in triplicate and results were expressed as mean ± SD. (*$P < 0.05$.)

MSCs. Among them, cell growth, proliferative response, type I collagen synthesis, and osteogenic differentiation are significantly affected [Rodríguez et al., 1999]. We have also reported that hO-MSCs have an altered capacity to differentiate into osteoblasts in vitro and preferentially respond to adipogenic stimuli [Rodríguez et al., 2000]. The study of the hO-MSCs may provide a better understanding of the mechanisms involved in the pathogenesis of osteoporosis.

The results presented here show that, as with the functional characteristics mentioned above, hO-MSCs also showed an altered behavior in the MAP kinase pathway. This fact is evidenced by the high level of p-ERK under basal conditions, the unresponsiveness to increase the p-ERK1,2 level with the osteogenic stimulus, and the insensitivity to the inhibitor PD98059. The fact that basal phosphorylation and activation of the ERK1,2 in hC-MSCs and hO-MSCs occur in a different manner provide the first evidence that differences in ERK1,2 activity could be implicated in the reduced ability of hO-MSCs to differentiate into the osteoblast lineage. Under basal conditions, hO-MSCs exhibit a constitutive level of p-ERK1,2 2–3 times higher than hC-MSCs (Fig. 1). Under osteogenic conditions, the ratio p-ERK1,2/ERK1,2 did not change in hO-MSCs but increased 2.5 times in hC-MSCs (Fig. 2). In this study, we also demonstrate differential effects of PD98059 in the inhibition of ERK phosphorylation in hC-MSCs and hO-MSCs. hO-MSCs were insensitive to the inhibitor PD98059, but it inhibited the phosphorylation of ERK in hC-MSCs (Fig. 3). At present, we do not known a mechanism explaining the slightly increase of ERK phosphorylation observed in hO-MSCs in the presence of the inhibitor PD98059. New experiments are necessary to dilucidate this point, and are currently in progress in our laboratory. However, this fact support our suggestion that a high level of p-ERK1,2 is not a sufficient signal to promote osteogenic differentiation in MSCs. Results obtained here relate with the activation of ERK1,2, in response to the osteogenic stimulus, in hC-MSCs closely agree with those reported by Jaiswal et al. [2000].

Our results suggest that a high level of p-ERK1,2 is not by itself a signal strong enough to promote appropriate osteogenic differentiation. This is supported by the fact that under basal conditions hO-MSCs exhibited a high level of p-ERK1,2 but a decreased ability to differentiate into the osteogenic lineage. It is known that a large number of signals can stimulate the MAP kinase cascade, but only a select number of these can affect osteoblast differentiation. Activation of MAP kinase signaling induces transcription of osteoblast-specific genes and this is accomplished with phosphorylation of core binding factor 1 (Cbfa1). It is likely that the activation of MAP kinase pathway must be present together with other appropriate stimuli for Cbfa1 activation to take place [Xiao et al., 2000].

In this study, we also report that increased alkaline phosphatase activity associated with the MEK1,2 inhibition by PD98059 in hC-MSCs cultured in osteogenic conditions correlates with a greater calcium deposition as compared with cells cultured in the absence of the inhibitor. This finding agrees with previously reported results showing that continuous inhibition of ERK1,2 MAP kinase signaling promotes early osteoblastic differentiation of human bone morphogenetic protein-2 (h-BMP-2) treated mesenchymal cells [Higuchi et al., 2002]. Our results are in agreement with those reported recently by Hu et al. [2003], indicating that the inhibition of ERK1,2 phosphorylation increases the expression of alkaline phosphatase activity and the calcium phosphate deposition in h-BMP-2-treated mouse calvarial osteoblasts. Conversely, in hO-MSCs cultured in osteogenic medium with PD98059 we did not observe significant changes in alkaline phosphatase expression nor in calcium deposition. The extent of the inhibition of ERK1,2 phosphorylation by PD98059 is related to the level of alkaline phosphatase activity and the amount of calcium deposited. The results presented here allow us to speculate that the induction of the alkaline phosphatase activity during the first stages (3 days) of osteogenic differentiation determines the extent of calcium deposition at the end of the test period (10 days).

The constitutive p-ERK1,2 and the low response of hO-MSCs to PD98059 inhibition reported in this study cannot be easily explained at this time; however, we can speculate that (a) an activated or overexpressed tyrosine kinase receptor could maintain high levels of p-ERK1,2, producing strong and rapid activation of MEK1,2 despite PD98059 treatment and, (b) a low activity of ERK1,2 specific phosphatase maintains higher cellular levels

of phosphorylated ERK1,2 bypassing MEK1,2 inhibition. Further studies of the upstream regulatory mechanism of ERK1,2 MAP kinase activation and/or dephosphorylation by specific ERK1,2 phosphatases are necessary to elucidate these phenomena.

Preliminary experiments showed no significant differences in the basal activation of the transcriptional factor smad1 by BMP in hO-MSC, which could explain the loss of osteogenic capacity by these cells (data no shown). It is possible that high ERK1,2 activity may suppress BMP signal by interfering with Smad1 transactivity. Because direct phosphorylation of Smad1 by ERKs is not required for inhibition, other transcriptional factors also regulated by ERKs might be involved in the regulation of osteoblastic differentiation [Nakayama et al., 2003].

It has been proposed [Higuchi et al., 2002] that MEK-1 inhibitors would be useful in vivo for promotion of bone formation, for instance, in cases of delayed fracture healing or focal osteoporotic changes around fracture sites. The usefulness of the MEK-1 inhibitor to promote bone formation seems to be evident according to the results presented here using hC-MSCs. However, the results obtained with hO-MSCs are different; in these cells, the MEK inhibitor was unable to promote a significant bone mineralization. This strongly suggests that inhibitors of the MAP kinase pathways would not be good candidates in the design of new therapeutic strategies for treatment of osteoporosis. Since the precise underlying action mechanism of these molecules is not completely understood, experiments are currently in progress in our laboratory to gain further knowledge about the cellular and molecular mechanisms involved in MSCs differentiation.

We conclude that the MAP kinase pathway is differently activated in hC-MSCs than in hO-MSCs, and we suggest that the high p-ERK1,2/ERK1,2 ratio observed in hO-MSCs under basal conditions, could determinate the unresponsiveness of MSCs derived from postmenopausal women to the osteogenic differentiation stimulus.

## ACKNOWLEDGMENTS

The authors thank Dr. O. Brunser and Dr. M. Llanos for critical review of the manuscript and valuable comments.

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