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FAST TRACK

The Cell Survival Signal Akt is Differentially Activated by PDGF-BB, EGF, and FGF-2 in Osteoblastic Cells

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Abstract Stimulation of osteoblast survival signals may be an important mechanism of regulating bone anabolism. Protein kinase B (PKB/Akt), a serine-threonine protein kinase, is a critical regulator of normal cell growth, cell cycle progression, and cell survival. In this study we have investigated the signaling pathways activated by growth factors PDGF-BB, EGF, and FGF-2 and determined whether PDGF-BB, EGF, and FGF-2 activated Akt in human or mouse osteoblastic cells. The results demonstrated that both ERK1 and ERK2 were activated by FGF-2 and PDGF-BB. Activation of ERK1 and ERK2 by PDGF-BB and FGF-2 was inhibited by PD 098059 (100 μM), a specific inhibitor of MEK. Wortmannin (500 nM), a specific inhibitor of phosphatidylinositol 3-kinase (PI 3-K), inhibited the activation of ERK1 and ERK2 by PDGF-BB but not by FGF-2 suggesting that PI 3-K mediated the activation of ERK MAPK pathway by PDGF-BB but not by FGF-2. Rapamycin, an inhibitor of p70 S6 protein kinase and a downstream target of ERK1/2 and PI 3-K, did not affect the activation of ERK1 and ERK2 by the growth factors. Furthermore, our results demonstrated that Akt, a downstream target of PI 3-K, was activated by PDGF-BB but not by FGF-2. Akt activation by PDGF-BB was inhibited by PI 3-kinase inhibitor LY294002. Rapamycin had no effect on Akt activation. Epidermal growth factor (EGF) also activated Akt in osteoblastic cells which was inhibited by LY294002 but not by rapamycin. Taken together, our data for the first time revealed that the activation of ERK1/2 by PDGF-BB is mediated by PI 3-K, and secondly, Akt is activated by PDGF-BB and EGF but not by FGF-2 in human and mouse osteoblastic cells. These results are of critical importance in understanding the role of these growth factors in apoptosis and cell survival. PDGF-BB and EGF but not FGF-2 may stimulate osteoblast cell survival. J. Cell. Biochem. 81:304–311, 2001.

Key words: Akt; protein kinase B; osteoblast; cell survival; PDGF-BB; FGF-2; EGF

During bone remodeling, the process of new bone formation involves the recruitment of osteoprogenitors which mature into bone forming osteoblasts that actively synthesize and mineralize bone matrix [Lian et al., 1999; Mundy, 1999]. Basic fibroblast growth factor (FGF-2) and platelet-derived growth factor (PDGF) play important roles in the regulation of osteoblastic cell proliferation and differentiation and bone formation, however, the molecular mechanisms of action of these growth factors that enable them to functionally affect the osteoblast development and maturation are not well understood. PDGF stimulates cell growth of normal human osteoblastic (HOB) cells [Zhang et al., 1991; Kim and Valentini, 1997] and MC3T3-E1 cells [Okazaki et al., 1992]. FGF-2 is a mitogen for mesenchymal cells including normal human bone marrow stromal cells [Robinson et al., 1995], osteoblasts [Rodan et al., 1989], and mouse MC3T3-E1 cells [Suzuki et al., 1996]. FGF-2 and PDGF stimulated bone formation in vivo [Mayahara et al., 1993; Nakamura et al., 1995; Mitlak et al., 1996], but inhibited collagen type I gene expression and alkaline phosphatase activity in osteoblastic cells [Hurley et al., 1993; Boudreaux and Towler, 1996; Chaudhary and Avioli, 2000] demonstrating differences between in vivo and in vitro systems. Also, over-expression of FGF-2 as well as disruption of FGF receptor causes skeletal defects: shortening and flattening of bones, severe and progressive bone dysplasia with enhanced and prolonged endochondral bone growth [Coffin et al., 1995; Deng et al., 1996]. Cell surface receptors for FGF-2 and PDGF possess intrinsic

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protein tyrosine kinase activity and transduce signals from the cell surface to the nucleus by activating mitogen-activated protein (MAP) kinase cascades: (1) the extracellular signal-regulated kinase (ERK), which utilizes the Ras /Raf-1 /MEK/ ERK cascade, (2) c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK), and (3) p38 MAP kinase [Su and Karin, 1996; Robinson and Cobb, 1997]. The ERK MAP kinase pathway is a three kinase cascade which is sequentially activated involving the activation of MAP kinase kinase kinase (MAPKKK/MEKK)/Raf-1, activated Raf-1 phosphorylates and activates MAP kinase kinase (MAPKK/MEK) which in turn phosphorylates and activates downstream ERK1 and ERK2 [Su and Karin, 1996; Robinson and Cobb, 1997]. We have demonstrated that FGF-2 and PDGF-BB activate the ERK MAPK cascade in osteoblastic cells [Chaudhary and Avioli, 1997, 1998].

Cell proliferation and programmed cell death (apoptosis) play a critical role in maintaining tissue homeostasis including bone. The imbalance in these two events may contribute to the pathogenesis of a number of human diseases including cancer, autoimmune diseases, neurodegenerative disorders, and osteoporosis [Thompson, 1995]. Decreased osteoblast cell survival has been implicated in the reduction of bone formation associated with glucocorticoid induced osteoporosis [Weinstein et al., 1998; Silvestrini et al., 2000]. A fraction of the initial population of osteoblasts (30–50%) become embedded in the bone as osteocytes and rest of the nonosteocytic osteoblasts (50–70%) are presumed to die by apoptosis [Parfitt, 1976] which is a significant number. Thus, a decrease and/or delay in osteoblast apoptosis may allow additional synthesis and deposition of more bone matrix by osteoblasts resulting in an increased bone formation and net bone mass. Growth factors are mainly cell proliferative agents, but some may also affect apoptosis by triggering specific cell survival signals. Protein kinase B (PKB/Akt), a serine-threonine protein kinase encoded by the c-akt proto-oncogene, has been implicated in normal cell growth, cell cycle progression and apoptosis, and is a critical mediator of cell survival [Datta et al., 1999]. Although PDGF and FGF-2 have been implicated in the regulation of bone formation, the molecular mechanisms of PDGF and FGF-2 action in bone metabolism and osteoblast survival remain to be established.

The goal of this study was to determine whether PDGF, EGF, and FGF-2 activated Akt and whether there were differences in signaling pathways triggered by these growth factors in human and mouse osteoblastic cells. Our data demonstrated for the first time that Akt was activated by PDGF-BB and EGF but not by FGF-2.

MATERIALS AND METHODS

Materials

Fetal bovine serum (FBS), α-minimum essential medium (α-MEM), Dulbecco's phosphate-buffered saline (PBS), crude bacterial collagenase, Dulbecco's modified Eagle medium (DMEM): Ham's F-12 medium (1:1) and trypsin-EDTA were purchased from Sigma Chemical Co. (St. Louis, MO). Recombinant human FGF-2 and PDGF-BB were obtained from R & D Systems, Inc. (Minneapolis, MN). Epidermal growth factor (EGF), PD098059 and rapamycin were purchased from Calbiochem (San Diego, CA). Affinity purified anti-sera for active-MAPK and pan-ERK were purchased from Promega (Madison, WI). Phospho-Akt and Akt antibodies were obtained from New England Biolabs (Beverly, MA). All other chemicals were of analytical grade. MC3T3-E1 cells were purchased from American Tissue Culture Collection (Rockville, MD).

Human Bone Cell Culture

Human ribs obtained from surgery patients/tissue donors shortly after death were transported to the laboratory in tissue culture flasks containing DMEM/Ham's F-12 medium and were processed immediately or after storage overnight in refrigerators as described previously [Chaudhary and Avioli, 1996]. Briefly, trabecular bone chips were scraped out with a size 4 bone currette. Bone chips were washed several times with DMEM:Ham's F-12 medium and digested with collagenase (Boehringer Mannheim, 250 U/ml) and DNase type I (Sigma, 1 μg/ml) in DMEM:F-12 medium for 2 h at 37°C. After digestion, chips were washed with DMEM:F-12 (calcium-free) containing 10% FBS and plated in calcium-free DMEM:F-12 medium containing 10% FBS and penicillin/streptomycin (100 U/ml and 100 μg/ml respectively) in T-175 culture flasks. Cloned osteoblast-like MC3T3-E1 cells derived from newborn mouse calvaria [Sudo et al., 1983]

were cultured in α-MEM containing 10 % fetal bovine serum (FBS). Cells were maintained in a humidified atmosphere of 95% air and 5% CO_2 at 37°C.

Cell Lysate Preparation

After cells reached confluency, normal HOB and MC3T3-E1cells were trypsinized and seeded in P-100 culture dishes at a density of one million cells/dish. Cells were made quiescent in α-MEM medium containing 0.3% FBS for 48 h. The required amounts of concentrated stock solutions of growth factors were directly added to the medium for specified time. Cells were rinsed with PBS and cell lysates were prepared by adding 0.5 ml of boiling lysis buffer containing 1% SDS, 10 mM Tris-HCl, pH 7.4, 100 μM sodium orthovanadate, 5 mM EGTA and 5 mM EDTA to each dish. Cell mixture was transferred to microcentrifuge tubes, boiled for 5 min, passed several times through a 25 gauge needle and centrifuged for 15 min at 14,000*g* in a microcentrifuge. The supernatants were removed and stored at − 80°C until used. Proteins in cell lysates were measured with the Bio-Rad DC protein assay kit (Bio-Rad, Hercules, CA).

Western Blotting (Immunoblotting)

Cell lysates were subjected to SDS-PAGE and proteins were electrophoretically transferred to polyvinylidene fluoride (PVDF)/Immobilin-P membrane (Millipore Corp., Medford, MA). The membranes were incubated in blocking buffer (Tris-buffered saline-0.1% Tween-20 (TBST) containing 1% BSA (anti-active MAPK antibody) or 5% Carnation milk (anti-phospho-Akt and anti-Akt antibodies) at room temperature for 2 h, then incubated with appropriate antibodies according to the protocols of the manufacturers (Promega and New England BioLabs) and washed with TBST. Antibody binding was detected by enhanced chemiluminescence (ECL) western blotting detection systems as directed by the manufacturer (Amersham, Arlington Heights, IL). Data shown are representative of at least two individual experiments with similar results.

RESULTS

Effects of MEK, PI 3-K, and $p70^{S6K}$ Inhibitors on the Activation of ERK1/2 by PDGF-BB and FGF-2 in Human Osteoblastic (HOB) Cells

To determine whether activation of ERK pathway by FGF-2 and PDGF-BB was mediated via phosphatidylinositol 3-kinase (PI 3-Kinase), experiments were designed to examine the effects of various specific inhibitors. Quiescent HOB cells were pretreated for 30 min with wortmannin (WM, 500 nM), an inhibitor of PI 3-kinase; PD098059 (PD, 100 μM), an inhibitor of MEK; or rapamycin (RP, 20 nM), an inhibitor of $p70^{S6K}$ followed by treatment with PDGF-BB (20 ng/ml) or FGF-2 (50 ng/ml) for additional 10 minutes. To determine activation of ERK1/2 we used anti-active MAPK antibody which specifically recognizes activated forms of ERK1 and ERK2 in response to growth factors. Results demonstrated that PD098059 inhibited ERK1/2 activation by FGF-2 and PDGF-BB (Fig. 1). Data also showed that wortmannin inhibited PDGF-BB activation of ERK1/2 but had no effect on FGF-2 stimulation of ERK1/2. Rapamycin, an inhibitor of $p70^{S6K}$ which may be a downstream target of ERK1/2 and PI 3-K, did not affect the ERK1/2 activation by FGF-2 and PDGF-BB (Fig. 1). The anti-pan ERK antibody which recognizes both activated and non-activated forms of ERK1/2, serves as an important control to demonstrate the amount of total ERK protein in each lane, thereby allowing accurate interpretation of the corresponding signal obtained with the anti-active MAPK antibody. As shown in Figure 1 (lower B panel), amounts of total ERK1/2 proteins were same in treated and non-treated samples.

Effects of PDGF-BB and FGF-2 on the Activation of Akt in Human Osteoblastic Cells

Both PDGF-BB and FGF-2 activated ERK1/2 in agreement with our previous reports [Chaudhary and Avioli, 1997, 1998], and MEK inhibition by PD098059 blocked ERK1/2 activation by both growth factors (Fig. 1). Furthermore, our data presented in Figure 1 indicated that PDGF-BB and FGF-2 differentially activated signaling pathways in osteoblastic cells. FGF-2 activation of ERK1/2 was not affected by PI 3-kinase inhibitor, wortmannin (Fig. 1). These observations suggested that PDGF activation of ERK1/2 pathway was mediated by PI 3-kinase and prompted us to further investigate possibility whether there were downstream differences in the signaling by FGF-2 and PDGF-BB. Since PI 3-kinase is known to activate Akt, experiments were designed to determine the effect of PDGF-BB and FGF-2 on Akt activation. As shown in Figure 2, PDGF-BB treatment activated Akt in HOB cells

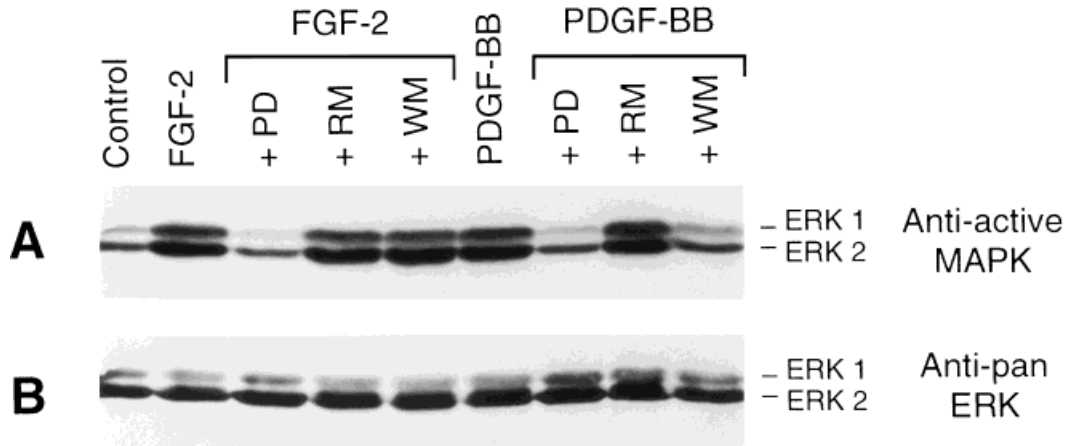

Fig. 1. Effects of MEK, PI 3-K, and $p70^{S6K}$ inhibitors on ERK1/2 activation by PDGF-BB and FGF-2 in HOB cells. Quiescent HOB cells were pretreated for 30 min with PD098059 (PD, 100 μM), an inhibitor for MEK; wortmannin (WM, 500 nM), an inhibitor for PI 3-K; or rapamycin (RM, 20 nM), an inhibitor for $p70^{S6K}$ and then with PDGF-BB (20 ng/ml) or FGF-2 (50 ng/ml) for additional 10 minutes. Cell lysates were subjected to SDS-PAGE followed by transfer of proteins to Immobilin-P membrane and then incubated with anti-active MAPK (ERK1/2) antibody. The same membrane was stripped and incubated with anti-pan ERK antibody which recognizes total activated and non-activated forms of ERK1/2. The binding of antibody was detected by ECL plus detection reagents. Data shown are representative of two individual experiments with similar results.

whereas FGF-2 had no effect on the activation of Akt. LY294002, a specific inhibitor of PI 3-kinase, significantly inhibited PDGF-BB activation of Akt. Rapamycin, an specific inhibitor of $p70^{S6K}$, had no effect on the activation of Akt by PDGF-BB. The amount of total Akt protein was same in treated and non-treated samples as determined by using anti-Akt antibody which recognizes both activated and non-activated forms of Akt 9 (Fig. 2).

Effects of PDGF-BB and FGF-2 on the Activation of Akt in MC3T3-E1 Cells

To assess whether there were differences across species in the activation of Akt by

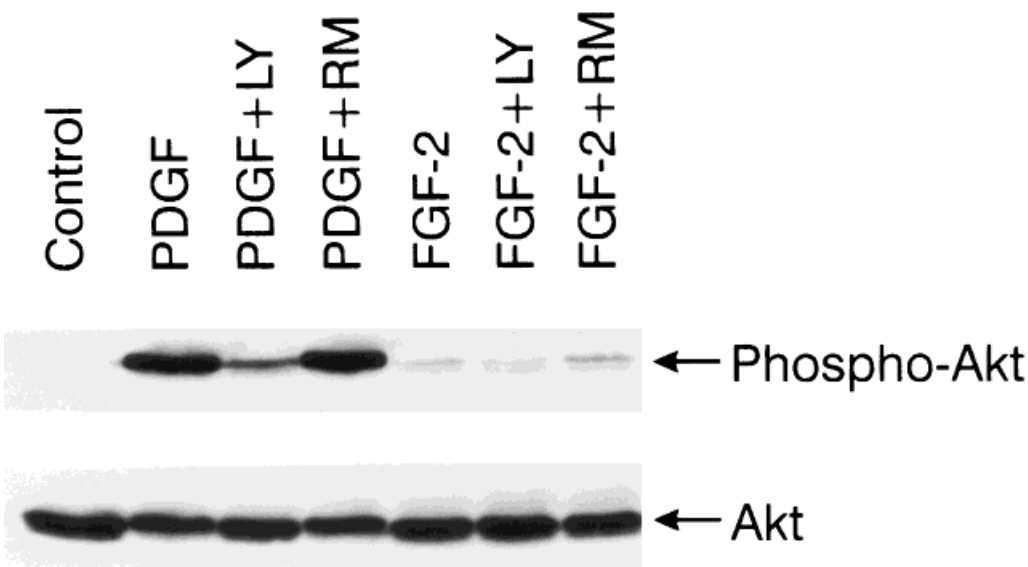

Fig. 2. Effects of PDGF-BB and FGF-2 on the activation of Akt in HOB cells. Quiescent HOB cells were pretreated for 30 min with LY294002 (LY, 50 μM) or rapamycin (RM, 50 nM) and then with PDGF-BB (30 ng/ml) or FGF-2 (30 ng/ml) for additional 10 minutes. Cell lysates were subjected to SDS-PAGE followed by transfer of proteins to Immobilin-P membrane and then incubated with anti-phospho-Akt antibody. The same membrane was stripped and incubated with anti-Akt antibody which recognizes total activated and non-activated forms of Akt.

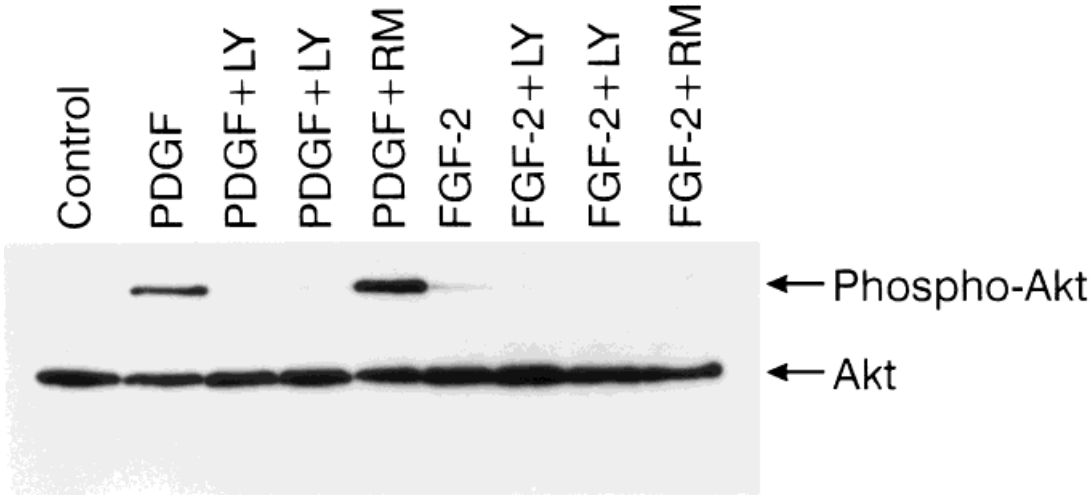

Fig. 3. Effects of PDGF-BB and FGF-2 on the activation of Akt in MC3T3-E1 cells. Quiescent MC3T3-E1 cells were pretreated for 30 min with LY294002 (LY, 50 μM) or rapamycin (RM, 50 nM) and then with PDGF-BB (30 ng/ml) or FGF-2 (30 ng/ml) for additional 10 minutes. Cell lysates were subjected to SDS-PAGE followed by transfer of proteins to Immobilin-P membrane and then incubated with anti-phospho-Akt antibody. The same membrane was stripped and incubated with anti-Akt antibody which recognizes total activated and non-activated forms of Akt.

PDGF-BB and FGF-2, the effects of growth factors on Akt were investigated in mouse osteoblastic cell line, MC3T3-E1 cells. As shown in Figure 3, PDGF-BB treatment of cells activated Akt whereas FGF-2 treatment did not activate Akt in MC3T3-E1 cells. PI 3-K inhibitor LY294002 inhibited Akt activation by PDGF-BB but rapamycin had no effect.

Effects of EGF on the Activation of Akt in MC3T3-E1 cells

Since FGF-2 and PDGF-BB receptors have intrinsic tyrosine kinase activity and since our data have demonstrated differences in the downstream signaling by these growth factors, we investigated the effects of another growth factor, epidermal growth factor (EGF), which elicits its biological action through protein tyrosine kinase receptor and activation of PI 3-K [Brown, 1995]. As represented in Figure 4, EGF treatment of MC3T3-E1 cells activated Akt which was inhibited by LY294002 but not by rapamycin.

DISCUSSION

In the present study, we investigated the downstream cell signaling pathways activated by PDGF, EGF, and FGF-2 in osteoblasts. The specific inhibitors: wortmannin and LY294002 for PI 3-kinase; PD098059 for MEK; and rapamycin for $p70^{S6K}$, have been extremely useful in explaining the roles of PI 3-kinase, ERK1/2 and $p70^{S6K}$ in cellular functions in response to growth factors/cytokines. By employing these inhibitors we demonstrated differences in signaling pathways activated by PDGF and FGF-2 in osteoblastic cells. We discovered that PDGF-BB activation of ERK MAPK pathway was mediated by PI 3-K whereas FGF-2 transduced signals from cell surface to the nucleus via Ras/Raf-1/MEK/ERK pathway without the involvement of PI 3-K. It is important to mention that the activation of ERK1/2 by PDGF-BB was not completely inhibited by PI 3-K inhibitor wortmannin (Fig. 1) suggesting that PDGF-BB activated ERK1/2 via two PI 3-K dependent and independent signaling pathways. However, the activation by PI 3-K independent pathway was a small part of the total stimulation and the PDGF activation of ERK pathway was mainly through the activation of PI 3-K. Our studies revealed that FGF-2 activated ERK1/2 via Ras/Raf-1/MEK/ERK pathway, whereas PDGF activated ERK1/2 by triggering Ras/Raf-1/MEK/ERK mediated by PI 3-K. These data prompted us to further investigate the possibility whether FGF-2 and PDGF activate cell survival signal Akt, a downstream target of PI 3-Kinase.

Our results demonstrated activation of Akt by PDGF-BB and EGF but not by FGF-2 in human

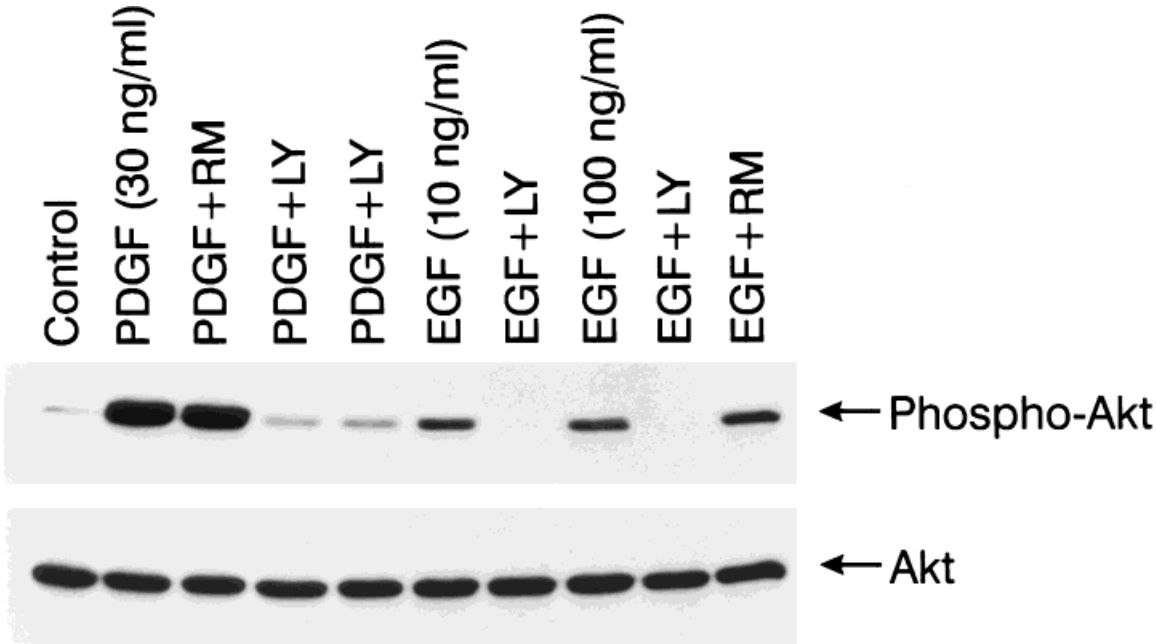

Fig. 4. Effects of EGF and PDGF-BB on the activation of Akt in MC3T3-E1 cells. Quiescent cells were pretreated for 30 min with LY294002 (LY, 50 μM) or rapamycin (RM, 50 nM) and then with EGF (10 or 100 ng/ml) and PDGF-BB (30 ng/ml) for additional 10 minutes. Cell lysates were subjected to SDS-PAGE followed by transfer of proteins to Immobilin-P membrane and then incubated with anti-phospho-Akt antibody. The same membrane was stripped and incubated with anti-Akt antibody which recognizes total activated and non-activated forms of Akt.

and mouse osteoblastic cells. Similarily, FGF-1 did not activate Akt in primary murine calvarial osteoblasts [Mansukhani et al., 2000] indicating that FGF-1 and FGF-2 utilized similar signaling pathway in human and mouse osteoblasts. One of the aims during the course of these studies was to define pathways downstream of growth factors which prevent apoptosis. Our findings that PDGF-BB and EGF but not FGF-2 provide a survival signal implicating PI 3-kinase and Akt as key molecules in the survival of osteoblasts. Our data suggest that the signaling pathways emanating from receptor activation are not necessarily linear but must be considered a network of interwoven events. Thus, our observations are of importance in clarifying the specific roles of PDGF and FGF-2 in osteoblast proliferation, differentiation, and in the prevention of apoptosis as well as in determining the relative importance of the various inputs on the resulting physiological responses.

The dual actions of PDGF in cell proliferation and survival may have advantages in increasing and maintaining bone mass over other growth factors which have only proliferative effects. The case in point is that although FGF-2 stimulates proliferation of osteoprogenitors but is a weak mitogen for differentiating and mature human osteoblasts [Debiais et al., 1998]. PDGF is more mitogenic for normal HOB cells than TGF-β, IGF-1, EGF [Piche and Graves, 1989] or FGF-2 [Kang et al., 1995]. Since PDGF and FGF-2 elicit their biological action through their respective protein tyrosine kinase receptors [Su and Karin, 1996; Robinson and Cobb, 1997] and activate ERK MAP kinase pathway in osteoblast [Chaudhary and Avioli, 1997, 1998], the downstream signaling pathways utilized by these growth factors differ. Taken together, differences in cell signaling demonstrated here may determine the final biological responses to growth factors. Further, this will allow to analyze the role of apoptosis in bone formation and to identify new signaling site (s) which could be targets for intervention and development of potential agents for the treatment of osteoporosis.

In summary, the present study is the first demonstration of Akt activation by PDGF-BB and EGF but not by FGF-2 in osteoblastic cells. We also report that ERK1/2 activation by PDGF

is mediated by PI 3-K but FGF-2 activation of ERK1/2 is independent of PI 3-K. Results suggest that PDGF and EGF but not FGF-2 may enhance osteoblast survival.

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REFERENCES

Boudreaux JM, Towler DA. 1996. Synergistic induction of osteocalcin gene expression. Identification of a bipartite element conferring fibroblast growth factor 2 and cyclic AMP responsiveness in the rat osteocalcin promoter. J Biol Chem 271:7508–7515.

Brown KD. 1995. The epidermal growth factor/transforming growth factor-alpha family and their receptors. Eur J Gastro Hepatol 7:914–922.

Chaudhary LR, Avioli LV. 1996. Regulation of interleukin-8 gene expression by interleukin-1β, osteotropic hormones, and protein kinase inhibitors in normal human bone marrow stromal cells. J Biol Chem 271:16591–16596.

Chaudhary LR, Avioli LV. 1997. Activation of extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2) by FGF-2 and PDGF-BB in normal human osteoblastic and bone marrow stromal cells: Differences in mobility and in-gel renaturation of ERK1 in human, rat and mouse osteoblastic cells. Biochem Biophys Res Comm 238:134–139.

Chaudhary LR, Avioli LV. 1998. Identification and activation of mitogen-activated protein (MAP) kinase in normal human osteoblastic and bone marrow stromal cells: attenuation of MAP kinase activation by cAMP, parathyroid hormone and forskolin. Mol Cell Biochem 178:59–68.

Chaudhary LR, Avioli LV. 2000. Extracellular-signal regulated kinase signaling pathway mediates down-regulation of type I procollagen gene expression by FGF-2, PDGF-BB, and okadaic acid in osteoblastic cells. J Cell Biochem 76:354–359.

Coffin JD, Florkiewicz RZ, Neumann J, Mort-Hopkinas T, Dorn II GW, Lightfoot P, Howles PN, Kier A, O'Toole BA, Sasse J, Gonzales AM, Baird A, Doetschman T. 1995. Abnormal bone growth and selective translational regulation in basic fibroblast growth factor (FGF-2) transgenic mice. Mol Biol Cell 6:1861–1873.

Datta SR, Brunet A, Greenberg ME. 1999. Cellular survival: a play in three Akts. Gene Dev 13:2905–2927.

Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. 1996. Fibroblast growth factor 3 is a negative regulator of bone growth. Cell 84:911–921.

Debiais F, Hott M, Graulet AM, Marie PJ. 1998. The effects of fibroblast groeth factor-2 on human neonatal calvaria osteoblastic cells are differentiation stage specific. J Bone Min Res 13:645–654.

Hurley MM, Abreu C, Harrison JR, Lichtler AC, Raisz LG, Kream BE. 1993. Basic fibroblast growth factor inhibits type I collagen gene expression in osteoblastic MC3T3-E1 cells. J Biol Chem 268:5588–5593.

Kang Y-M, Leh Y-L, Graves DT. 1995. Interleukin-1 modulates phosphorylation of proteins in human osteoblastic cells. J Bone Min Res 10:96–105.

Kim HD, Valentini RF. 1997. Human osteoblast response *in vitro* to platelet-derived growth factor and transforming growth factor-β delivered from controlled-release polymer rods. Biomaterials 18:1175–1184.

Lian JB, Stein GS, Canalis E, Robey PG, Boskey AL. 1999. Bone formation: osteoblast lineage cells, growth factors, matrix proteins, and the mineralization process. In: Fabus MJ, editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 4th edition. Philadelphia: Lippincot Williams & Wilkins. p. 14–29.

Mansukhani A, Bellosta P, Sahni M, Basilico C. 2000. Signaling by fibroblast growth factor (FGF) and fibroblast growth factor receptor 2 (FGFR2)-activating mutations blocks mineralization and induces apoptosis in osteoblasts. J Cell Biol 149:1297–1308.

Mayahara H, Ito T, Nagai H, Miyajima H, Tsukuda R, Taketomi S, Mizoguchi J, Kato K. 1993. In vivo stimulation of endosteal bone formation by basic fibroblast growth factor in rats. Growth Factors 9:73–80.

Mitlak BH, Finkelman RD, Hill EL, Li J, Martin B, Smith T, D'Andrea M, Antoniades HN, Lynch SE. 1996. The effects of systemically administered PDGF-BB on the rodent skeleton. J Bone Min Res 11:238–247.

Mundy GR. 1999. Bone remdeling. In: Fabus MJ, editor. Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism, 4th edition. Philadelphia: Lippincot Williams & Wilkins. p. 30–38.

Nakamura T, Hanada K, Tamura M, Shibanushi T, Nigi H, Tagawa M, Fukumoto S, Matsumoto T. 1995. Stimulation of endosteal bone formation by systemic injections of recombinant basic fibroblast growth factor in rats. Endocrinology 136:1276–1284.

Okazaki R, Ikeda K, Sakamoto A, Nakano T, Morimoto K, Kikuchi T, Urakawa K, Ogata E, Matsumoto T. 1992. Transcriptional activation of c-fos and c-jun proto-oncogenes by serum growth factors in osteoblast-like MC3T3-E1 cells. J Bone Min Res 7:1149–1155.

Parfitt AM. 1976. The actions of parathyroid hormone on bone: relation to bone remodeling and turnover, calcium homeostasis, and metabolic bone disease. Part III. PTH and osteoblasts, the ralationship between bone turnover and bone loss, and the state of the bones in primary hyperparathyroidism. Metabol Clin Exp 25:1033–1069.

Piche JE, Graves DT. 1989. Study of the growth factor requirements of human bone-derived cells: a comparison with human fibroblasts. Bone 10:131–138.

Robinson D, Bab I, Nevo Z. 1995. Osteogenic growth peptide regulates proliferation and osteogenic maturation of human and rabbit bone marrow stromal cells. J Bone Min Res 10:690–696.

Robinson MJ, Cobb MH. 1997. Mitogen-activated protein kinase pathways. Curr Opin Cell Biol 9:180–186.

Rodan SB, Wesolowski G, Thomas KA, Yoon K, Rodan GA. 1989. Effects of acidic and basic fibroblast growth factors on osteoblastic cells. Conn Tiss Res 20:283–288.

Silvestrini G, Ballanti P, Patacchioli FR, Mocetti P, Di Grezia R, Wedard BM, Angelucci L, Bonucci E. 2000. Evaluation of apoptosis and the glucocorticoid receptor in

the cartilage growth plate and metaphyseal bone cells of rats after high-dose treatment with cortisone. Bone 26:33–42.

Su B, Karin M. 1996. Mitogen-activated protein kinase cascades and regulation of gene expression. Curr Opin Immunol 8:402–411.

Sudo H, Kodama HA, Amagai Y, Yamamoto S, Kasai S. 1993. In vitro differentiation and calcification in a new clonal osteogenic cell line derived from newborn mouse calvaria. J Cell Biol 96:191–198.

Suzuki A, Shinoda J, Kanda S, Oiso Y, Kozawa O. 1996. Basic fibroblast growth factor stimulates phosphatidylcholine-hydrolyzing phospholipase D in osteoblast-like cells. J Cell Biochem 63:491–499.

Thompson CB. 1995. Apoptosis in the pathogenesis and treatment of disease. Science 267:1456–1462.

Weinstein RS, Jilka RL, Parfitt AM, Manolagas. 1998. Inhibition of osteoblastogenesis and promotion of apoptosis of osteoblasts and osteocytes by glucocorticoids. J Clin Invest 102:274–282.

Zhang L, Leeman E, Carnes DC, Graves DT. 1991. Human osteoblasts synthesize and respond to platelet-derived growth factor. Am J Physiol 261:C348–C354.