Fibroblast Growth Factor (FGF)-2 Directly Stimulates Mature Osteoclast Function through Activation of FGF Receptor 1 and p42/p44 MAP Kinase*

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We previously reported that fibroblast growth factor-2 (FGF-2) acts not only on osteoblasts to stimulate osteoclastic bone resorption indirectly but also on mature osteoclasts directly. In this study, we investigated the mechanism of this direct action of FGF-2 on mature osteoclasts using mouse and rabbit osteoclast culture systems. FGF-2 stimulated pit formation resorbed by isolated rabbit osteoclasts moderately from low concentrations (≥10^{-12} M), whereas at high concentrations (10^{-9} M) it showed stimulation on pit formation resorbed by unfractinated bone cells very potently. FGF-2 (10^{-10}–10^{-12} M) also increased cathepsin K and MMP-9 mRNA levels in mouse and rabbit osteoclasts. Among FGF receptors (FGFR1 to 4) only FGFR1 was detected on isolated mouse osteoclasts, whereas all FGFRs were identified on mouse osteoclasts. FGF-2 (10^{-12} M) up-regulated the phosphorylation of cellular proteins, including p42/p44 mitogen-activated protein (MAP) kinase, and increased the kinase activity of immunoprecipitated FGFR1 in mouse osteoclasts. The stimulation of FGF-2 on mouse and rabbit osteoclast functions was abrogated by PD-98059, a specific inhibitor of p42/p44 MAP kinase. These results strongly suggest that FGF-2 acts directly on mature osteoclasts through activation of FGFR1 and p42/p44 MAP kinase, causing the stimulation of bone resorption at physiological or pathological concentrations.

Among many growth factors regulating bone metabolism, fibroblast growth factor-2 (FGF-2 or basic FGF) is recognized as a potent mitogen for a variety of mesenchymal cells (1). Several genetic diseases with severe impairment of bone and cartilage formation, such as achondroplasia (2–4) and thanatophoric dysplasia type II (5), have recently been shown to be caused by mutations of FGF receptors (FGFRs). In bone tissues, FGF-2 is produced by cells of osteoblastic lineage, is accumulated in bone matrix, and acts as an autocrine/paracrine factor for bone cells (6–10). We and others have reported that the exogenous application of FGF-2 has stimulatory effects on bone formation in several in vivo models as a pharmacological action of FGF-2 (11–17). On the other hand, in vitro studies revealed that high concentrations of FGF-2 (10^{-8}–10^{-10} M) stimulated osteoclastogenesis in bone marrow culture (18) and bone resorption in bone organ cultures (19, 20). This stimulatory effect of FGF-2 on bone resorption is known to be mediated at least in part by cyclooxygenase-2 (COX-2) induction and pros-taglandin production (18, 20), which cause the expression of osteocalcin differentiation factor (RANKL/ODF), a key membrane-associated molecule that regulates osteoclast differentiation, in osteoclastic cells (21). Other than this indirect action through the mediation of osteoblasts, we recently reported that FGF-2 acts directly on mature osteoclasts to stimulate bone resorption (22).

There are four structurally related high affinity receptors (FGFR1 to 4) belonging to receptor tyrosine kinases (RTKs) that have an intrinsic protein-tyrosine kinase activity and elicit tyrosine autophosphorylation of the receptor (23, 24). Because it is located downstream of the autophosphorylation of FGFRs, mitogen-activated protein (MAP) kinase has been reported to be the major signaling pathway in neuronal and endothelial cells (25–27). In osteoblasts, MAP kinase activation followed by the autophosphorylation of FGFR1 and 2 is also involved in FGF-2 signaling (28, 29). Osteoclastic bone resorption is regulated by two different steps: one is the recruitment and differentiation of osteoclasts and the other is the activation of mature osteoclast function. Although a number of signaling pathways through the mediation of osteoblasts for osteoclast differentiation have been clarified, little is known about the signaling to stimulate mature osteoclast function directly. A recent study of random sequence analysis of PCR-amplified cDNA clones identified 14 distinct kinase-related genes in purified rabbit mature osteoclasts (30). Eight of these genes were identified as RTKs: Tie, Tie2/tylpyrazolo[3,4-d]pyrimidine, PAGE, polyacrylamide gel electrophore-sis; M-BMM, M-CSF-dependent bone marrow macrophages; TNF, tumor necrosis factor; PD-98059, 2’-amino-3’-methoxyflanalone; SB-203580, 4-(4-fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)-1H-imidazole.
c-Kit, Fms, Met, Axl, Tyro3, INS-R, and FGFR1.

In this study, we investigated the molecular mechanism whereby FGF-2 stimulates mature osteoclast function using mouse and rabbit osteoclast culture systems. Studies on the signaling pathway were performed using isolated mouse osteoclasts; however, for those on the resorbing activity, isolated rabbit osteoclasts were used because mouse osteoclasts do not have enough potency to resorb bone after being isolated.

**EXPERIMENTAL PROCEDURES**

**Materials**—Neonatal, 5-week-old, and 8-week-old male ddY mice were purchased from the Shizuoka Laboratories Animal Center (Shizuoka, Japan). 10-day-old male Japanese white rabbits were purchased from the Shizuoka Laboratories Animal Center (Saitama, Japan). Human recombinant FGF-2 was kindly provided by Kaken Pharmaceutical Co. Ltd. (Chiba, Japan) and NS-388 by Taisho Pharmaceutical Co. Ltd. (Tokyo, Japan). α-modified minimum essential medium (αMEM) was purchased from Life Technologies, Inc. (Rockville, MD), and fetal bovine serum (FBS) was from the Cell Culture Laboratory (Cleveland, OH). Macrophage colony-stimulating factor (M-CSF) was from Austrail Biologicals (San Ramon, CA). Bacterial collagenase, 1,25(OH)2 vitamin D3, and ISOGEN were purchased from Wako Pure Chemicals Co. (Osaka, Japan), and dispase from Nitta Gelatin Co. (Osaka, Japan). Polyclonal rabbit antibody against phosphotyrosine was obtained from UBI (Lake Placid, NY), and monoclonal mouse antibody against p60v-src (monoclonal rabbit antibody against phosphotyrosine was obtained from OncoGene Research Products (Osaka, Japan), and dispase from Nitta Gelatin Co. (Osaka, Japan). Polyclonal rabbit antibody against p60v-src (monoclonal antibody 327) was obtained from Oncogene Research Products (Osaka, Japan), and monoclonal mouse antibody against p60v-src (monoclonal antibody 327) was obtained from Oncogene Research Products (Cambridge, MA). This antibody recognizes specifically both p60v-src and p66v-src and has been used to determine the expression of p60v-src in various primary cells and clonal cell lines. Polyclonal rabbit antibodies against mouse FGFR1 through 4 and nonimmune IgG as well as blocking peptides for respective antibodies, were obtained from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Polyclonal rabbit antibodies against phospho-p44/42 MAP kinase, phospho-p38 MAP kinase, phospho-e-c-Jun N-terminal protein kinase (JNK), and 2-amin-o-3-methoxyflanone (PD-98059) were obtained from New England Biolabs, Inc. (Beverly, MA). 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyrone sulfonyl)benzene (SB-203580) and Pronase were purchased from Calbiochem-Novobiochem Co. (La Jolla, CA). 32P-labeled cDNA probes for FGFR1, 2, 3, and 4 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Fms-ligand (FL) was obtained from Sigma Chemical Co. (St. Louis, MO). PD-98059 (1, 3, 10, and 30 μM), and SB-203580 (30 μM) were purchased from Calbiochem-Novobiochem Co. (La Jolla, CA). [32P]dCTP and [3H]-thymidine (2 μCi) were purchased from Amer sham-Burroughs (Bucks, UK). 4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyrone sulfonyl)benzene (SB-203580) and Pronase were purchased from Calbiochem-Novobiochem Co. (La Jolla, CA). 32P-labeled cDNA probes for FGFR1, 2, 3, and 4 were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA). Fms-ligand (FL) was obtained from Sigma Chemical Co. (St. Louis, MO).

**Resorbed Pit Formation Assay by Purified Mature Osteoclasts and Unfractionated Bone Cells from Rabbit Long Bones**—Purified mature osteoclasts and bone marrow cells were prepared using 10-day-old ddY mice as described previously (31). Briefly, long bones from 10-day-old rabbits were minced with scissors and agitated with a vortex mixer. An aliquot of unfractionated bone cells was seeded onto 0.24% collagen gel (Nitta Gelatin) in 100-mm tissue culture dishes at 106 living cells per dish. After an overnight culture, the cells were cultured in the presence or absence of FGF-2 (10-10 to 10-8 M) or M-CSF (200 units/ml) for various periods up to 48 h, fixed with citrate-acetone-formaldehyde fixative for 30 s, and stained with TRAP and trypan blue and TRAP. Trypan blue-negative and TRAP-positive osteoclasts were counted.

**RT-PCR for FGF Receptors**—Total RNA was extracted from mouse osteoclasts and osteoblasts using ISOGEN following the manufacturer’s instructions, and 2 μg of RNA was reverse-transcribed and amplified by PCR. The primers for FGFR1 through 4 were as follows: FGFR1: sense, 5'-TGAGTTTCTCTGGAAAGGTG3'; antisense, 5'-ATAGAGGAGGCATCTTGGT3'; antisense, 5'-GAATACGAAATCTCCAAAC3'; antisense, 5'-GCCGCTTCTCCATCTTCTT3'; FGFRII: sense, 5'-ACTGTTCTACTGTGG3'; antisense, 5'-GTCGGGAGGCTGTCG3'; FGFRIII: sense, 5'-GCGGGTCTCTGTGTCG3'; antisense, 5'-GTCGGGGAGGCTGTCG3'; FGFRIV: sense, 5'-CTTGTGGACATCCTTGGG3'; antisense, 5'-CTGGGAAGGCTGTCGTC-3'; PCR consisted of 40 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 45 s, and extension at 72°C for 60 s. The PCR products for FGFR1, 2, 3, and 4 were 856, 266, and 145 bp, respectively. After purification, osteoclasts were cultured in the presence or absence of FGF-2 (10-10 to 10-8 M) or M-CSF (200 units/ml) for various periods up to 48 h, fixed with citrate-acetone-formaldehyde fixative for 30 s, and stained with trypan blue and TRAP. Trypan blue-negative and TRAP-positive osteoclasts were counted.

**RT-PCR for the Total RNA from Mouse Osteoclasts and Osteoblasts**—Total RNA was extracted from mouse osteoclasts and osteoblasts using ISOGEN following the manufacturer’s instructions, and 2 μg of RNA was reverse-transcribed and amplified by PCR. The primers for FGFR1 through 4 were as follows: FGFR1: sense, 5'-TGAGTTTCTCTGGAAAGGTG3'; antisense, 5'-ATAGAGGAGGCATCTTGGT3'; antisense, 5'-GAATACGAAATCTCCAAAC3'; antisense, 5'-GCCGCTTCTCCATCTTCTT3'; FGFRII: sense, 5'-ACTGTTCTACTGTGG3'; antisense, 5'-GTCGGGAGGCTGTCG3'; FGFRIII: sense, 5'-GCGGGTCTCTGTGTCG3'; antisense, 5'-GTCGGGGAGGCTGTCG3'; FGFRIV: sense, 5'-CTTGTGGACATCCTTGGG3'; antisense, 5'-CTGGGAAGGCTGTCGTC-3'; PCR consisted of 40 cycles of denaturation at 94°C for 45 s, annealing at 53°C for 45 s, and extension at 72°C for 120 s. The PCR products for FGFR1, 2, 3, and 4 were 856, 266, and 145 bp, respectively. After purification, osteoclasts were cultured in the presence or absence of FGF-2 (10-10 to 10-8 M) or M-CSF (200 units/ml) for various periods up to 48 h, fixed with citrate-acetone-formaldehyde fixative for 30 s, and stained with trypan blue and TRAP. Trypan blue-negative and TRAP-positive osteoclasts were counted.

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bated with polyclonal anti-mouse FGFR1, 2, 3, and 4 or nonimmune IgG. Immunoreactive bands were stained using the ECL chemiluminescence reaction (Amersham Pharmacia Biotech) following the manufacturer’s instructions. After this visualizing, the antibodies on the membrane were stripped in a buffer consisting of 62.5 mM Tris-HCl (pH 6.7), 2% SDS, and 100 mM 2-mercaptoethanol at 50 °C for 40 min. To ascertain the specificity of these blots, the stripping membrane was further immunoreacted with each polyclonal anti-FGFR and respective blocking peptide, and the immunoreactive bands were again visualized under the same conditions as above. The immunoreactivity to each anti-FGFR was not lost by this stripping procedure.

Assays for Tyrosine Phosphorylation of Cellular Proteins—Osteoclast cells and bone marrow cells were co-cultured on 100-mm tissue culture dishes in αMEM containing 10% FBS and 1,25(OH)2 vitamin D3 (10−8 M) for 6 days, with a medium change at 2 days, and then for 1 more day in αMEM/0.1% FBS. After mouse and rabbit osteoclasts were isolated as described above, they were precultured for 2 h with αMEM/0.1% FBS and treated with FGF-2 (10−12 M) for various periods (2–30 min). The cells were quickly washed twice with ice-cold PBS and lysed with TNE buffer. Cell lysates containing equal amounts of protein (10 μg) were subjected to 8% SDS-PAGE, and proteins separated in the gel were subsequently electrotransferred onto nitrocellulose membranes. After blocking with 5% bovine serum albumin, the membranes with mouse osteoclast lysates were incubated with polyclonal rabbit antibody against phospho-p44/42 MAP kinase, -JNK, and -p38 MAP kinase, and the immunoreactive bands were visualized using the ECL chemiluminescence reaction following the manufacturer’s instructions. After the antibody was stripped from the membrane, to block nonspecific binding, membranes were incubated with 5% skim milk and then with monoclonal mouse antibody against p60v-src, polyclonal rabbit antibodies against phospho-p44/42 MAP kinase, -JNK, and -p38 MAP kinase, and the immunoreactive bands were visualized as described above.

In Vitro Kinase Assay—Isolated mouse osteoclasts were incubated with and without FGF-2 (10−12 M) for various periods (1–10 min). The cells were quickly washed twice with ice-cold PBS and lysed with TNE buffer, and equal amounts of protein (100 μg) were immunoprecipitated with 1 μg of polyclonal rabbit anti-FGFR1. The immune complex was washed three times with TNE buffer and three times with kinase buffer (20 mM HEPES-NaOH (pH 7.4), and 10 mM MgCl2); the samples were then resuspended in 60 μl of kinase buffer with 1 μCi (37 kBq) of [γ-32P]ATP and incubated for 15 min at 30 °C. The reaction was stopped by adding 20 μl of 4× sample buffer (250 mM Tris-HCl (pH 6.8), 8 mM EDTA, 12% SDS, 500 mM 2-mercaptoethanol, 15% glycerol, and 0.01% bromophenol blue) and subjected to 10% SDS-PAGE under reducing conditions followed by autoradiography.

Statistical Analysis—Means of groups were compared by ANOVA, and significance of differences was determined by post-hoc testing using Bonferroni’s method.

RESULTS

Direct and Indirect Effects of FGF-2 on Isolated Rabbit Osteoclast Function—To examine the direct action of FGF-2 on osteoclasts, the effect of FGF-2 on resorbed pit formation on a dentine slice by purified osteoclasts was compared with that by unfractionated bone cells from rabbit long bones (Fig. 1). FGF-2 at 10−12–10−8 M stimulates resorbed pit formation by isolated mature osteoclasts with a maximal effect of 1.9-fold at 10−8 M, and no further stimulations were observed at higher concentrations (Fig. 1A). This stimulation was not due to the increase in the number of osteoclasts but to the activation of each osteoclast function, because the area of each pit (the total pit area per number of pits) was similarly increased by FGF-2 (>10−12 M, data not shown). Because previous reports have shown that the bone resorptive effect of FGF-2 is mediated at least in part by COX-2 induction (22), the contribution of COX-2 to the direct action was examined by adding NS-398 (1 μM), a specific inhibitor of COX-2, to the culture of isolated osteoclasts. NS-398 did not alter the FGF-2 action on isolated osteoclasts, indicating that the direct action is not mediated by COX-2 induction or by PG production (Fig. 1A). On the other hand, FGF-2 at 10−8 and 10−6 M further stimulated resorbed pit formation by unfractionated bone cells up to 7.5-fold (Fig. 1B). This stimulatory effect of high concentra-
Mouse and rabbit cells were ascertained to be osteoclasts by TRAP were from 10-day-old rabbit long bones. More than 99% of isolated osteoclasts. Cathepsin K, MMP-9, and MMP-14 in isolated mouse and rabbit that of G3PDH measured by densitometry.

Is the treated/control ratio of the intensity of each band normalized to that of G3PDH measured by densitometry.

is examined by Northern blot analysis. The number under each band is the treated/control ratio of the intensity of each band normalized to that of G3PDH measured by densitometry.

Effects of FGF-2 on the Survival of Isolated Mouse Osteoclasts—To investigate the effect of FGF-2 (10^{-11} M) on their survival, isolated mouse osteoclasts were cultured in a plastic dish for up to 48 h (Fig. 3). The survival rates decreased with time similarly in the control and FGF-2-treated cultures. At 24 h 27% and 32% of initially surviving cells still adhered to the dish in control and FGF-2-treated cultures, respectively, and by 48 h all cells had died in both cultures. Similar results were seen when a higher concentration of FGF-2 (10^{-8} M) was used (data not shown). On the contrary, M-CSF (2000 units/ml), a positive control, maintained the survival of osteoclasts: the survival rates were 76% at 24 h and 21% at 48 h, as reported previously (42).

FGF Receptors (FGFR1–4) on Mouse Osteoclasts and Osteoblasts—The molecular mechanism of the signal transduction of FGF-2 in osteoclasts was further investigated using isolated mouse osteoclasts. mRNA and protein levels of FGFRs on osteoclasts were studied and compared with those on osteoblasts from neonatal mouse calvariae by RT-PCR and Western blotting analyses, respectively. Only FGFR1 was detected on osteoclasts, whereas all FGFR1 through 4 were identified on osteoblasts both in mRNA and protein levels (Fig. 4). This difference in distribution of FGFRs between osteoclasts and osteoblasts might explain the difference of affinities and concentrations of FGF-2 affecting these cells as seen in bone resorptive activity in rabbit cell cultures (Fig. 1, A and B).

Phosphorylation of FGFR1 and Intracellular Proteins in Mouse and Rabbit Osteoclasts—Fig. 5A shows the time course of effects of FGF-2 on tyrosine phosphorylation of cellular proteins in isolated mouse osteoclasts. Several proteins were selectively phosphorylated by FGF-2 (10^{-12} M) as early as 2 min.

The c-Src signal in each lane indicates a quantitative internal control. Western blot analyses using antibodies against specific proteins related to MAP kinase revealed that phosphorylation of p42/p44 MAP kinase was induced at 5 min, reached maximum at 10 min, and was maintained for more than 30 min (Fig. 5A). Phosphorylations of p38 and JNK MAP kinases were slightly induced just at 10 min. To investigate the autophosphorylation of FGFR1 by FGF-2, kinase activity of immunoprecipitated FGFR1 was examined by in vitro kinase assay. FGF-2 induced the kinase activity of FGFR1 at 1 min, which reached maximum at 2 min, and decreased considerably after 10 min (Fig. 5B). Similar regulation of tyrosine phosphorylation of cellular proteins by FGF-2 was observed in isolated rabbit osteoclasts, and intracellular proteins were phosphorylated at 2 min (Fig. 5C). However, further studies on signaling molecules in rabbit osteoclasts could not be carried out, because antibodies against rabbit proteins were not available.

Functional Relevance of MAP Kinase Activation in Rabbit and Mouse Osteoclasts—To examine the functional relevance of
the activation of p42/p44 and p38 MAP kinases by FGF-2 in osteoclasts, PD-98059, a specific inhibitor of the upstream kinase of p42/p44 MAP kinase (43, 44), and SB-203580, a specific inhibitor of p38 MAP kinase (45, 46), were added to the cultures of rabbit and mouse osteoclasts. PD-98059 dose dependently inhibited the stimulation of FGF-2 on pit formation resorbed by isolated rabbit osteoclasts to the levels of the control culture, while SB-203580 (30 μM) did not affect the FGF-2 stimulation (Fig. 6A). PD-98059 also inhibited the FGF-2 stimulation on cathepsin K and MMP-9 mRNA levels in isolated mouse osteoclasts, and this inhibition was not seen by SB-203580 (Fig. 6B). Although PD-98059 at the highest concentration (30 μM) did not decrease the resorbed pit formation or proteinase mRNA levels in the control culture, inhibitors of src kinase, herbimysin (1 μM) and PP-1 (10 μM), abrogated both of these osteoclast functions not only in FGF-2-stimulated cultures but also in control cultures (data not shown), suggesting the essential role of src kinase signaling in the basal function of osteoclasts.

**DISCUSSION**

In the present study, we confirmed our previous report that FGF-2 directly stimulated the bone resorptive activity of rabbit osteoclasts and further demonstrated that it induced the expression of proteinases in mouse and rabbit osteoclasts. These actions were mediated by the autophosphorylation of FGFR1, the only subtype of FGFRs expressed on osteoclasts, and the subsequent phosphorylation of cellular proteins, including p42/p44 MAP kinase.

Although it is ideal to use a single culture system for functional and molecular analyses, two different osteoclast cultures were employed in this study: one is the culture of isolated rabbit osteoclasts and the other is that of mouse osteoclasts. The isolated rabbit osteoclasts are capable of resorbing dentine and maintaining their survival on dentine slices even in the absence of bone-derived osteoblastic/stromal cells (31).
Thereby, direct actions of osteotropic hormones and local factors on mature osteoclasts in vitro can be precisely estimated without the influence of nonosteoclastic cells. However, the lack of molecular information about nucleotide and protein sequences expressed in rabbits and antibody availability for rabbit proteins restricts studies in the rabbit osteoclast culture system. To overcome this disadvantage, the mouse osteoclast culture system may aid researchers, because much molecular information has already been accumulated. Isolated mouse osteoclasts, on the other hand, essentially require the presence of bone-derived osteoblastic/stromal cells for their bone resorbing activity and survival. Indeed, as shown in Fig. 3, only a part of isolated mouse osteoclasts remained alive after 24 h of culture. Recently, mouse osteoclasts formed from cultured bone marrow cells in the presence of M-CSF: M-CSF-dependent bone marrow macrophages (M-BMMs) (47) and M-CSF-dependent bone marrow cells (MDBM cells) (48), have been reported to exhibit the potency to resorb the dentine slice even in the absence of osteoblastic/stromal cells. However, tumor necrosis factor-α (TNF-α) or RANKL/ODF, in addition to M-CSF, is essential for these mouse osteoclasts to form resorbed pits. Although our preliminary studies revealed that FGF-2 (10−11 M) increased the resorbed pit formation by mouse osteoclasts both in M-BMMs and MDBM cell cultures in the presence of TNF-α and M-CSF, the effects in both cultures were weaker (1.3- to 1.4-fold over the culture with TNF-α and M-CSF alone) than seen in the rabbit osteoclast culture. Because both M-BMMs and MDBM cell cultures still contain osteoclast precursors in much higher concentrations than the rabbit osteoclast culture, we cannot deny the possibility that these stimulations by FGF-2 may not be due to the direct action on mature osteoclast function but to the action on osteoclast differentiation. In addition, FGF-2 might affect the signaling of M-CSF through some cross-talk mechanism, because both their receptors, FGFR1 and Fls, are RTKs. In fact, we have reported that the direct action of FGF-2 on osteoclast precursor differentiation was inhibitory and that the tyrosine phosphorylation of several cellular proteins induced by M-CSF was inhibited by FGF-2 using the osteoclast precursor cell line C7 cell culture (49). Hence, these mouse osteoclast culture systems appear not to be suitable for this study that investigated the direct action of FGF-2 on mature osteoclasts. Given the above circumstances, we properly used the rabbit and mouse osteoclast cultures to study the function of FGF-2 on bone resorbing activity and its molecular mechanism, respectively.

Another issue is the relationship between the stimulation of pit formation and the up-regulation of proteinases. Because a previous report showed that cathepsin K antisense oligodeoxynucleotide inhibited the resorbed pit formation by isolated rabbit osteoclasts using the same system as this study (36), the induction of cathepsin K in osteoclasts is likely to contribute to the FGF-2 stimulation of osteoclastic bone resorption. On the other hand, BB94, a nonselective MMP inhibitor (50), did not affect the FGF-2 stimulation on resorbed pit formation on the dentine slice by rabbit osteoclasts but inhibited that on the dentine slice coated with collagen.2 These results indicate that MMPs are important for the migration of osteoclasts through the unmineralized osteoid to reach the mineralized bone surface, but not for the bone resorbing activity of osteoclasts as previously reported (41).

Signaling pathways through RTKs on osteoclasts were studied on M-CSF, which stimulates motility and cytoplasmic spreading in osteoclasts (51). FGF-2 and M-CSF, although receptors of both are RTKs expressed on osteoclasts (30), showed different actions on osteoclast function. FGF-2 did not maintain the survival of osteoclasts but M-CSF did (Fig. 3). Contrarily, M-CSF itself did not stimulate resorbing activity of osteoclasts but FGF-2 did. Regarding signal transduction, M-CSF induced the autophosphorylation of its receptor, Fls, and c-src-dependent tyrosine phosphorylation of selected proteins, including Grb2-binding protein. c-Src, a ubiquitous cellular tyrosine kinase, which is highly expressed in osteoclasts, is essential for osteoclasts to form a ruffled border and to resorb bone (52), and the contribution of c-src kinase to FGF-2 signaling has been suggested in endothelial cells and fibroblasts (27, 53, 54). In this study, inhibitors of the src family kinases, herbimycin and PP1, abrogated the osteoclast function in control cultures as well as in FGF-2-stimulated cultures. Hence, we assume that the src kinase signal may be essential for the basal osteoclast function, whereas p42/p44 MAP kinase is the major pathway for the FGF-2 action. To our knowledge, this study is the first indicating that the activation of p42/p44 MAP kinase causes the stimulation of osteoclast function.

Regarding the physiological relevance of the direct action of FGF-2 on osteoclasts, we recently reported that endogenous FGF-2 in the synovial fluid contributes to joint destruction in rheumatoid arthritis patients (55). The concentration of FGF-2 in the synovial fluid was positively correlated to the severity of joint destruction in these patients. However, the concentrations of FGF-2 were lower, on the order of 10−13–10−14 M, than other cytokines such as interleukin-6 and soluble interleukin-6 receptor, on the order of 10−11–10−10 M. These levels of FGF-2 are not enough to induce COX-2 in osteoblastic cells (18–22) but possibly affect mature osteoclasts directly. Although these effects are small compared with the COX-2-mediated effects, they occur at a concentration of FGF-2 that is likely to be important in vivo. Thus, FGF-2 in the synovial fluid might play a role in the final step of osteoclastic bone resorption in rheumatoid arthritis joint destruction that is preceded by recruitment and differentiation of osteoclasts by other factors. Other than the well-known pharmacological action of FGF-2 on bone formation, endogenous FGF-2 might function in the pathogenesis of bone resorptive diseases through its direct action on osteoclasts. Further studies will reveal the contribution of FGF-2 to the pathophysiology of osteopoenic disorders like rheumatoid arthritis.

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