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ERK5/BMK1 Is Indispensable for Optimal Colony-Stimulating Factor 1 (CSF-1)-Induced Proliferation in Macrophages in a Src-Dependent Fashion

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CSF-1, by binding to its high-affinity receptor CSF-1R, sustains the survival and proliferation of monocyte/macrophages, which are central cells of innate immunity and inflammation. The MAPK ERK5 (also known as big MAPK-1, BMK1, or MAPK7) is a 98-kDa molecule sharing high homology with ERK1/2. ERK5 is activated by oxidative stress or growth factor stimulation. This study was undertaken to characterize ERK5 involvement in macrophage signaling that is elicited by CSF-1. Exposure to the CSF-1 of primary human macrophages or murine macrophage cell lines, as well as murine fibroblasts expressing ectopic CSF-1R, resulted in a rapid and sustained increase of ERK5 phosphorylation on activation-specific residues. In the BAC1.2F5 macrophage cell line, ERK5 was also activated by another mitogen, GM-CSF, while macrophage activators such as LPS or IFN-γ and a number of nonproliferative cytokines failed. Src family kinases were found to link the activation of CSF-1R to that of ERK5, whereas protein kinase C or the serine phosphorylates PP1 and PP2A seem not to be involved in the process. Treatment of macrophages with ERK5-specific small interfering RNA markedly reduced CSF-1-induced DNA synthesis and total c-Jun phosphorylation and expression, while increasing the expression of the cyclin-dependent kinase inhibitor p27. Following CSF-1 treatment, the active form of ERK5 rapidly translocated from cytosol to nucleus. Taken together, the results reported in this study show that ERK5 is indispensable for optimal CSF-1-induced proliferation and indicate a novel target for its control. The Journal of Immunology, 2008, 180: 4166–4172.

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3 Abbreviations used in this paper: SFK, Src family kinase; EGF, epidermal growth factor; MEKK, MEK kinase; PKC, protein kinase C; PTP, protein tyrosine phosphatase; siRNA, small interfering RNA; TPA, 12-O-tetradecanoylphorbol-13-acetate.

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Materials and Methods

Cells and cell culture

Murine macrophages of the BAC1.2F5 cell line, which depends on CSF-1 for survival and proliferation (19), were cultured in DMEM supplemented with 4 mM glutamine, 10% FBS, and 10% L cell-conditioned medium (20) as a source of CSF-1 ("complete medium"). The J774 murine macrophage cell line, derived from a reticular cell sarcoma, and the NIH/3T3 murine fibroblast cell line expressing ectopic human CSF-1R (21, 22) were cultured in DMEM supplemented with 10% FBS. All cells were incubated at 37°C in a humidified atmosphere containing 5% CO2. Human primary macrophages were obtained with informed consent from healthy donor volunteers. Leukocyte-enriched buffy coat, diluted 1/1 with PBS (35 ml), was overlaid on Lympholyte (15 ml) (Cederlane Laboratories; catalog no. CL5015) and centrifuged at 2200 rpm for 15 min without brake. Interphase...
cells (lymphocytes and monocytes) were then washed twice with PBS by centrifugation. Cells were then resuspended in RPMI 1640 supplemented with 10% FBS and allowed to adhere for 2–3 h in flasks before removing the nonadherent cells. Cells were then differentiated into macrophages by culturing for 7 days in RPMI 1640 supplemented with 10% FBS and 30 ng/ml recombinant human M-CSF (PeproTech).

**Total cell lysates**

BAC1.2F5 cells and primary macrophages were incubated for 16–18 h in the absence of CSF-1, whereas J774 cells and the NIH/3T3 cell line were exposed to ectopic human CSF-1R were incubated in the absence of FBS for 24 h before being stimulated with the appropriate stimuli. Culture plates were then plated on ice; cell monolayers were rapidly washed three times with ice-cold PBS containing 100 mM orthovanadate. Cells were lysed by scraping in Laemmli buffer (62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 0.005% bromophenol blue, SDS 2%) and incubating at 95°C for 10 min. Lysates were then clarified by centrifugation (13,000 rpm for 10 min at room temperature).

**Cytosol-nucleus fractionation**

BAC1.2F5 cells were incubated for 16–18 h in DMEM supplemented with 10% FBS before being stimulated or not with CSF-1. Cells were then washed twice with ice-cold PBS and lysed by incubating for 5 min on ice in hypotonic buffer (10 mM HEPES, 10 mM NaCl, 5 mM NaHCO₃, 1 mM CaCl₂, 0.5 mM MgCl₂, 5 mM Na₂EDTA, 1 mM sodium orthovanadate, 20 mM NaF, 10 mM sodium pyrophosphate, 10 mg/ml leupeptin, 20 mg/ml aprotinin, and 1 mM PMSF) supplemented with 0.5% Nonidet P-40. Nuclei were then separated by centrifugation (for 1 min at 2000 rpm) and the cytosolic fractions contained in the supernatant were recovered. Nuclei were washed by vortexing with hypotonic buffer and lysed with Laemmli buffer as described above.

**Western blotting and immunoblotting**

Protein concentration was determined by the bicinchoninic acid method and 30–50 μg aliquots of each sample were incubated at 95°C for 10 min in the presence of 100 mM 2-ME. Proteins were then separated by SDS-PAGE (10%) and electrophorized onto nitrocellulose membranes (Hybond-ECL; Amersham Biosciences) by electroblotting. Membranes were incubated (for 3 h at room temperature) first in PBS containing 0.1% Tween 20 and 1–5% BSA (blocking buffer), then with primary Ab in blocking buffer (for 16–18 h at 4°C), and finally in the presence of HRP-conjugated secondary Ab in blocking buffer (for 1 h at 4°C). Ab-coated protein bands were visualized by the ECL detection system (Amersham Biosciences). When needed, membrane stripping was performed by incubation (for 3 x 10 min at 50°C) in a stripping buffer (62.5 mM Tris-HCl (pH 6.7), 2% SDS, 100 mM 2-ME) followed by extensive washing with PBS and 0.1% Tween 20.

**Abs, cytokines, and other reagents**

The following Abs were used according to the manufacturer’s specifications: rabbit anti-phospho-T218/Y220-ERK5 (Cell Signaling Technology; catalog no. 3371), rabbit anti-ERK5 (Cell Signaling Technology; catalog no. 3372), rabbit anti-phospho-T202/Y204-ERK1/2 (Cell Signaling Technology; catalog no. 9101), rabbit anti-ERK1 (Santa Cruz Biotechnology; catalog no. sc-93), rabbit monoclonal anti-phospho-Y723-Fms (Cell Signaling Technology; catalog no. 3372), rabbit anti-phospho-T202/Y204-ERK5 (Cell Signaling Technology; catalog no. 3371), rabbit anti-phospho-Y723-Fms (Cell Signaling Technology; catalog no. 3372), goat anti-phospho-T218/Y220-ERK5 (Cell Signaling Technology; catalog no. 3371), mouse anti-vinulsin (Sigma-Aldrich; catalog no. V9131), rabbit anti-p27 (Santa Cruz Biotechnology; catalog no. sc-528), rabbit anti-phospho-S663/73-cJun (Santa Cruz Biotechnology; catalog no. sc-66312), rabbit anti-c-Jun (Santa Cruz Biotechnology; no. sc-45), HPR-conjugated anti-mouse IgG, and anti-rabbit IgG (Sigma-Aldrich; catalog nos. A-6165 and A-4416, respectively). The cytokines and growth factors used, all acquired from PeproTech, were human recombinant CSF-1 (1 ng/ml corresponding to 5 ng/ml murine rL-3, murine recombinant GM-CSF, murine recombinant IFN-y, human rL-6, and human rL-1. LPS was from Sigma-Aldrich. The inhibitors used (manufacturer; substrate; time of pretreatment) were PP1 and PP2 (Calbiochem; SFK; 30 min), U0126 (Calbiochem; SFK; 30 min), sodium orthovanadate (Sigma-Aldrich; protein tyrosine phosphatases (PTP); 30 min), calybiecanum (PeproTech; PKC); Rp33712 (PeproTech; PKC); SB 203580 (Calbiochem; PKC), several isoflavons (30 min), rottlerin (Calbiochem; PKC), 30, 12-O-tetradecanoylphorbol-13-acetate (TPA) (Sigma-Aldrich; PKC; 10 min), PD98059 (Calbiochem; MEK1/2; 30 min), U0126 (Cell Signaling Technology; MEK1/2; 30 min), and CI-1040 (PD184352) (gift of Pfizer; MEK1/2; 30 min). Oxidative stress was induced by incubating cells for 10 min with 5 μM glucose oxidase (Sigma-Aldrich), which, in the presence of glucose, produces H₂O₂ at a continuous rate.

**ERK5 silencing with small interfering RNA (siRNA) and measurement of DNA synthesis**

Cells were removed by trypsinization, seeded in complete medium without antibiotics, and incubated for 24 h until they reached 50% confluence. Transfection was then performed with Lipofectamine 2000 (Invitrogen Life Technologies) and 100 nM SMARTpool siRNA for ERK5 (a mix of four oligos) directed to different parts of human ERK5 (catalog no. NM_002729) or murine (GenBank accession no. NM_011841) ERK5 mRNA (Dharmacon; catalog no. M-003513-02 and M-040333-00, respectively), 100 nM siCONTROL nontargeting siRNA no. 1 (Dharmacon; catalog no. D-001206-13) following the manufacturer’s instructions. Transfection efficiency was 90%, as assessed in parallel with Cy3-labeled siGLO RISC-free siRNA. Mock transfection was conducted with Lipofectamine 2000 alone. One day after transfection, macrophages were washed with PBS and incubated for 16–18 h in the absence of CSF-1 before being stimulated or not with human recombinant CSF-1 for 24 h. During the last 4 h of incubation [³H]thymidine was added to the culture at 1 μCi/ml final concentration. Thymidine incorporation was stopped by incubating the cells for 1 h in the presence of 10% trichloroacetic acid. After extensive washing, cells were harvested in 0.5 N NaOH and radioactivity was measured by a liquid scintillation counter.

**Immunofluorescence**

BAC1.2F5 macrophages were plated on glass coverslips in complete medium and incubated for 16–18 h in DMEM supplemented with 10% FBS before being stimulated or not by CSF-1. Cells were washed once with PBS and fixed with 4% paraformaldehyde in PBS for 10 min at room temperature. Coverslips were washed once with PBS and then permeabilized by a 5-min-long incubation with 0.2% Triton X-100 in PBS. After three washes in PBS, protein binding sites were saturated by incubation with 10% horse serum in PBS and 1% BSA for 45 min. Cells were then washed in PBS and incubated overnight at 4°C in a 1/50 dilution of a goat anti-ERK5 primary Ab (Santa Cruz Biotechnology; catalog no. sc-1284) in PBS with 1% BSA. Cells were washed three times with PBS and immunocomplexes were revealed with anti-goat Cy3-labeled secondary Abs (AP180C; Chemicon). Cells were washed once in PBS and incubated with the Hoechst 33258 (Sigma-Aldrich; catalog no. B-1155) nuclear dye in PBS at 10 min for 37°C. Following two washes in PBS, coverslips were mounted with propylthiogallate on glass slides and the cells were observed in an inverted confocal Nikon Eclipse TE2000 microscope equipped with a Nikon S Fluor x60 oil immersion lens, a violet diode laser (408 nm), an argon laser (488 nm), and a helium-neon laser (543 nm). The C1 software was used for image acquisition and the Adobe Photoshop software for image size setting. Incubation with the secondary Ab only did not produce any significant fluorescence.

**Results**

**CSF-1 stimulates ERK5 activation in macrophages**

BAC1.2F5 cells routinely cultured in the presence of CSF-1 showed a slight constitutive ERK5 dual phosphorylation (not shown). When BAC1.2F5 cells were deprived of CSF-1 for 18 h, ERK5 phosphorylation was almost undetectable (Fig. 1A, lane 1). CSF-1 stimulated ERK5 activation in a dose-dependent manner. Doses as low as 2.5 ng/ml (Fig. 1A, lane 2) were able to increase ERK5 phosphorylation, which was further increased with 5 ng/ml (lane 3) and reached maximal levels with 25–50 ng/ml CSF-1 (lanes 4 and 5). In BAC1.2F5 cells ERK5 migrates as a 115-kDa major band, as determined by means of two different Abs directed to the dual-phosphorylated form of ERK5, as well as two Abs directed to different epitopes of ERK5 protein (Fig. 1 and data not shown). The kinetics of CSF-1-induced ERK5 activation was very rapid, as ERK5 phosphorylation was detectable as early as 1 min after CSF-1 administration (Fig. 1B, lane 2) and reached a peak of activation after 3–10 min (Fig. 1, B and C, see also Fig. 2B)
that CSF-1 activated ERK5 in J774 cells (Fig. 1). When murine macrophage cell lines other than BAC1.2F5 were tested, we found results were obtained in NIH/3T3 cells expressing ectopic CSF-1R (lanes 1–3). When murine macrophage cell lines other than BAC1.2F5 were tested, we found that CSF-1 activated ERK5 in J774 cells (Fig. 1D, lane 6 and 7) but not RAW cells (lanes 8 and 9). CSF-1 was also markedly activated following CSF-1 treatment in murine NIH/3T3 fibroblasts expressing ectopic human CSF-1R (Fig. 5B, lanes 1 and 2).

Proliferative, rather than activating, signals stimulate ERK5 in macrophages

CSF-1 turned out to be a strong activator of ERK5 when compared with oxidative stress, a stimulus known to markedly activate ERK5 in a number of cell types, which is generated following glucose oxidase administration to cells (Fig. 2A, lane 12 vs lane 2). Similar results were obtained in NIH/3T3 cells expressing ectopic CSF-1R or when H2O2 was directly administered to cells (not shown). In contrast, potent macrophage activators such as LPS (at 10 ng/ml, a standard macrophage-activating dose) or IFN-γ, as well as other inflammatory cytokines such as IL1 or IL6, failed to activate ERK5. In Fig. 2B LPS was used at a higher dose (200 ng/ml) that is known to activate ERK1/2. In these experiments, ERK1/2 was activated by LPS (Fig. 2B, lanes 6–9) and CSF-1 (lanes 2–5) with the different kinases previously reported (23, 24), whereas ERK5, here again, was insensitive to LPS at any time of treatment. Although CSF-1 is the main stimulator of proliferation, GM-CSF also induces proliferative signals in macrophages. GM-CSF induced ERK5 activation (Fig. 2C, lanes 6 and 7), although to levels markedly lower than those induced by CSF-1 (lane 2). Moreover, IL-3, which also stimulates macrophage proliferation and was able to activate ERK1/2 in BAC1.2F5 cells (not shown), failed to activate ERK5 (Fig. 3C, lanes 3–5). Taken together, the results of Fig. 2 indicate that ERK5 is activated in macrophages by inducers of proliferation but not activation and, among the former, CSF-1 is the most potent activator of ERK5.

Tyrosine phosphatases, the Ser/Thr phosphatases PPI and PP2A, and PKC are not involved in CSF-1-induced ERK5 activation

Okadaic acid, a specific inhibitor of the PPI and PP2A Ser/Thr phosphatases, has been demonstrated to block the activation of ERK5 that occurs in HeLa cells exposed to EGF or H2O2, as well as in PC12 cells stimulated by nerve growth factor or H2O2 (25). As shown in Fig. 3A, the treatment of BAC1.2F5 cells with orthovanadate, a PTP inhibitor, or okadaic acid did not affect CSF-1-induced ERK5 phosphorylation or modify its kinetics (data not shown), suggesting that okadaic acid-sensitive phosphatases or PTP are not involved in ERK5 dephosphorylation. PKC has been demonstrated to act as a negative regulator of the ERK5 activation pathway (25). CSF-1 has been reported to activate PKC, or at least PKC-α and -δ (26, 27), although PKC is not involved in CSF-1-induced ERK1/2 activation (28). Fig. 3B shows that the treatment with several inhibitors to target different PKC isoforms did not interfere with the CSF-1-induced ERK5 activation (lanes 4, 6, and 8 vs lane 2). It should be noted that PKC is also not involved in CSF-1-induced ERK1/2 activation (Fig. 3B, lanes 4, 6, and 8 vs lane 2), as already reported (28). In contrast, direct activation of PKC, elicited by treating cells with TPA (Fig. 3B, lane 9), determined massive activation of ERK1/2 but not ERK5, in keeping with the results of Fig. 2B relative to treatment with LPS, which is a known PKC activator in macrophages (29). Consistent with the previously reported TPA-induced cleavage of CSF-1R (30), when CSF-1 was administered to cells pretreated with TPA, ERK5 phosphorylation did not occur. We can therefore conclude that PKC is
neither a positive nor a negative regulator of CSF-1-induced ERK5 activation, confirming that ERK5 is not downstream of PKC in macrophages. We also found that inhibition of the PI3K pathway by wortmannin or LY294002 did not decrease the level of CSF-1-induced ERK5 activation (not shown).

**CSF-1-induced ERK5 activation is not sensitive to MEK1/2 inhibitors**

It has been previously reported that MEK1/2 inhibitors (namely, PD98059 and U0126) affect the ERK5 pathway as well (11, 31). In BAC1.2F5 cells, CI-1040 (32), considered the most specific inhibitor of ERK1/2 phosphorylation, did not alter CSF-1-induced ERK5 phosphorylation (Fig. 4, lanes 3–5). On the contrary, ERK5 phosphorylation was sensitive to 10 μM UO126, the concentration normally used to inhibit ERK1/2 phosphorylation (Fig. 4, lanes 6–8), as well as PD98059 at standard doses (lanes 9–11). All of the above is in keeping with data collected in a number of cell systems (11, 31) and indicates the absence, to date, of specific ERK5 inhibitors.

**CSF-1 induced ERK5 activation is dependent on SFK**

ERK5 activation has been demonstrated to be SFK-dependent in other cell systems (33, 34). We therefore assessed this possibility for ERK5 activation in response to CSF-1, as CSF-1R is known to transduce SFK-dependent as well as SFK-independent signals (Fig. 5). To this purpose, we used two different concentrations of different SFK inhibitors, PP1 and PP2, and the unrelated SU6656. The treatment of BAC1.2F5 cells with 10 μM PP1 or PP2 strongly inhibited, and that with 3 μM SU6656 suppressed, CSF-1-induced ERK5 phosphorylation (Fig. 5A, lanes 4, 7, and 10 vs lane 2). These treatments, except for that with SU6656, did not significantly affect CSF-1R expression (not shown) or the activation of other signaling pathways, such as that of PI3K, as shown by using AKT phosphorylation as readout (Fig. 5A, lane 7) is due to a slight effect of this inhibitor on CSF-1R phosphorylation (not shown). SFK are known to be recruited by binding to the tyrosine residue 561 located in the juxtamembrane domain of CSF-1R. Therefore, we used murine fibroblasts expressing the wild-type or a mutated form of ectopic human CSF-1R to confirm the involvement of SFK in CSF-1-induced ERK5 activation. When Y561 was mutated to prevent the docking of SFK, CSF-1 was unable to activate ERK5 (Fig. 5B, lane 4 vs lane 2). ERK1/2 activation, which is partially SFK-dependent and -independent, still underwent a massive phosphorylation in response to CSF-1.
ERK5 is necessary for CSF-1-induced macrophage proliferation

We showed above that proliferative, rather than macrophage-activating, signals stimulate ERK5 phosphorylation in macrophages and that, among the former, CSF-1 is the most potent activator of ERK5. We therefore assessed the involvement of ERK5 in CSF-1-induced proliferation by silencing ERK5 with specific siRNA (Fig. 6). In BAC1.2F5 macrophages, siRNA directed to ERK5 resulted in a decrease of 50% of ERK5 protein level, as assessed by Western blotting (Fig. 6A). Data reported in the graph are average ± SEM of data obtained from five independent experiments, each performed in triplicate. Phosphorylated Jun (p-Jun) and Jun densitometric values in NT and ERK5 siRNA-transfected cells are shown in B. Indeed, we found that the level of c-Jun protein was increased after ERK5 silencing and was reduced almost by half (0.48 vs 0.7, p < 0.05) following ERK5 silencing, as determined by averaging the values obtained from three different experiments (not shown). Accordingly, total c-Jun phosphorylation decreased after ERK5 silencing and was reduced almost by half (0.7 vs 1.3; see Fig. 6C, lane 4 vs lane 2). These effects may explain the impairment of CSF-1-induced cell proliferation following ERK5 silencing.

In primary human macrophages, ERK5 silencing resulted in an inhibition of [3H]thymidine uptake that was even more pronounced than that observed in BAC1.2F5 cells (Fig. 7A). Indeed, when ERK5 expression was reduced to 40% by treatment with specific siRNA, the effect of CSF-1 treatment was abrogated. Moreover, as reported above for BAC1.2F5 cells, ERK5 silencing resulted in a marked decrease of c-Jun phosphorylation. This may be due in part to a markedly decreased c-Jun protein (Fig. 7B). The results shown in Figs. 6 and 7 suggest a central role of ERK5 in macrophage proliferation.
exposure). p-ERK5, Phosphorylated ERK5.

kinase (MEKK) 2 appears to be the most potent ERK5 activator, as previously reported in the case of the EGF receptor (37). MEK

that shows ERK5 to be involved in CSF-1R signaling. Moreover, by

show that ERK5 activation is seemingly dependent on SFK, which

are known to be activated following CSF-1 administration. The accuracy of the cell fractionation technique was validated by immunoblotting for vinculin and histone H4 as markers for the cytosol and the nucleus, respectively. These results confirm those of a recent study in which the nuclear translocation of ERK5 was demonstrated to be dependent on its activating phosphorylation by MEK5 (36).

Discussion

In this work we present data that demonstrate the ability of mitogens such as CSF-1 and GM-CSF to activate ERK5. Compared with many other stimuli, CSF-1 appears to be the strongest activator of ERK5 in macrophages, and ERK5 appears to be required for CSF-1-induced proliferation of these cells. This is the first report, to our knowledge, that shows ERK5 to be involved in CSF-1R signaling. Moreover, by characterizing the initial events for CSF-1-induced ERK5 activation, we show that ERK5 activation is seemingly dependent on SFK, which are known to be activated following CSF-1 treatment (3). It is possible that CSF-1R recruits ERK5 via an adaptor/MEKK2/MEK5 complex, as previously reported in the case of the EGF receptor (37). MEK kinase (MEKK) 2 appears to be the most potent ERK5 activator, although both MEKK2 and MEKK3 potentially activate the MEK5/ERK5 module (38).

CSF-1-induced ERK5 activation is rapid and sustained, with a time course typical of MAPK response to proliferative signals. Specifically, CSF-1-induced ERK5 activation is more rapid than that of ERK1/2, which is involved in macrophage proliferation. However, whereas ERK1/2 also participates in the transduction of macrophage-activating signals, LPS or IFN-γ failed to activate ERK5. This result is in apparent contrast with a previous report describing LPS-mediated ERK5 phosphorylation in RAW 264.7 macrophages (39). Our combined data are consistent with a role for ERK5 in macrophages essentially related to the control of proliferation. Indeed, ERK5 activation was paralleled by the migration of the active form into the nucleus. This is important in view of the fact that many substrates of ERK5, especially those involved in cell proliferation, are nuclear proteins. Accordingly, when ERK5 expression was decreased following siRNA transfection, CSF-1-induced proliferation was markedly decreased in BAC1.2F5 macrophages and almost completely suppressed in human primary macrophages. Moreover, following ERK5 silencing in NIH/3T3 cells expressing ectopic CSF-1R, we found a 20% reduction of CSF-1-induced mitogenesis (not shown).

Studies of the effects of ERK5 silencing on ERK5 downstream targets showed that c-Jun expression and phosphorylation following CSF-1 administration were decreased in ERK5 siRNA-transfected cells, while the expression of p27 was increased. Specifically, absolute c-Jun phosphorylation, i.e., c-Jun phosphorylation normalized for c-Jun content, did not seem to be affected by ERK5 silencing (not shown). The effect of ERK5 on c-Jun expression is not unique to macrophages, as we recently showed in hepatic stellate cells (40), and may be due to mechanisms similar to those recently reported by Ren and colleagues (41). The ERK5 silencing effects on c-Jun and p27 expression levels may well explain the impairment of CSF-1-induced mitogenesis. This conclusion is supported by the fact that an inhibitory effect of p27 accumulation on CSF-1-induced proliferation has been previously described (42). In contrast, neither p27 nor c-Jun appeared to be involved in basal [3H]thymidine incorporation in the absence of CSF-1. No effect of transfection with ERK5 siRNA was detected on cyclin D1 and c-Myc (not shown), although their transcription depends on c-Jun (43, 44).

Previous reports showed that the CSF-1-dependent proliferation of myeloid cells depends on ERK1/2 and PI3K (6). ERK5 does not seem to be directly upstream of PI3K or ERK1/2 in CSF-1- or platelet-derived growth factor (PDGF)-stimulated cells (this work and Ref. 40, as silencing of ERK5 does not alter the activation of PI3K, at least on the basis of AKT phosphorylation, or ERK1/2. Furthermore, as neither PI3K nor ERK1/2 inhibition resulted in the impairment of ERK5 activation, ERK5 does not appear to act downstream of either kinase. The down-regulation of c-Jun following ERK5 silencing may well explain the impairment of the ERK1/2-dependent, c-Jun-mediated, CSF-1-induced proliferation. In contrast, cross-talk between the ERK5 and the PI3K pathways has not been reported. However, we cannot exclude the possibility that PI3K-dependent, CSF-1-induced proliferation could be masked by the down-modulation of a common target of PI3K and ERK5.

Our studies have been performed on mesenchymal cells such as macrophages where CSF-1R is physiologically expressed at the highest levels or fibroblasts expressing ectopic CSF-1R. The characterization of CSF-1-elicited signals in macrophages is of great interest, as these are cells central to innate immunity and inflammation (45). Moreover, the importance of the so-called tumor-associated macrophages in the context of a developing neoplasia.
18. English, J. M., G. Pearson, R. Baer, and M. H. Cobb. 1998. Identification of CSF-1 and its receptor correlates with tumor cell invasiveness and adverse clinical prognosis (46). Recent findings also implicate tumor-produced CSF-1 as a promoter of bone metastasis in breast cancer (46). Therefore, the study of CSF-1-elicited signals is needed to characterize the behavior of both tumor-associated macrophages and tumor cells themselves, i.e., to increase our understanding of the pathogenesis of several neoplastic diseases.

Our results also introduce future perspectives in the treatment of CSF-1-related neoplasia. Targeting ERK5 affords the possibility of inhibiting CSF-1-dependent proliferation of neoplastic cells, but not macrophage activation. Therefore, the use of specific ERK5 inhibitors may have, when they become available, significant clinical value.

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Disclosures

The authors have no financial conflict of interest.

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