Colony-stimulating factor-1–induced oscillations in phosphatidylinositol-3 kinase/AKT are required for caspase activation in monocytes undergoing differentiation into macrophages

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Introduction

Monocytes have the unique property among peripheral blood cells to migrate into tissues and to further differentiate into morphologically and functionally heterogeneous cells that include macrophages, myeloid dendritic cells, and osteoclasts. In contrast with the differentiation of bone-marrow progenitors and precursors, this development generally proceeds in the absence of proliferation. The differentiation of peripheral blood monocytes into resident tissue macrophages can be recapitulated ex vivo by incubation in the presence of macrophage colony-stimulating factor, also known as colony-stimulating factor-1 (CSF-1). This homodimeric glycoprotein stimulates the proliferation, differentiation, and survival of multipotential, bipotential, and unipotential bone marrow progenitors as they progress throughout the monocyte differentiation pathway.1 CSF-1 may be sufficient in physiologic conditions to provoke the formation of the caspase-8–activating molecular platform. CSF-1 and its receptor are both required for oscillations in AKT activation to occur, and expression of a constitutively active AKT mutant prevents the macrophage differentiation process. The extracellular receptor kinase 1/2 pathway is activated with a coordinated oscillatory kinetics in a CSF-1R–dependent manner but plays an accessory role in caspase activation and nucleophosmin cleavage. Altogether, CSF-1 stimulation activates a molecular clock that involves phosphatidylinositol-3 kinase and AKT to promote caspase activation. This oscillatory signaling pathway, which is coordinated with extracellular receptor kinase 1/2 oscillatory activation, involves CSF-1 and CSF-1R and controls the terminal differentiation of macrophages. (Blood. 2009;114:3633-3641)
molecule that may couple the activated CSF-1R to MAPK activation, whereas PI3K can be activated by direct binding of its p85 regulatory subunit to phosphorylated tyrosine 723 of CSF-1R or through interaction of p85 with a Src family kinase (SKF) member that binds phosphorylated tyrosine 559 of the CSF-1R. Active PI3K, which is formed by recruiting one of the p110 isoforms of the class I A catalytic subunit to p85, stimulates downstream targets that include the serine/threonine kinase AKT. Negative regulators of PI3K pathways include inositol phosphatases such as phosphatase and tensin homologs and the Src homology 2 domain-containing inositol 5′-phosphatase SH2 inositol 5′-phosphatase 1 and SH2 inositol 5′-phosphatase 2. These negative regulators of CSF-1 signaling reduce AKT activity and inhibit nuclear factor (NF-κB)–regulated gene transcription in CSF-1–stimulated cells. ERK1/2-negative regulators in monocytes may include protein phosphatase 2A, T-cell protein tyrosine phosphatase, and some of the MAPK phosphatase family members (MKP1, 2, and 4). Both the MAPK and the PI3K/AKT pathways are required for the commitment of monocytes to macrophages, but the interplay between them in this specific context remains unclear.

Transient activation of NF-κB is part of the transcriptional program that drives CSF-1–induced macrophage differentiation. We have shown that CSF-1–mediated differentiation requires the activation of caspase-8 in a multimolecular platform whose formation culminates 2 to 3 days after CSF-1 stimulation. As a consequence, the serine/threonine kinase RIP1 is cleaved, allowing the release of a cleavage fragment involved in the down-regulation of NF-κB activity that characterizes terminal macrophage differentiation. Caspase-8 activates downstream caspase-3 that cleaves a limited number of intracellular proteins. Here, we demonstrate that the synchronized oscillatory activation of PI3K/AKT and ERK1/2 amplifies these signaling pathways and that PI3K/AKT waves end with caspase-8 activation, suggesting that a molecular clock is involved in the control of caspase activity along CSF-1–driven macrophage differentiation.

Methods

Reagents and antibodies

CSF-1 was purchased from PromoCell; kinase inhibitors MG132 and epoxomicin from Calbiochem; and Q-VD-OPH from MBL. Mouse monoclonal antibodies (Abs) used include anti–human heat shock cognate (HSC) 70, CD71, HCK, LYN, and FYN (from Santa Cruz Biotechnology); caspase 8 (from BML); and Rieske iron-sulfur protein (RIP1) and phospho-IκBα, phospho-ERK1/2, AKT, phospho-AKT, p38 MAPK, phospho-p38 MAPK, CSF-1R, phospho-CSF-1R, phospho-p70 S6 kinase, IκBα, phospho-IκBα, and IκBα, nucleophosmin (NPM), caspase-1, and peroxidase-conjugated anti–rabbit Abs were from Cell Signaling Technology. Secondary Abs included horseradish peroxidase–conjugated goat anti–mouse IgG1 and IgG2b (Southern Biotechnology). In flow cytometric experiments, we used APC-conjugated anti-CD71, -CD16, and isotype IgG1-matched control; PE-conjugated anti-CD11b, -CD14, and -CD115 (CSF-1R); and IgG1 isotype control (PharMingen). A goat anti–caspase-8 (Santa Cruz Biotechnology) was used for immunoprecipitation experiments.

Cell culture and differentiation

Human peripheral blood monocytes were obtained from healthy donors with informed consent according to recommendations of an independent scientific review board, in accordance with the Declaration of Helsinki. The ethical board of Dijon Hospital approved this protocol. Cells were enriched by the use of a monocYTE isolation kit with autoMACS Separator according to the manufacturer’s instructions (Miltenyi Biotec), grown in RPMI 1640 medium with glutamax-I (Lonza) supplemented with 10% or 2% (vol/vol) fetal bovine serum (Lonza) in standard conditions, and exposed to 100 ng/mL CSF-1. Macrophage differentiation (adhesion to culture flasks and fibroblast-like shape) was visualized by the use of an inverted microscope (Axiovert 25; Zeiss) equipped with an AxioCam HRm camera (Zeiss). Phase images of culture were recorded with a 20×/0.30 PH objective with AxioVision 4.7 software (Zeiss). Trypan blue was used to check cell viability.

Flow cytometry

To detect caspase activity, we used FAM-IEDT-fmk and FAM-DEVD-fmk detection kits (PromoCell). The expression of CD115 was studied on cells washed with ice-cold phosphate-buffered saline (PBS), incubated at 4°C for 1 hour in PBS/volume serum albumin (bovine serum albumin 0.1%) with a phycoerythrin-conjugated CD115 or an isotype control, washed, and fixed in 2% paraformaldehyde. Macrophage differentiation was studied by use of the same protocol with anti-CD71, -CD163, -CD14, and -CD16 Abs. Exposure of phosphatidylinerine on the outer plasma membrane leaflet was identified by annexin V–FITC staining in the presence of propidium iodide according to the manufacturer’s instruction (annexin V–FITC apoptosis detection kit I; Pharmingen). Fluorescence was measured by the use of an LSRII flow cytometer (Becton Dickinson).

Immunoblot assays

Cells were lysed for 30 minutes at 4°C in lysis buffer (50 mmol/L HEPES, pH 7.4; 150 mmol/L NaCl; 20 mmol/L ethylene diamine tetraacetic acid; 100 mmol/L NaF; 10 mmol/L Na3VO4; complete protease inhibitor mixture; and 1% Triton X-100). Lysates were centrifuged at 20 000g (15 minutes, 4°C), and supernatants were supplemented with concentrated sodium dodecyl sulfate. Then, 50 μg of proteins were separated and transferred following standard protocols before analysis with chemiluminescence detection kit (Santa Cruz Biotechnology).

Transient transfection and siRNA knockdown

Small interfering (si) RNAs were introduced into monocytes by nucleoforation of 5 μg of siRNA. Cells were incubated overnight with 4 mL of prewarmed complete medium, and CSF-1 was subsequently added. We used siRNAs (Eurogentec) targeting AKT (AKT1: 5′-CACCGCGCAAuUAaUaUCGGAGAU-CACtAa-3′ and AKT2: 5′-CGCCGACaUaUaAgucGAGAU-CACtAa-3′), p85 PI3K subunit (PI3K1: 5′-CG CCPCCGCACCUACUUUt-3′ and PI3K2: 5′-CGCCGCGCuUUACAGUAaAaAa-3′), and luciferase as a negative control (Sense: 5′-CUAcGUAGCUAUCUCC-3′). Other siRNA sequences are accessible upon request (Invitrogen). Transient transfections were performed by nucleoforation of 5 × 10⁶ monocytes in 100 μL of nucleofector solution with 150 ng of pcdNA4 or pcdNA4-Myr-AKT plasmid (a kind gift of Dr Jean-François Tanti, Inserm U895).

Reverse-transcription and real-time polymerase chain reaction

Total RNA was isolated with TRizol (Invitrogen) and reverse transcribed by Moloney murine leukemia virus reverse transcriptase (Promega) with random hexamers (Promega). Specific forward and reverse primers are accessible upon request. Real-time polymerase chain reaction (PCR) was performed with AmpliTaq Gold polymerase in an Applied Biosystems 7500 Fast thermocycler by use of the standard SyBr Green detection protocol (Applied Biosystems). In brief, 12 ng of total complementary DNA, 50 mmol/L (each) primers, and 1× SyBr Green mixture were used in a total volume of 20 μL.

Immunoprecipitation

Monocytes (50 × 10⁶) were stimulated with 100 ng/mL CSF-1 in the presence or absence of kinase inhibitor, washed with cold PBS, and lysed for 30 minutes on ice in 1 mL of lysis buffer containing 1% NP-40, 20 mmol/L Tris-HCl, pH 7.5; 150 mmol/L NaCl; 10% glycerol; and...
complete protease inhibitor mixture. After centrifugation at 20,000 g (4°C, 30 minutes), 6 mg of supernatant proteins were precluded in the presence of Sepharose 6B (Sigma-Aldrich, Sigma), centrifuged, and incubated with an anti-caspase 8 antibody (1 g/mL) at 4°C for 20 hours in the presence of 60 g/mL of protein G (Amersham). Beads were washed 4 times with the lysis buffer, and proteins were analyzed by immunoblotting.

Densitometry and statistical analysis

The densitometry of blots was realized by the use of ImageJ software (National Institutes of Health). The statistical analysis was performed by the use of the Student $t$ test, and significance was considered when $P$ values were lower than .05. Results are expressed as mean plus or minus SEM.

Results

CSF-1–induced caspase activation is sensitive to specific kinase inhibitors

We and others have shown that CSF-1–induced differentiation of peripheral blood monocytes into macrophages required the activation of caspase-8 and downstream caspase-3. Figure 1A shows the time-dependent accumulation of FAM-IETD-fmk and FAM-DEVD-fmk cleavage activities, which may indicate caspase-8 and caspase-3 activation, along the differentiation process. These activities were detected at day 2 to 3 after the beginning of cell exposure to CSF-1 and increased with time (Figure 1A). The differentiation of monocytes into macrophages is assessed by the increased expression of CD71 and CD163 at the cell surface, as well as the increase fraction of CD14-positive cells that express CD16 (Figure 1A). Immunoblot analysis of monocytes exposed to CSF-1 for 4 days demonstrated the differentiation-associated procaspase-8 and -3 cleavages into intermediate fragments that precede the formation of active enzymes and the downstream proteolysis of the 38-kDa NPM, whereas transferrin receptor (CD71) accumulated in the cells (Figure 1B).

To identify the signaling pathways connecting CSF-1R interaction with its ligand to downstream caspase activation, we tested a series of kinase inhibitors, including SFK inhibitors PP1 and PP2, mitogen-activated protein/extracellular signal-regulated kinase inhibitor U0126, PI3K inhibitors LY294002 and wortmannin, p38 MAPK inhibitor SB202190, and protein kinase C inhibitor GF109203X. The concentration chosen depended on preliminary experiments demonstrating the kinase inhibitory activity of these inhibitors.
The interaction of CSF-1 with CSF-1R rapidly and transiently activates, within the first 30 minutes, a series of signaling events starting with an auto-/trans-phosphorylation of CSF-1R (seen in Figure 1C-D) and wortmannin (not shown) all prevented pro-caspase-8 and -3 and NPM cleavage, similarly to Q-VD-OPH. The ERK kinase inhibitor U0126 demonstrated a limited effect, and the ERK1/2 reappeared between 1 and 2 hours after the beginning of cell treatment, disappeared at 2 hours to reappear at 3 hours, with a peak at 5 hours and a subsequent dephosphorylation at 6 hours. These 3 first synchronizes waves (in the first 30 minutes, then between 0.5 and 2.5 hours, and then between 2.5 and 6 hours) were identified with a remarkably similar kinetics in 5 independent experiments. A new wave of phosphorylation of AKT and ERK1/2 appeared at 7 hours, then decreased progressively during a 48-hour period with some variations that could indicate that additional periods of phosphorylation/dephosphorylation could have been missed by these experiments (Figure 2B,E). These later waves of AKT and ERK1/2 activation preceded the appearance of CD71, a marker of monocyte differentiation into macrophages, and the proteolytic cleavage of NPM by caspases (Figure 2E). The
interplay between PI3K/AKT and ERK1/2 activation in CSF-1–stimulated human primary monocytes is only partly understood. Here, we show that U0126 added at the beginning of cell culture completely blocks the phosphorylation of ERK1/2 but marginally affects the oscillatory AKT phosphorylation. Conversely, LY294002 added at the beginning of cell culture inhibits AKT phosphorylation and marginally affects oscillations in ERK1/2 phosphorylation (Figure 2F). These results suggested that oscillations in both pathways were synchronized but independent.

CSF-1R is required for the oscillatory activation of ERK1/2 and AKT

In response to CSF-1, phosphorylated CSF-1R dimers are ubiquitinated by the E3 ligase c-Cbl and rapidly degraded in the lysosomes. An alternative intramembrane proteolysis of CSF-1R also was described in CSF-1–stimulated cells. Here, we found a rapid 30% decrease in CSF-1R expression at the surface of monocytes exposed to CSF-1 (Figure 3A-B). This decrease remained unaffected by 2 proteasome inhibitors, MG132 and epoxomicin (supplemental Figure 2). Thirty minutes later, the receptor was re-expressed at its initial level, although the total level of cellular protein explored by immunoblot had decreased. CSF-1R expression at the cell surface then diminished in a time-dependent manner (Figure 3A). The phosphorylation of tyrosine 723, used as a marker for CSF-1R activation, was detected 5 minutes after exposure to CSF-1 and decreased, to reappear with a lower intensity at 4 and 8 hours after cell exposure to the cytokine, suggesting oscillatory activation (Figure 3C). GW2580, an ATP-competitive inhibitor of CSF-1R, completely inhibited the phosphorylation of the receptor on tyrosine 723 as well as that of ERK1/2 and AKT when added to the culture with CSF-1 (Figure 3E). GW2580 also prevented the following waves of phosphorylation of ERK1/2 and AKT when added 30 minutes, 2 hours, or 6 hours after the beginning of cytokine treatment (Figure 3C). These results suggested that, despite partial down-regulation, CSF-1R was required for the oscillatory activation of ERK1/2 and AKT pathways. Accordingly, when CSF-1 was removed from the culture medium 2 hours after the beginning of cell stimulation, the amplitude of the following waves of AKT phosphorylation was decreased, and no subsequent wave of AKT activation could be detected (Figure 3D).

The PI3K/AKT pathway plays a key role in macrophage differentiation-associated caspase activation

To further confirm the role of the PI3K/AKT pathway in caspase activation that occurs along CSF-1–driven macrophage differentiation of monocytes, we used siRNA targeting AKT and the p85 regulatory subunit of PI3K. We selected 2 siRNAs that efficiently decreased the targeted mRNA level in monocytes (Figure 4A). As observed with LY294002, down-regulation of AKT or p85 prevented the differentiation of monocytes into macrophages, as indicated by the decreased number of cells with a fibroblast-like shape and the appearance of CD163 at the cell surface (Figure 4B). The down-regulation of AKT and PI3K also limited the differentiation associated appearance of a DEVD-fmk cleavage activity, as observed with LY294002 (Figure 4C). The down-regulation of targeted kinases was confirmed by immunoblot together with NPM cleavage inhibition (Figure 4D-E).

Because the role of the other signaling pathways initially was explored with pharmacologic compounds of limited specificity, we also tested a series of siRNA targeting specific SFK, including...
SRC, HCK, LYN, and FYN, as well as ERK1 and ERK2 or the combination of both. Schematically, down-regulation of HCK and, to a lesser extent, down-regulation of LYN negatively interfered with NPM proteolysis into a 30-kDa fragment by caspase-3. Down-regulation of FYN, SRC, ERK1, and ERK2 did not demonstrate any effect, although they could interfere with other aspects of macrophage differentiation such as the expression of some cell-surface markers, and the combination of ERK1 and ERK2 siRNA had limited efficacy (supplemental Figure 3). We then transfected primary monocytes with a constitutively active mutant of AKT (Myr-AKT). The efficacy of the transfection was checked by quantitative PCR (qPCR), and the kinase activity was demonstrated by identification of the phosphorylated form of p70 S6K. The efficacy of the transfection was checked by quantitative PCR (qPCR), and the kinase activity was demonstrated by identification of the phosphorylated form of p70 S6K.

Figure 4. PI3K and AKT down-regulation prevents CSF-1-mediated caspase activation. (A) Monocytes were transfected with siRNA targeting Luciferase (SC) or AKT1(AKT or AKT2) or the p85 subunit of PI3K (PI3K1 or PI3K2) 2 days before real-time qPCR analysis of the corresponding mRNAs (mean ± SD of 3 independent experiments). (B) Monocytes were exposed for 2 days to CSF-1 after indicated siRNA transfection. Macrophage differentiation was examined morphologically (fibroblast-like shape) and by 2-color flow cytometric analysis. Percentages indicate cells that express both CD71 and CD163 or both CD14 and CD16. Similar results were obtained with the 2 siRNAs targeting indicated genes. (C) FAM-DEVD cleavage activity was studied by flow cytometry in monocytes treated for 2 days with CSF-1 in the absence or presence of 10 μmol/L LY added 30 minutes before CSF-1 or in cells transfected at day 0 with siRNA targeting AKT (AKT1 and AKT2) or p85 PI3K subunit (PI3K1 and PI3K2). Numbers: percentages of cells with DEVD activity. One representative of at least 3 independent experiments is shown. (D-E) Expression of p85 PI3K subunit, NPM, and its cleavage fragment (NPM*) in cells transfected with indicated siRNA and treated for 2 days with CSF-1 as above. HSC70 indicates loading control. Molecular weight (MW) is in kilodaltons. In panel D, vertical line indicates repositioned gel lanes caused by the removal of an inefficient control.

Discussion

CSF-1 regulates the development of macrophages found in tissues undergoing active morphogenesis and/or tissue remodeling. The activation of caspase-8 and -3 is part of the specific differentiation program activated in monocytes by CSF-1.20,21 The present study demonstrates that CSF-1 interaction with its receptor is connected to procaspase-8 interaction with FADD by the PI3K/AKT pathway and, to a much lower extent, the ERK1/ERK2 pathway. Importantly, amplification of the PI3K/AKT signaling pathway through waves of phosphorylation/dephosphorylation is required for caspase-8 recruitment in the multimeric platform in which the protease is activated. The oscillatory activation of AKT signaling pathway may act as a molecular clock that accompanies the differentiation process.
Molecular clocks regulate several biologic processes. One is the segmentation that establishes in vertebrate embryo during somitogenesis, which is associated with the periodic activation of 3 major signaling pathways, Notch, fibroblast growth factor, and Wnt.25 Another is the circadian clock that involves transcriptional/posttranscriptional feedback loops together with cytosolic rhythms in small signaling molecules.26 Oscillations in Ras and ERK activities do exist in some cell systems through negative feedback phosphorylation of Sos by ERK27 or the Raf kinase inhibitor protein.28 Analysis of a murine myeloid cell line ectopically expressing CSF-1R demonstrated that CSF-1 could induce 2 temporally distinct phases of MAPK activation.16 Such oscillations have not been described for the PI3K/AKT pathway. Here, we demonstrate that, in primary human monocytes exposed to CSF-1, several waves of phosphorylation/activation of AKT with increasing amplitude and duration are required to activate caspases. In turn, these enzymes cleave specific proteins19 and modulate the transcription program that drives the differentiation process.17 Expression of a constitutively activated kinase abolishes CSF-1–driven macrophage differentiation, which suggests that oscillations in the kinase activity are an absolute requirement for the differentiation process to progress.

Figure 5. A constitutively activated AKT inhibits macrophage differentiation. Monocytes were transfected with an empty vector (pcDNA) or the vector encoding a constitutively active mutant of AKT (Myr-AKT). Cells were then treated for 3 days with CSF-1 before qPCR analysis of indicated gene expression (mean ± SD of triplicates; A), morphologic examination of cells (B), and immunoblot analysis of indicated proteins (C). Molecular weight (MW) stated as kilodaltons. *Cleavage fragment. pp70 S6K is used as a marker of AKT activity.

Figure 6. CSF-1–mediated caspase-8 interaction with FADD and RIP1 depends on PI3K. (A) Monocytes were exposed for 4 days in the absence or presence of Y294002 (LY, 10 μmol/L) or U0126 (U0, 10 μmol/L) added either 30 minutes before CSF-1 (P) and at indicated times after the beginning of CSF-1 treatment and the expression of procaspase-3 and caspase-3 cleavage (C3*) was analyzed by immunoblot. HSC70 indicates loading control. (B) Monocytes were analyzed before any treatment or after exposure to CSF-1 for 72 hours in the presence or absence of LY (10 μmol/L) added 24 hours after the beginning of CSF-1 treatment. Cell lysates were used for immunoblotting before (lysates) or after (IP: caspase 8) immunoprecipitation with an anti-caspase-8 antibody. Molecular weight (MW) is given in kilodaltons. One representative of 3 independent experiments is shown.
The Src family of kinases plays an essential role in CSF-1 signaling, but the kinases involved are still a matter of debate. CSF-1 stimulation results in the phosphorylation of SRC, FYN, and YES, whereas LYN and HCK could negatively regulate CSF-1 signaling in hematopoietic cells because their loss skew HSC differentiation toward monocytes. SFK inhibitors PP1 and PP2 were observed to prevent caspase activation in primary monocytes exposed to CSF-1. Because SFK connects the activated CSF-1R to the PI3K/AKT pathway, PP1 and PP2 effects could be related to the inhibition of downstream waves of AKT inactivation. Accordingly, down-regulation of HCK could play a role in the initiation of this oscillatory signaling pathway, whereas neither SRC nor LYN nor FYN appear to be involved, which indicates distinct roles of SFK in HSC differentiation toward monocytes and monocyte differentiation into resident macrophages. Class I PI3Ks play an essential role at various stages of hematopoietic cell differentiation.

In normal human peripheral blood monocytes, PI3K regulates differentiation through activation of AKT and hexokinase II-mediated glycolysis, and AKT activation was proposed to up-regulate Mcl-1 to prevent caspase-dependent cell death. Here, we show that suppression of AKT1 or PI3K in human primary monocytes actually prevents the activation of caspases required for CSF-1–induced differentiation into macrophages.

The interplay between PI3K/AKT and ERK1/2 pathways in CSF-1–stimulated human primary monocytes remains poorly understood. The ERK1/2 activation was suggested to depend on both PI3K products and the production of radical oxygen species, but a Raf-dependent, PI3K-independent pathway of ERK1/2 activation could exist. The parallel but independent oscillations in PI3K/AKT and ERK1/2 phosphorylation identified in the present study led us to explore whether CSF-1R was a common starting point for these oscillations. CSF-1 was reported to stabilize noncovalent dimers of CSF-1R, which precedes the receptor activation waves and prevents any subsequent activation of the regulatory subunit of PI3K and with c-Src, which induces its phosphorylation on tyrosine 380. This phosphorylation prevents the conversion of procaspase-8 to proteolytically active enzyme and confers to the protein the ability to control calpain activation, lamellipodial assembly, cell interaction with the extracellular matrix, and cell migration. A distinct mechanism may operate in monocytes undergoing differentiation into macrophages in which a series of intracellular proteins are cleaved by proteolytically active caspases, indicating the serine/threonine kinase RIP1, whose caspase-mediated cleavage contributes to the down-regulation of NF-κB activity. Similarly, the proteolytic activity of caspase-3 appears to be required for optimal erythroid cell differentiation. Thus, procaspase-8 could contribute to hematopoietic progenitor-cell maturation through nonproteolytic functions, whereas monocytes maturation into macrophages as well as terminal erythroid differentiation may require the proteolytic activity of one or several caspases.

Altogether, our results demonstrate that CSF-1 interaction with CSF-1R activates oscillatory and coordinated AKT and ERK1/2 signaling pathways, probably through a HCK-dependent mechanism. The waves of phosphorylation appear to be reactivated at the CSF-1R level by interaction with the cytokine. After 2 to 3 days, oscillations in AKT phosphorylation end with caspase-8 and caspase-3 activation, whereas the functions of ERK1/2 waves remain elusive. Tight regulation of these molecular clocks that could involve one or several phosphatases may be required for appropriate renewal of tissue macrophages, and the deregulation of these finely regulated oscillations could account for the pathogenesis of various human diseases.

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Authorship

Contribution: A.J. performed the experimental work and data analysis; N.B., J.P., N.L., L.G., E.K.D., M.C., and C.R. contributed to some experiments; O.M., L.D., and N.D. participated with helpful discussion; and E.S. directed the work and wrote the paper. All authors discussed the results and commented on the manuscript. Conflict-of-interest disclosure: The authors declare no competing financial interests.

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References


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