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MAPKAPK2-mediated LSP1 phosphorylation and FMLP-induced neutrophil polarization ☆

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Abstract

In neutrophils, the major substrate of MAPKAPK2 (MK2) is an F-actin binding protein LSP1. Studies using mutants of the two potential Serine phosphorylation sites in LSP1 C-terminal F-actin binding region indicated that the major phosphorylation site for MK2 is Ser243 in murine neutrophils (Ser252 in humans).

Human phosphoLSP1 antibodies that recognize phosphoSer252 site were prepared and revealed fMLP-induced neutrophil LSP1 phosphorylation. The phosphorylation was inhibited by p38 MAPK (upstream kinase for MK2) inhibitor SB203580. The antibodies also detect LSP1 phosphorylation in murine neutrophils. Immunostaining revealed that in WT murine neutrophils phosphoLSP1 was localized in F-actin enriched lamellipodia and oriented toward the fMLP gradient while non-phosphoLSP1 failed to colocalize with F-actin. In suspension, WT neutrophils exhibited persistent F-actin polarization following fMLP stimulation, while *MK2*$^{-/-}$ neutrophils exhibited transient F-actin polarization. These studies suggest that MK2-regulated LSP1 phosphorylation is involved in stabilization of F-actin polarization during neutrophil chemotaxis.

Keywords: Neutrophil; Chemotaxis; Phosphorylation; MAPKAPK2; LSP1

LSP1, despite its name, is expressed in lymphocytes, macrophages, neutrophils, and endothelial cells [1–6]. It is an F-actin-binding protein with a highly acidic N-terminal domain and a basic C-terminal domain [2]. The LSP1 C-terminal domain has two caldesmon homologous domains (CI and CII) and two villin headpiece homologous regions (VI and VII) for F-actin binding [7,8]. In lymphocytes, LSP1 is a major substrate for protein kinase C (PKC) [9,10], and is found in the cytoskeleton of lymphocytes and cocaps with the IgM receptor [11]. Most recent studies suggest that LSP1 could interact with DC-SIGN in dendritic cells to facilitate virus transport into proteasome [12]. Neutrophils from human patients overexpressing LSP1 have deficiencies in chemotaxis, phagocytosis, and cell spreading [4]. In these neutrophils, thin-hairlike-F-actin structures are projected from cell surface, which was also shown in hairy leukemia cells [13]. While LSP1$^{-/-}$ mice showed normal T and B cell development, they exhibited an enhanced influx of neutrophils and macrophages into the peritoneum upon thioglycollate (TG) challenge. In vitro studies suggested that the negative regulatory roles of LSP1 on neutrophil adhesion/polarization/migration occurred in an integrin-dependent manner

Abbreviations: MAPK, mitogen-activated protein kinase; MK2, MAPKAPK2 (MAPK-activated protein kinase 2); LSP1, leukocyte-specific protein 1; GST, glutathione *S*-transferase; fMLP, fMet-Leu-Phe.

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[14]. LSP1$^{-/-}$ neutrophils showed impairments in both migration speed and chemotaxis direction during chemokine KC-induced chemotaxis, while morphologically exhibiting discontinuous primary actin-rich cortexes [15]. Recent intravital microscopy studies indicated that the observed impaired neutrophil transmigration in LSP1$^{-/-}$ mice was solely due to the reduction of endothelial barrier permeability in these mice [6].

Besides being a target site of the endogenous anti-inflammatory agent lipoxin [16], LSP1 is the major substrate for MK2 in neutrophils [17]. MK2 is a specific downstream target of p38 MAPK. Treatments of cells with chemotactic factors, endotoxin, and proinflammatory cytokines activate p38 MAPK and MK2 [18]. The inhibitor of p38 MAPK, SB203580, blocks chemotactic factor-induced neutrophil chemotaxis [19,20]. *MK2*$^{-/-}$ neutrophils showed a partial loss of directionality but a higher migration speed in Zigmond chambers containing an fMLP gradient [21]. These studies suggest that LSP1, as the major substrate for MK2 and a potential regulator for F-actin structure, may play an important role in mediating neutrophil activation and chemotaxis. The function of LSP1 may be regulated through the phosphorylation of LSP1 by MK2. In this paper, we identified the major phosphorylation site for MK2 on LSP1, and the role of this MK2-mediated LSP1 phosphorylation in fMLP-stimulated neutrophil activation and chemotaxis was studied.

Materials and methods

Materials. Neutrophils were isolated from human blood using Ficoll/Hypaque gradient as described [22], or isolated from murine bone marrow using Ficoll gradient [23]. *N*-Formyl-Met-Leu-Phe (fMLP), protein kinase A (PKA), protein kinase C (PKC), and calcium–calmodulin-dependent kinase II (CAPMPKII) were obtained from Sigma (St. Louis, MO). The autoactive truncated MK2 mutant T334A was prepared as described [24]. Antibodies to non-phosphoLSP1 (Ser252) were purchased from AnaSpec (San Francisco, CA). Antibodies to LSP1 were a generous gift from Dr. Jongstra. MK2$^{-/-}$ mice were generated at Martin Luther University (Halle, Germany; Ref. [25]) and maintained at University of Connecticut Health Center. C57BL6/J WT mice were purchased from the Jackson Laboratory (Bar Harbor, ME).

Site-directed mutagenesis of LSP1. Oligonucleotides for site-directed mutagenesis were as follows. S243A LSP1 mutant: forward primer is 5′GCTGTCCCGCCAGCCCGCCATAGAGCTG3′, and reverse primer is 5′CAGCTCTATGGCGGGCTGGCGGGACAGC3′. S243E LSP1 mutant: forward primer is 5′GTCCCGCCAGCCCGAGATAGAGCTG CCCAG3′, and reverse primer is 5′CTGGGCAGCTCTATCTCGGGC TGGCGGGAC3′, S195A LSP1 mutant: forward primer is 5′TAGGAC CGAGGCCCTGAACCG3′, and reverse primer is 5′CGGTTCAGGGC CTCGGTCCTA3′, 5′GCGACCATCCTCCAAAATCGG3′, and 5′TCA CGATGCGGCCGCTCGAG3′ were used as outside primers. Two rounds of PCR are performed in a PTC-100 thermal cycler (MJ Research, Inc., Watertown, MA) for 30 cycles at 92 °C for 30 s, 55 °C for 30 s and 72 °C for 1 min. The final PCR fragment was eluted from the gel, purified with DNA purification kits (Promega, Madison, WI) and subsequently treated with restriction enzymes to form the two required ends so that the DNA fragment would be cloned into corresponding restriction sites of pGEX-4T2 expression vector. The pGEX-4T2 mutation vectors were transformed directly into competent *Escherichia coli* strain UT481 cells. Mutant colonies were selected randomly and confirmed by DNA sequencing.

Phosphorylation of various forms of GST-fusion proteins of LSP1 by MK2 (T334A) and other kinases. GST fusion proteins of murine LSP1 were prepared as described previously [17]. Purified GST fusion proteins of the recombinant LSP1 were phosphorylated by MK2 mutant (T334A) according to the procedure described [26]. Reaction buffer contains 20 mM Hepes (pH 7.3), 10 mM $MgCl_2$, 1 mM EGTA, 5 mM sodium orthovanadate, 5 μM okadaic acid, 2 mM DTT, 20 mM H-7, and 0.2 mM [γ-^{32}P]ATP (10^5 c.p.m./pmol). Phosphorylation of LSP1 by PKA was performed by incubating 2 μg protein with 180 ng of PKA kinase in 40 μl reaction buffer at RT for 30 min. Phosphorylation by PKC was carried out by incubating 2 μg protein with 10.5 ng of PKC in 40 μl reaction buffer, plus 100 ng/ml PMA, 100 ng/ml PS at 30 °C for 30 min. The phosphorylation of LSP1 by CAMPKII was done by incubating 2 μg protein with 25 U of CAMPKII in 40 μl reaction buffer (20 mM Tris–HCl, pH 7.5, 10 mM $MgCl_2$, 0.5 mM DTT, 0.1 mM EDTA, 1 mM $CaCl_2$, 2.4 μM calmodulin, and 0.2 mM [γ-^{32}P]ATP (10^5 c.p.m./pmol), at 30 °C for 30 min.

Preparation of antibodies to phophoLSP1. Antisera specific for phosphoserine 252 of human LSP1 was generated by Chiron Technologies (Clayton Victoria, Australia) in New Zealand white rabbits. The rabbits were immunized with the phosphopeptide RTPKLARQAS(PO_4)IELPSM conjugated to diphtheria toxoid. The amino acid sequence of this phosphopeptide corresponds to the amino acid 243–258 of human LSP1. Antibodies were purified from the antisera by affinity chromatography. This antibody also recognize the corresponding phosphorylation sequence RTPKLSRQPS(PO_4)IELPSM in murine LSP1.

Immunoblots. Protein obtained from cell lysates was analyzed by SDS–PAGE (10%) gels and detected using antibodies against LSP1 (1:2000 dilution), antibodies against phosphoLSP1 (1:1000 dilution), and non-phosphoLSP1 (1:1000 dilution).

Confocal microsopy of fMLP-stimulated neutrophils in suspension and chemotaxing neutrophils in Zigmond chambers containing the fMLP gradients. Cell suspensions of neutrophils derived from mouse bone marrow were stimulated with fMLP (10^{-5} M) at 37 °C for the indicated time. Following stimulation they were fixed using 4% paraformaldehyde. The fixed cells were cytospun onto coverslips (1000 rpm, 3 min, RT) and permeabilized with 1% Triton lysis buffer for 15 min at 4 °C. After incubated with the blocking reagent (2% BSA) for 2 h, the neutrophils were stained with Rhodamine-conjugated Phalloidin (Molecular Probes, Eugene, OR). To examine the intracellular localization of phosphoLSP1/non-phosphoLSP1 in chemotaxing neutrophils, the neutrophils were added into Zigmond chambers for chemotaxis as described previously [21]. Anti-phophoLSP1/anti-nonphosphoLSP1 (1:50 dilution) followed by FITC-conjugated donkey anti-rabbit IgG (1:100 dilution) and Rhodamine-conjugated Phalloidin (Molecular Probes, Eugene, OR) were added sequentially to stain the neutrophils. Slides were finally mounted with slowfade mounting reagent (Molecular Probes, Eugene, OR).

Slides were examined using a Zeiss (Oberkochen, Germany) confocal imaging system with excitation wavelengths of 488 and 568 nm and emission filters.

Results

Identification of the major phosphorylation site for MK2 on LSP1

As we have reported earlier [17], the amino acid sequences surrounding the two serine residues at position 195 and 243 in mouse (204 and 252 in human) LSP1 are in agreement with the minimal sequence required for efficient phosphorylation by MK2, Xaa-Xaa-Xaa-Arg-Xaa-Xaa-Ser-Xaa-Xaa- [27]. To test whether one or both of the two serine residues are recognized by MK2, three mutants of GST-LSP1 with point mutations, S243A, S243E, and S195A were prepared and tested for phosphorylation by various kinases. As shown in Fig. 1, mutation at

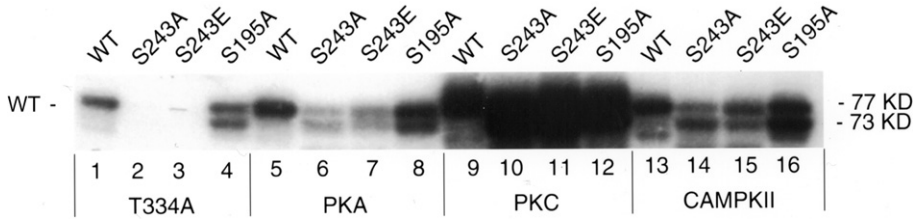

Fig. 1. Identification of Ser243 as the major phosphorylation site for MK2 on LSP1. Phosphorylation of purified GST/LSP1 WT and mutant fusion proteins by active MAPKAP kinase 2 mutant T334A, PKA, PKC, and CAMPKII was measured. Lanes 1, 5, 9, and 13 were WT fusion protein, lanes 2, 6, 10, and 14 were S243A mutant fusion protein, lanes 3, 7, 11, and 15 were S243E mutant fusion protein, lanes 4, 8, 12, and 16 were S195A mutant fusion protein. Lanes 1–4 show the autoradiograph of the corresponding proteins after phosphorylation by T334A kinase mutant, lanes 5–8 show phosphorylation by PKA, lanes 9–12 show phosphorylation by PKC, lanes 14–16 show phosphorylation by CAMPKII.

Ser243 (S243A or S243E), but not Ser195 (S195A) resulted in the complete loss of phosphorylation of the LSP1 by the MK2 (T334A) kinase and a partial loss of phosphorylation by the PKA, but not by PKC or CAMPKII. The result indicates that Ser243, but not Ser195, is the major phosphorylation site recognized by the MK2 in murine cells. This result is consistent with the previous report that the major phosphorylation site is localized within the amino acid residues 231–330 of C-terminal, but not N-terminal of LSP1 [17].

The polyclonal affinity-purified anti-human phopshoLSP1 antibody can detect the LSP1 phosphorylation in fMLP-stimulated human and murine neutrophils

Antiphosphopeptide antibodies have been widely used to detect the site-specific phosphorylation of proteins [28]. We prepared antibodies to a phosphopeptide with the sequence RTPKLARQAS(PO_4)IELPSM which contains the amino acid residues surrounding the phosphorylation site Ser252 of human LSP1 (PhosphoLSP1) for MK2. The antibodies were able to detect an increase of the phosphoLSP1 level in fMLP (0.1 μM) stimulated human neutrophils. The fMLP-induced LSP1 phosphorylation was inhibited by pretreatment with SB203580, an inhibitor for p38 MAPK [29] (Fig. 2B).

The phosphoLSP1 antibody can also detect the phosphorylation of LSP1 in WT murine neutrophils but the immunoblotting pattern of LSP1 in murine cells differs from that of human cells (could be due to different alternative splicing isoforms of LSP1 existent in human and murine cells [1]). The site-specific LSP1 phosphorylation was reduced in *MK2*$^{-/-}$ neutrophils (Fig. 2C). In addition, a decrease of non-phosphoLsp1 was shown in fMLP-stimulated murine WT neutrophils by using site-specific antibodies (Fig. 3A).

Since LSP1 antibody used here is a polyclonal rabbit antibody, it gives rise to strong intensity of blotted LSP1 band. However, phospho- and non-phosphoLSP1 antibodies are both site-specific antibodies, which exhibit much less band intensities in immunoblots. Therefore, the percentage of LSP1 phosphorylation cannot be simply deduced from dividing phosphoLSP1 band intensity by total LSP1 band intensity.

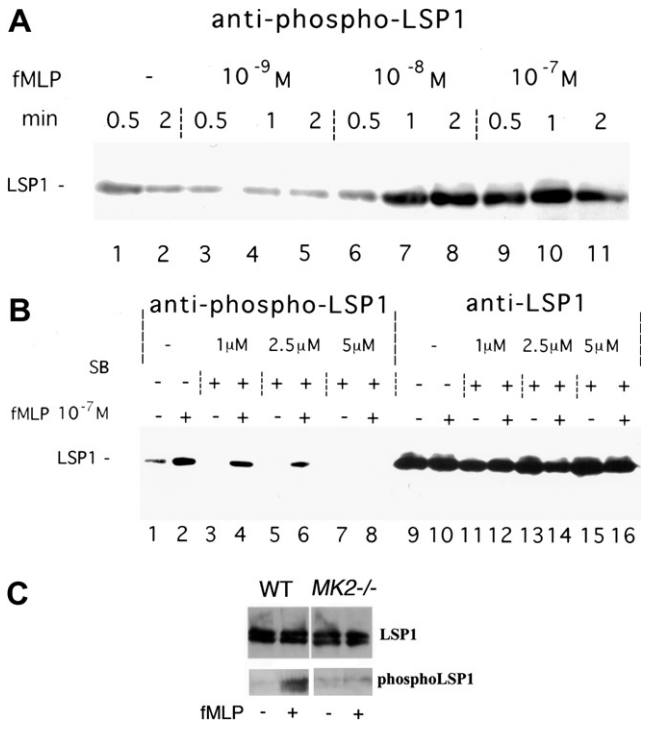

Fig. 2. Detection of LSP1 phosphorylation in fMLP-stimulated human and murine neutrophils using our phospho-Ser252 specific human LSP1 antibody. (A) The dose-course and time-course of LSP1 phosphorylation in fMLP-stimulated human neutrophils. Lanes 1 and 2 were control cells. Lanes 3–5, 6–8, and 9–11 were neutrophils treated with different dosages of fMLP at different time lengths. (B) The inhibitory effects of SB203580 on LSP1 phosphorylation. Lanes 1–8 were immunoblots with phosphoLSP1 antibody. Neutrophils were pretreated without (lanes 1 and 2) or with (lanes 3–8) SB203580 of different dosages, then simulated without (lanes 1, 3, 5, and 7) or with (lanes 2, 4, 6, and 8) 10^{-7} M fMLP. Lanes 9–16 were reblots using human LSP1 antibody. (C) *MK2*$^{-/-}$ mice neutrophils have reduced LSP1 phosphorylation. Purified bone marrow neutrophils from WT and *MK2*$^{-/-}$ mice were stimulated without or with 10^{-5} M fMLP. The cell lysates were used for immunoblots with phosphoLSP1 antibody (lower panel) and reblots with mouse LSP1 antibody (upper panel).

Intracellular staining of phosphoLSP1 and non-phosphoLSP1 in chemotaxing neutrophils

To examine the polarization pattern of phsphoLSP1 in chemotaxing neutrophils, we placed murine neutrophils in the Zigmond Chambers containing the fMLP gradient

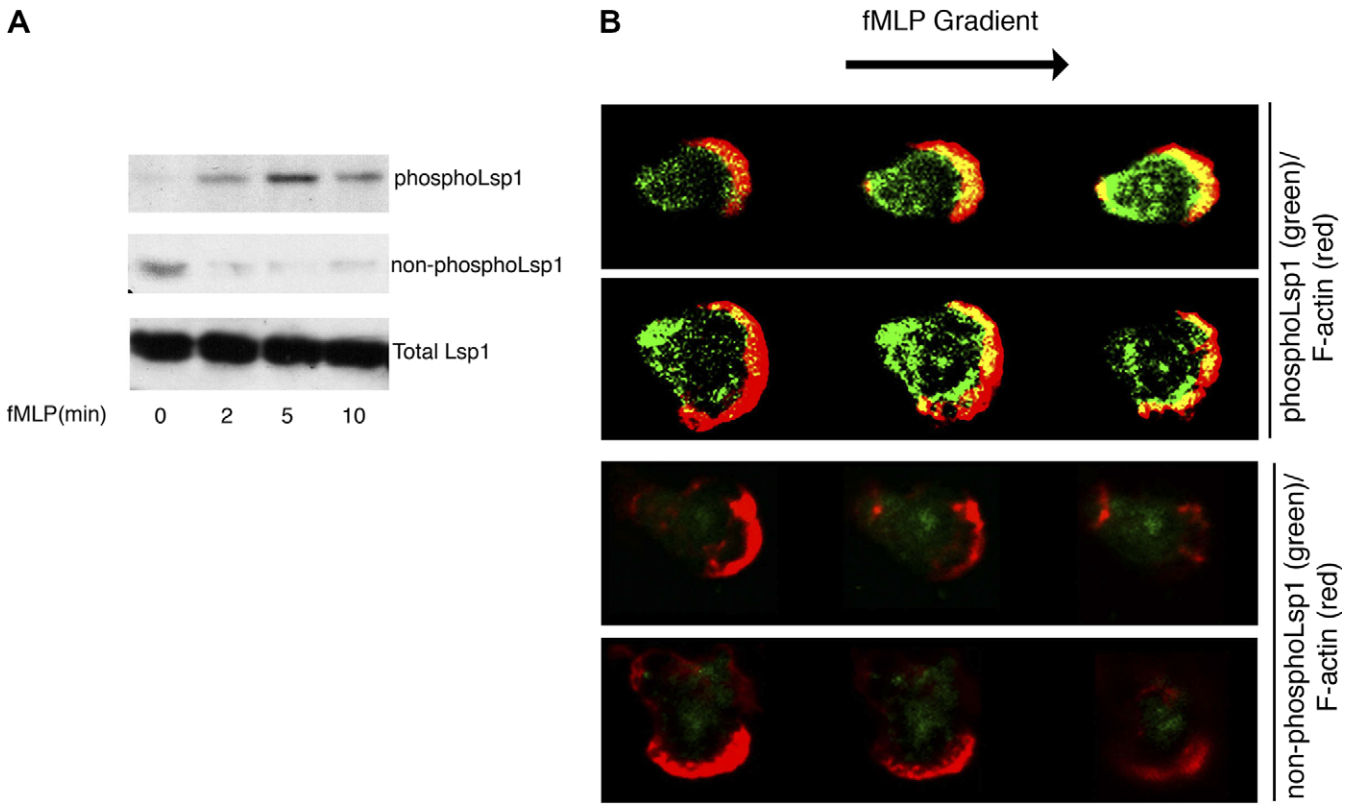

Fig. 3. PhosphoLsp1 and non-phosphoLsp1 have different subcellular localizations in chemotaxing WT neutrophils. (A). Immunoblotting analysis of phosphoLsp1 and non-phosphoLsp1 amounts in fMLP-stimulated WT neutrophils for the indicated time. (B) Neutrophils were incubated in Zigmond chambers containing the fMLP gradient for 10 min, and then immunostained for phosphoLsp1 (green) and F-actin (red) (upper panel). Non-phosphoLsp1 (green) and F-actin (red) immunostaining were in the lower panels.

(10 μM) prior to immunostaining. In chemotaxing WT neutrophils most of phosphoLSP1 accumulated at the lamellipodia region enriched with F-actin and oriented toward the fMLP gradient (Fig. 3B, upper 2 panels). In contrast, non-phosphoLsp1 did not colocalize with F-actin in neutrophils undergoing chemotaxis (Fig. 3B, lower 2 panels). This suggested that phosphoLSP1 could, in part, regulate the neutrophil chemotaxis.

Failure of maintaining polarization in fMLP-stimulated $MK2^{-/-}$ neutrophils

Neutrophils can have F-actin polarized at the presumptive leading edge even in a suspension state that lacks chemotactic gradient, which will prepare them for responding rapidly to the chemoattractant stimuli [30]. In order to identify whether F-actin is polarized, we per-

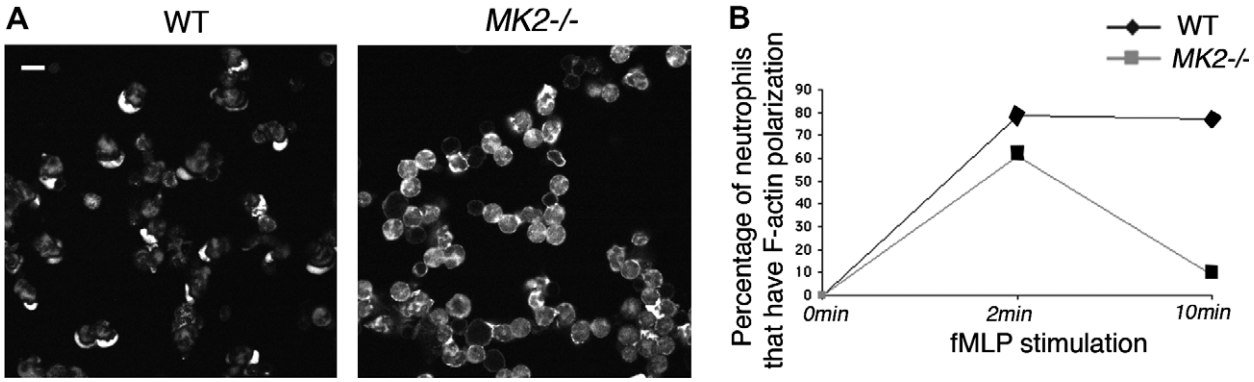

Fig. 4. Failure of F-actin polarization and stabilization in fMLP-stimulated $MK2^{-/-}$ neutrophils in suspension. (A) Whole-field pictures showing F-actin staining pattern of WT (left) and $MK2^{-/-}$ (right) neutrophils at 10 min after fMLP (10^{-5} M) stimulation. (B) The percentage of neutrophils that have F-actin polarization at different time points after fMLP stimulation. 100 individual neutrophils obtained from WT and $MK2^{-/-}$ mice, respectively, were examined at different time points after fMLP stimulation. The data shown are representatives of two similar experiments.

formed F-actin (red) staining. Both WT and *MK2*$^{-/-}$ neutrophils exhibited F-actin polarization immediately after fMLP stimulation (data not shown). But at 10 min after stimulation, while WT neutrophils still showed the polarized morphologies with F-actin accumulated at the presumptive leading edge, *MK2*$^{-/-}$ neutrophils had lost polarity (Fig. 4A). The percentage of neutrophils that had F-actin polarization was 78% and 12% in WT and *MK2*$^{-/-}$, respectively (Fig. 4B).

Discussion

The C-terminal domain of LSP1 is more conserved across species compared with its N-terminal domain and includes several threonine/serine residues. GST-LSP1 can be phosphorylated by various kinases at Ser/Thr residues (Fig. 1). Only the point mutation of Ser243 totally abolished the phosphorylation of GST-LSP1 induced by MK2, which indicates that Ser243 of mouse LSP1 is the only phosphorylation site on LSP1 recognized by MK2 in vitro. In contrast, PKA, PKC, and CAMPKII can bypass the Ser243 mutation and phosphorylate GST-LSP1 at other Ser/Thr residues.

The Ser252 phosphorylation site-specific antibody can detect phosphorylation mediated by MK2 in both human and murine neutrophils, although the amino acid residues around this phosphorylation site are not identical between human and murine LSP1. When comparing the LSP1 Ser243 phosphorylation level between WT and MK2$^{-/-}$ murine neutrophils, *MK2*$^{-/-}$ exhibited less but not absent LSP1 phosphorylation. This may be attributable to the existence of other kinases, such as MK3 [31] and 3PK [32], which share the high amino acid identity with that of MK2 and whose activities can be blocked by the p38 MAP kinase-inhibitor SB203580 [31]. In addition, the concentration of fMLP used for murine neutrophil stimulation is 100 times higher than that for human cells (10^{-5} M vs. 10^{-7} M), which is due to the weak fMLP receptor affinity in murine neutrophils [21].

LSP1 246–295 (CII domain) is homologous to the caldesmon F-actin binding site and its binding capability with F-actin has been proven [8]. Ser243 is located right before this CII domain, thus its phosphorylation may affect the affinity of LSP1 for F-actin. Using neutrophils from *MK2*$^{-/-}$ mice, we assessed the effects of LSP1 Ser243 phosphorylation defects on cytoskeleton F-actin in fMLP-stimulated neutrophils. Intracellular F-actin staining revealed that F-actin was well polarized in WT neutrophils at 10 min after stimulation while this polarization was absent in MK2$^{-/-}$ neutrophils. We speculate there is an interrelationship between MK2-regulated LSP1 phosphorylation and F-actin polarization/stabilization. Intracellular phosphoLSP1 staining indicates that most of the phosphorylated LSP1 was localized at the leading front of the polarized neutrophils and slightly behind the polarized F-actin at the leading edge. However, there is still a relative amount of phosphoLSP1 localized in the posterior of neutrophils, which could be due to its association with other LSP1 target proteins. F-actin assembly is a dynamic process, with actin monomer added to the newly formed plus ends of actin filaments [33]. It is presumed that the location of polarized phosphoLSP1 represents the sites at which the minus ends of F-actin exist. Therefore, phosphoLSP1 would help to maintain the polarized F-actin in place by stabilizing F-actin minus ends and preventing actin monomer dissociation from the actin filaments. In addition, our finding that non-phosphoLSP1 was not colocalized with F-actin suggests that the F-actin—bound LSP1 identified in vitro most likely is phosphorylated. Although in Fig. 3B, more phosphoLSP1 staining was observed compared to that of non-phosphoLSP1, suggesting that most LSP1 is phosphorylated upon fMLP stimulation, this difference in staining intensities may be due, in part, to potential different sensitivities of the phosphoLSP1 and non-phosphoLSP1 antibodies used in this study. Among three major families of MAP kinases, p38 MAPK can be activated by cellular stress, such as inflammatory cytokines, and triggers a variety of proinflammatory events including chemotaxis [34]. Therefore, the role of p38 MAPK—MK2-mediated LSP1 phosphorylation involved in neutrophil activation and chemotaxis is most likely a stress response. This response would serve to maintain and possibly reinforce neutrophil polarity during chemotactic migration.

References

[1] J. Jongstra-Bilen, A.J. Young, R. Chong, J. Jongstra, Human and mouse LSP1 genes code for highly conserved phosphoproteins, J. Immunol. 144 (1990) 1104–1110.
[2] J. Jongstra-Bilen, P.A. Janmey, J.H. Hartwig, S. Galea, J. Jongstra, The lymphocyte-specific protein LSP1 binds to F-actin and to the cytoskeleton through its COOH-terminal basic domain, J. Cell Biol. 118 (1992) 1443–1453.
[3] Y. Li, A. Guerrero, T.H. Howard, The actin-binding protein, lymphocyte-specific protein 1, is expressed in human leukocytes and human myeloid and lymphoid cell lines, J. Immunol. 155 (1995) 3563–3569.
[4] T. Howard, Y. Li, M. Torres, A. Guerrero, T. Coates, The 47-kD protein increased in neutrophil actin dysfunction with 47- and 89-kD protein abnormalities is lymphocyte-specific protein, Blood 83 (1994) 231–241.
[5] J. Jongstra, M.E. Ittel, N.N. Iscove, G. Brady, The LSP1 gene is expressed in cultured normal and transformed mouse macrophages, Mol. Immunol. 31 (1994) 1125–1131.
[6] L. Liu, D.C. Cara, J. Kaur, E. Raharjo, S.C. Mullaly, J. Jongstra-Bilen, J. Jongstra, P. Kubes, LSP1 is an endothelial gatekeeper of leukocyte transendothelial migration, J. Exp. Med. 201 (2005) 409–418.
[7] Q. Zhang, Y. Li, T.H. Howard, Human lymphocyte-specific protein 1, the protein overexpressed in neutrophil actin dysfunction with 47-kDa and 89-kDa protein abnormalities (NAD 47/89), has multiple F-actin binding domains, J. Immunol. 165 (2000) 2052–2058.
[8] Q. Zhang, Y. Li, T.H. Howard, Hair-forming activity of human lymphocyte specific protein 1 requires cooperation between its caldesmon-like domains and the villin headpiece-like domains, Cell Motil. Cytoskeleton 49 (2001) 179–188.
[9] E. Carballo, D. Colomer, J.L. Vives-Corrons, P.J. Blackshear, J. Gil, Characterization and purification of a protein kinase C substrate in

human B cells. Identification as lymphocyte-specific protein 1 (LSP1), J. Immunol. 156 (1996) 1709–1713.
[10] N. Matsumoto, S. Kojima, T. Osawa, S. Toyoshima, Protein kinase C phosphorylates p50 LSP1 and induces translocation of p50 LSP1 in T lymphocytes, J. Biochem. (Tokyo) 117 (1995) 222–229.
[11] D.P. Klein, S. Galea, J. Jongstra, The lymphocyte-specific protein LSP1 is associated with the cytoskeleton and co-caps with membrane IgM, J. Immunol. 145 (1990) 2967–2973.
[12] A.L. Smith, L. Ganesh, K. Leung, J. Jongstra-Bilen, J. Jongstra, G.J. Nabel, Leukocyte-specific protein 1 interacts with DC-SIGN and mediates transport of HIV to the proteasome in dendritic cells, J. Exp. Med. 204 (2007) 421–430.
[13] E.K. Miyoshi, P.L. Stewart, P.W. Kincade, M.B. Lee, A.A. Thompson, R. Wall, Aberrant expression and localization of the cytoskeleton-binding pp52 (LSP1) protein in hairy cell leukemia, Leuk. Res. 25 (2001) 57–67.
[14] C. Wang, H. Hayashi, R. Harrison, B. Chiu, J.R. Chan, H.L. Ostergaard, R.D. Inman, J. Jongstra, M.I. Cybulsky, J. Jongstra-Bilen, Modulation of Mac-1 (CD11b/CD18)-mediated adhesion by the leukocyte-specific protein 1 is key to its role in neutrophil polarization and chemotaxis, J. Immunol. 169 (2002) 415–423.
[15] M. Hannigan, L. Zhan, Y. Ai, C.K. Huang, Leukocyte-specific gene 1 protein (LSP1) is involved in chemokine KC-activated cytoskeletal reorganization in murine neutrophils in vitro, J. Leukoc. Biol. 69 (2001) 497–504.
[16] T. Ohira, G. Bannenberg, M. Arita, M. Takahashi, Q. Ge, T.E. Van Dyke, G.L. Stahl, C.N. Serhan, J.A. Badwey, A stable aspirin-triggered lipoxin A4 analog blocks phosphorylation of leukocyte-specific protein 1 in human neutrophils, J. Immunol. 173 (2004) 2091–2098.
[17] C.K. Huang, L. Zhan, Y. Ai, J. Jongstra, LSP1 is the major substrate for mitogen-activated protein kinase-activated protein kinase 2 in human neutrophils, J. Biol. Chem. 272 (1997) 17–19.
[18] J. Rouse, P. Cohen, S. Trigon, M. Morange, A. Alonso-Llamazares, D. Zamanillo, T. Hunt, A.R. Nebreda, A novel kinase cascade triggered by stress and heat shock that stimulates MAPKAP kinase-2 and phosphorylation of the small heat shock proteins, Cell 78 (1994) 1027–1037.
[19] D.C. Cara, J. Kaur, M. Forster, D.M. McCafferty, P. Kubes, Role of p38 mitogen-activated protein kinase in chemokine-induced emigration and chemotaxis in vivo, J. Immunol. 167 (2001) 6552–6558.
[20] J.A. Nick, S.K. Young, P.G. Arndt, J.G. Lieber, B.T. Suratt, K.R. Poch, N.J. Avdi, K.C. Malcolm, C. Taube, P.M. Henson, G.S. Worthen, Selective suppression of neutrophil accumulation in ongoing pulmonary inflammation by systemic inhibition of p38 mitogen-activated protein kinase, J. Immunol. 169 (2002) 5260–5269.
[21] M.O. Hannigan, L. Zhan, Y. Ai, A. Kotlyarov, M. Gaestel, C.K. Huang, Abnormal migration phenotype of mitogen-activated protein kinase-activated protein kinase 2−/− neutrophils in Zigmond chambers containing formyl-methionyl-leucyl-phenylalanine gradients, J. Immunol. 167 (2001) 3953–3961.
[22] Y.L. Zu, Y. Ai, A. Gilchrist, M.E. Labadia, R.I. Sha'afi, C.K. Huang, Activation of MAP kinase-activated protein kinase 2 in human neutrophils after phorbol ester or fMLP peptide stimulation, Blood 87 (1996) 5287–5296.
[23] C.A. Lowell, L. Fumagalli, G. Berton, Deficiency of Src family kinases p59/61hck and p58c-fgr results in defective adhesion-dependent neutrophil functions, J. Cell Biol. 133 (1996) 895–910.
[24] Z. Xia, M. Dickens, J. Raingeaud, R.J. Davis, M.E. Greenberg, Opposing effects of ERK and JNK-p38 MAP kinases on apoptosis, Science 270 (1995) 1326–1331.
[25] A. Kotlyarov, A. Neininger, C. Schubert, R. Eckert, C. Birchmeier, H.D. Volk, M. Gaestel, MAPKAP kinase 2 is essential for LPS-induced TNF-alpha biosynthesis, Nat. Cell Biol. 1 (1999) 94–97.
[26] Y.L. Zu, Y. Ai, C.K. Huang, Characterization of an autoinhibitory domain in human mitogen-activated protein kinase-activated protein kinase 2, J. Biol. Chem. 270 (1995) 202–206.
[27] D. Stokoe, B. Caudwell, P.T. Cohen, P. Cohen, The substrate specificity and structure of mitogen-activated protein (MAP) kinase-activated protein kinase-2, Biochem. J. 296 (Pt 3) (1993) 843–849.
[28] J.P. Blaydes, B. Vojtesek, G.B. Bloomberg, T.R. Hupp, The development and use of phospho-specific antibodies to study protein phosphorylation, Methods Mol. Biol. 99 (2000) 177–189.
[29] Y.L. Zu, J. Qi, A. Gilchrist, G.A. Fernandez, D. Vazquez-Abad, D.L. Kreutzer, C.K. Huang, R.I. Sha'afi, p38 mitogen-activated protein kinase activation is required for human neutrophil function triggered by TNF-alpha or FMLP stimulation, J. Immunol. 160 (1998) 1982–1989.
[30] C.Y. Chung, S. Funamoto, R.A. Firtel, Signaling pathways controlling cell polarity and chemotaxis, Trends Biochem. Sci. 26 (2001) 557–566.
[31] M.M. McLaughlin, S. Kumar, P.C. McDonnell, S. Van Horn, J.C. Lee, G.P. Livi, P.R. Young, Identification of mitogen-activated protein (MAP) kinase-activated protein kinase-3, a novel substrate of CSBP p38 MAP kinase, J. Biol. Chem. 271 (1996) 8488–8492.
[32] G. Sithanandam, F. Latif, F.M. Duh, R. Bernal, U. Smola, H. Li, I. Kuzmin, V. Wixler, L. Geil, S. Shrestha, 3pK, a new mitogen-activated protein kinase-activated protein kinase located in the small cell lung cancer tumor suppressor gene region, Mol. Cell Biol. 16 (1996) 868–876.
[33] H.L. Yin, T.P. Stossel, Control of cytoplasmic actin gel-sol transformation by gelsolin, a calcium-dependent regulatory protein, Nature 281 (1979) 583–586.
[34] J. Han, J.D. Lee, L. Bibbs, R.J. Ulevitch, A MAP kinase targeted by endotoxin and hyperosmolarity in mammalian cells, Science 265 (1994) 808–811.