14-3-3-epsilon inhibits MK5-mediated cell migration by disrupting F-actin polymerization

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Abstract

The signal pathway by which 14-3-3ε inhibits cell migration induced by MAPK-activated protein kinase 5 (MK5) was investigated in cultured HeLa cells. Both in vivo and in vitro analyses have revealed that 14-3-3ε interacts with MK5. 14-3-3ε bound to MK5 inhibits the phosphorylation of HSP27, a known substrate of MK5. Disturbance of actin cytoskeleton organization by 14-3-3ε was shown in transfected cells transiently expressing 14-3-3ε as well as established cells stably expressing 14-3-3ε. Moreover, overexpression of 14-3-3ε resulted in the inhibition of cell migration induced by MK5 overexpression or TNFα treatment. Our results suggest that 14-3-3ε bound to MK5 inhibits cell migration by inhibiting the phosphorylation of HSP27 whose phosphorylation regulates F-actin polymerization, actin cytoskeleton organization and subsequent actinfilament dynamics.

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1. Introduction

14-3-3 proteins constitute a family of acidic dimeric proteins with a subunit mass of 28–33 kDa and are widely expressed in all eukaryotic cells [1–3]. There are seven mammalian isotypes of 14-3-3 proteins denoted by β, γ, ε, σ, η, θ/τ and ζ. 14-3-3 proteins work as regulatory proteins that interact with other proteins in a phosphorylation dependent [4,5] or independent manner [6]. Most notably 14-3-3 proteins are known to bind multiple cellular protein ligands through an amphipathic binding cleft that preferentially recognizes the phosphorylated Ser/Thr motifs in a sequence specific manner [4,5]. As many as 200 cellular proteins have been found to associate with 14-3-3 proteins. Numerous signaling proteins including MEKK1 [7], Raf1 [8], PI3 kinase [9], BAD [10] and ASK1 [11] associate with 14-3-3 proteins. Accordingly 14-3-3 proteins have been implicated to be involved in a variety of cell signal pathways leading to cell cycling, apoptosis, stress responses, cytoskeleton organization, cell migration and malignant transformation [8,11–14].

We have identified mitogen-activated protein kinase (MAPK)-activated protein kinase 5 (MK5) as one of the binding proteins that interact with 14-3-3ε in yeast two-hybrid genetic system. MK5, which is also known as p38-regulated/activated kinase (PRAK), is a member of MAPK-activated protein kinase subfamily (MAPKAPKs or MKs) and expressed in all vertebrates so far investigated. MK5 is a 472 amino acid protein and displays 20–30% sequence identity with the known MAPK-regulated protein kinases including RSK1/2/3, MNK1/2 and MK2/3. MK5 is activated in response to cellular stresses such as heat shock, UV light, oxidation and proinflammatory cytokines. MK5 activity as a protein kinase is regulated by p38 [15] and ERK3/4 [16–18], but not by JNKs [19]. The only regulatory phosphorylation site within the catalytic domain has been known to exist at Thr182 [15].

Activated MK5, in turn, phosphorylates its target proteins HSP25 and HSP27. Small heat shock protein HSP27 is also known to be a downstream target protein of MK2 and 3. HSP27 is believed to play a role in the regulation of F-actin polymerization. Nonphosphorylated HSP27 has been described as a potential
inhibitor of F-actin polymerization. Nonphosphorylated HSP27 functions as F-actin capping protein capable of inhibiting the addition of monomer and subsequent filament growth [20–22]. Upon stimulation by growth factors and oxidative stresses, HSP27 is rapidly activated by phosphorylation [23,24]. Phosphorylated HSP27 participates in the cellular processes that require the changes in the actin filament dynamics and stabilization of actin cytoskeleton. Thus, the state of HSP27 phosphorylation contributes to the changes in cell shape, cell migration and cell motility. The present study evaluates the roles of 14-3-3ɛ on HSP27 phosphorylation stimulated by MK5 activation and subsequent actin cytoskeleton organization in cultured human cells. Here, we present a new biochemical pathway in which 14-3-3ɛ bound to MK5 inhibits HSP27 phosphorylation, disrupts F-actin polymerization and inhibits cell migration.

2. Materials and methods

2.1. Reagents and antibodies

Tumor necrosis factor-α (TNFα), anti-FLAG antibody, anti-tubulin antibody and anti-FLAG M2-Agarose were from Sigma. Human recombinant HSP27 protein and polyclonal rabbit antibody against HSP27 were from Calbiochem. Polyclonal rabbit antibody against phospho-HSP27 (Ser82) was from Cell Signaling. Polyclonal rabbit antibody against MK5 (H-180) and monoclonal mouse antibody against 14-3-3ɛ (8C3) were from Santa Cruz. Antibody against HA was also supplied by Santa Cruz. [γ-32P]ATP and Protein G-Sepharose Fast Flow Resin were provided by Amersham Biosciences. Rhodamine-conjugated phalloidin was from Invitrogen.

2.2. Expression vectors

cDNA encoding MK5 was cloned into HindIII and BamHI sites of pFLAG-CMV-2 cloning vector (Sigma) to generate FLAG-MK5 expression vector. FLAG-tagged MK5 deletion mutants were cloned into HindIII and BamHI sites of pFLAG-CMV-2 cloning vector to generate expression vectors for FLAG-MK5-N1, FLAG-MK5-N2 and FLAG-MK5-C1. MK5 single mutant, MK5-T182A, was created by substituting Thr at 182 by Ala using a site-directed mutagenesis and cloned into HindIII and BamHI sites of pFLAG-CMV-2 cloning vector to generate expression vector for FLAG-MK5-T182A. cDNA encoding 14-3-3ɛ was cloned into BamHI and EcoRV sites of pcDNA3-HA cloning vector to generate pcDNA3-HA-14-3-3ɛ expression vector. The entire coding sequence of 14-3-3ɛ cDNA was cloned into the XhoI and BamHI sites of retroviral pMFG vector containing a puromycin-resistant gene to generate retroviral pMFG-14-3-3ɛ expression vector. HeLa cells were transfected with indicated vector constructs using Lipofectamine reagent (Invitrogen) according to the manufacturer’s procedures.

2.3. Establishment of MFG-14-3-3ɛ HeLa cells

293T cells were cotransfected with retroviral pMFG-14-3-3ɛ expression vector and pG expression vector encoding MuLV Gag-Pol (Takara) together with pVPack-VSV-G expression vector encoding VSV-G protein ( Stratagene) using Lipofectamine reagent. As a control, 293T cells were also cotransfected with pMFG empty vector and expression vectors for pG and pVPack-VSV-G. After incubation for 48 h, the culture medium containing viral particles was collected, filtered through a 0.22 μm pore size filter membrane and used to infect HeLa cells in the presence of 8 μg/ml polybrene. At 6 h after infection, the

Fig. 1. 14-3-3ɛ interacts with MK5. (A) Cell lysate proteins from HeLa cells were immunoprecipitated using anti-MK5 antibody and separated by 10% SDS-PAGE. 14-3-3ɛ bound to MK5 was detected by immunoblot analysis using anti-14-3-3ɛ antibody. (B) Cells (2 × 10^6) were cotransfected with expression vectors for HA-tagged 14-3-3ɛ (1 μg) and FLAG-tagged MK5-T182A (1 μg). Cell lysates were immunoprecipitated using anti-HA antibody and immunoprecipitates were resolved on 10% SDS-PAGE. Interaction between HA-14-3-3ɛ and FLAG-MK5-T182A was analyzed by immunoblot analysis using anti-Flag antibody. Cell lysates were examined by immunoblot analysis using anti-HA or anti-FLAG antibody for the expression of HA-14-3-3ɛ or FLAG-MK5-T182A. (C) Cells (2 × 10^6) were cotransfected with expression vectors for HA-14-3-3ɛ (1 μg) and FLAG-MK5 deletion derivatives (1 μg). Cell lysates were immunoprecipitated using anti-Flag antibody and immunoprecipitates were resolved on 12% SDS-PAGE. Interaction between HA-14-3-3ɛ and FLAG-MK5 deletion mutants was analyzed by immunoblot analysis using anti-HA antibody. Cell lysates were examined by immunoblot analysis using anti-FLAG or anti-HA antibody for the expression of FLAG-MK5 fragments or HA-14-3-3ɛ.
culture medium was replaced by the fresh medium and infected cells were incubated for 2 more days. Puromycin-resistant populations were selected by culture medium was replaced by the fresh medium and infected cells were assayed for the high expression of 14-3-3ɛ protein by Northern blot analysis. Briefly, total RNAs from HeLa cells stably expressing 14-3-3ɛ were extracted using easy-BLUE solution (Intron Biotechnology) according to the manufacturer’s instruction. RNA samples (6 μg each) were fractioned on 1% agarose–formaldehyde gel and transferred to positively charged nylon membrane. The primers specific for 14-3-3ɛ gene (forward primer: 5′-ATGGGATGATCGAGAGG-3′, reverse primer: 5′-TCACT-GATTCCATCTTC-3′) were used for PCR to produce DIG-labeled probe. The levels of 14-3-3ɛ mRNA were detected using DIG Luminescent Detection Kit (Roche Molecular Biochemicals).

2.4. Immunoprecipitation

HeLa cells were lysed in EBC lysis buffer (120 mM NaCl, 0.5% NP-40, 50 mM Tris, pH 8.0, 100 mM NaF, 200 mM sodium orthovanadate) containing 1 mM PMSF. Cell lysates were incubated with appropriate antibody or agaroase beads conjugated with anti-FLAG antibody at 4 °C. After incubation for 16 h, the immunocomplexes were eluted from the resin by boiling in SDS sample buffer (250 mM Tris, pH 6.8, 40% glycerol, 8% SDS, 4% 2-mercaptoethanol, 0.002% bromophenol blue). The immunocomplexes were analyzed by SDS-PAGE followed by immunoblot analysis using antibodies against respective epitopes.

2.5. Immunoprecipitation kinase assay

Transfected HeLa cells overexpressing FLAG-tagged MK5 with or without 14-3-3ɛ were washed with PBS and lysed in EBC lysis buffer. Cell lysates were centrifuged for 10 min at 10 000 × g at 4 °C. FLAG-MK5 in the supernatants was immunoprecipitated using anti-FLAG M2-Agarose. Beads were mixed for 16 h at 4 °C, washed three times with 500 mM NaCl in PBS and washed again three times with PBS. Human recombinant HSP27 substrate protein (3 μg) and beads containing immunoprecipitated FLAG-MK5 were added to the total volume of 30 μl of reaction mixture containing 50 mM Tris, pH 7.5, 10 mM MgCl2, 0.1 mM ATP, 1 μg/μl BSA and 10 μCi [γ-32P]ATP. The reaction mixture was incubated for 20 min with shaking at 30 °C. MK5 kinase activity was determined by examining the phosphorylation of HSP27 substrate. Phosphorylated proteins were detected by autoradiography following by 12% SDS-PAGE.

2.6. RNA interference

HeLa cells were transfected with 0.03 μM 14-3-3ɛ siRNA or control siRNA (Santa Cruz) using Lipofectamine reagent. Experiments were carried out 24 h after siRNA transfection.

2.7. Immunofluorescence microscopy

F-actin staining for immunofluorescence microscopy was performed as described with slight modifications [25]. Transfected or nontransfected HeLa cells were fixed with 3.7% formaldehyde in PBS at room temperature for 15 min, permeabilized in PBS containing 0.1% Triton X-100 at −20 °C for 5 min and
blocked with 1% bovine serum albumin (BSA) in PBS for 30 min. F-actin was stained with rhodamine-conjugated phalloidin (3 μg/ml) in PBS containing 1% BSA for 20 min. F-actin polymerization was examined by monitoring stress fiber formation under a confocal laser scanning microscope LSM510 (Carl-Zeiss) equipped with FITC and TRITC filter sets.

2.8. Cell migration assay

Cell migration assay was performed employing QCM Cell Migration Assay (Chemicon). HeLa cells at 80% confluence were placed in the serum free medium for 24 h prior to cell migration assay. Cells were harvested and plated on the upper face of polycarbonate membrane (8.0 μm pore) separating two chambers of 6.5 mm transwell culture plates. After incubation for 24 h, nonmigratory cells on the upper face of the membrane were removed using a cotton-tipped swab. Cells that have migrated through the lower face of polycarbonate membrane were stained with Cell Stain Solution. The stain from the lower face of the membrane was extracted using Extraction Buffer. Cell migration was then measured colorimetrically on a standard microplate reader (560 nm).

3. Results

3.1. 14-3-3ε interacts with MK5

To confirm whether 14-3-3ε interacts with MK5, coimmunoprecipitation experiment was carried out. The results in Fig. 1A show a high affinity interaction between the two endogenous cellular proteins in HeLa cells. Although many 14-3-3 proteins favor to bind to phosphorylated motifs of target proteins [4,5], the interaction between 14-3-3ε and MK5 appears to be irrelevant to the state of MK5 phosphorylation. MK5 mutant FLAG-MK5-T182A, in which the only regulatory phosphorylation site at Thr182 was replaced by Ala, interacts with 14-3-3ε as well (Fig. 1B). Intermolecular association between FLAG-tagged MK5 and 14-3-3ε disrupts MK5-mediated actin cytoskeleton organization. (A) HeLa cells (5 × 10⁵) were transiently transfected with expression vector for GFP (1 μg) alone. (B) Cells were cotransfected with expression vectors for GFP (0.1 μg) and 14-3-3ε (0.9 μg). (C) Cells were cotransfected with expression vectors for GFP (0.1 μg) and FLAG-MK5 (0.9 μg). (D) Cells were cotransfected with expression vectors for GFP (0.1 μg), FLAG-MK5 (0.9 μg) and 14-3-3ε (1 μg). (E) Cells were cotransfected with expression vectors for GFP (0.1 μg), FLAG-MK5 (0.9 μg) and 0.03 μM control siRNA. (F) Cells were cotransfected with expression vectors for GFP (0.1 μg), FLAG-MK5 (0.9 μg) and 0.03 μM 14-3-3ε siRNA. Transfected or cotransfected cells were incubated for 24 h, fixed, permeabilized, blocked, stained with rhodamine-conjugated phalloidin for F-actin and were imaged by confocal microscopy. The expressions of transfected or cotransfected genes were confirmed by detecting intrinsic fluorescence of GFP.

Fig. 3. 14-3-3ε inhibits MK5-mediated cell migration. (A) HeLa cells (2 × 10⁶) were transfected with empty vector (1 μg), expression vector for FLAG-MK5 (1 μg) or 14-3-3ε (1 μg), or cotransfected with expression vectors for FLAG-MK5 (1 μg) and 14-3-3ε (1 μg). (B) Cells were transfected with empty vector (1 μg), expression vector for FLAG-MK5 (1 μg) alone or together with 0.03 μM control siRNA or 0.03 μM 14-3-3ε siRNA (□). Cells were transfected with empty vector (1 μg), expression vector for FLAG-MK5-T182A (1 μg), MK5 mutant lacking protein kinase activity, alone or together with 0.03 μM control siRNA or 0.03 μM 14-3-3ε siRNA (■). After incubation for 24 h, cells were starved for 24 h before cell migration experiment. Cell migration was expressed as a Migration Index (Chemicon). Data represent the results from at least three independent experiments.
deletion derivatives and HA-tagged 14-3-3ɛ was also examined (Fig. 1C). The results suggest that the N-terminal region of MK5 which contains the catalytic domain is relevant to its interaction with 14-3-3ɛ.

3.2. 14-3-3ɛ inhibits MK5 kinase activity

To investigate the biological consequences of 14-3-3ɛ interaction with MK5, we examined the effects of 14-3-3ɛ expression on the phosphorylation of HSP27, a known substrate of MK5. Immunoblot analysis shows that 14-3-3ɛ expression does not affect the cellular levels of HSP27 but inhibits the phosphorylation of HSP27 in cotransfected HeLa cells (Fig. 2A). The effects of 14-3-3ɛ expression on the enzymatic activity of MK5 as a protein kinase were evaluated by immunoprecipitation kinase assay. Cell lysate proteins obtained from HeLa cells overexpressing FLAG-MK5 were examined for the enzymatic activity of MK5 which phosphorylates HSP27 substrate. Increased phosphorylation of HSP27 substrate by the immunoprecipitated proteins obtained from cells overexpressing FLAG-

Fig. 5. 14-3-3ɛ inhibits TNFα-induced actin cytoskeleton organization and cell migration. (A) HeLa cells (2 × 10⁶) were transiently transfected with expression vector for 14-3-3ɛ (2 μg), 0.03 μM control siRNA or 0.03 μM 14-3-3ɛ siRNA. After incubation for 24 h, transfected cells were starved for 3 h and were untreated or treated with 100 ng/ml TNFα for 20 min. Cellular levels of endogenous HSP27, phosphorylated HSP27, 14-3-3ɛ and tubulin were examined by immunoblot analysis using respective antibodies. (B) Cells (5 × 10⁵) were transfected with empty vector (1 μg) (a) or expression vector for 14-3-3ɛ (1 μg) (b). Transfected cells were incubated for 24 h. Cells were starved for 3 h and treated with 100 ng/ml TNFα for 20 min. F-actin was visualized after staining cells with rhodamine-conjugated phalloidin as in Fig. 3. (C) HeLa cells (2 × 10⁶) were transiently transfected with expression vector for 14-3-3ɛ (2 μg), 0.03 μM control siRNA or 0.03 μM 14-3-3ɛ siRNA. After incubation for 24 h, cells were starved for 24 h. Cells were untreated or treated with 100 ng/ml TNFα for 20 min. Cell migration was expressed as a Migration Index as in Fig. 4.
MK5 was evident. The kinase activity of ectopically expressed MK5 was significantly interfered by 14-3-3ɛ expression. Data also demonstrate that MK5 mutant, MK5-T182A in which activation phosphorylation site at Thr 182 was substituted with Ala, lacks the ability to phosphorylate HSP27 (Fig. 2B). In addition, 14-3-3ɛ silencing by 14-3-3ɛ siRNA transfection enhanced MK5 kinase activity as shown by the increased phosphorylation of MK5 and HSP27 in cells cotransfected with MK5 expression vector and 14-3-3ɛ siRNA. The control siRNA had no effects on the enzymatic activity of MK5 (Fig. 2C).

3.3. 14-3-3ɛ disrupts MK5-mediated actin cytoskeleton organization

Since phosphorylated HSP27 confers the stabilization of actin cytoskeleton under stress conditions [23,24], 14-3-3ɛ which inhibits HSP27 phosphorylation is anticipated to interfere with actin cytoskeleton organization. The effects of 14-3-3ɛ expression on actin cytoskeleton organization in HeLa cells were monitored by immunofluorescence microscopy (Fig. 3). It was evident that actin cytoskeleton organization leading to stress fiber formation was induced by MK5 expression (Fig. 3C). No apparent alteration of actin cytoskeleton organization was observed in transfected cells overexpressing 14-3-3ɛ alone (Fig. 3B). However, data reveal that 14-3-3ɛ disrupts actin cytoskeleton organization in cells coexpressing MK5 with 14-3-3ɛ (Fig. 3D). We sought to confirm the disruption of actin cytoskeleton organization by 14-3-3ɛ in 14-3-3ɛ knockdown cells. The silencing of 14-3-3ɛ by 14-3-3ɛ siRNA transfection led to the increase in MK5-mediated actin cytoskeleton organization, but the treatment of control siRNA did not affect actin cytoskeleton organization (Fig. 3E, F).

3.4. 14-3-3ɛ inhibits MK5-mediated cell migration

Cell migration is a cellular process that requires F-actin polymerization and actin cytoskeleton stabilization [26]. Therefore, it is reasonable to speculate that 14-3-3ɛ which inhibits MK5 kinase activity may participate in cell migration since MK5 induces HSP27 phosphorylation and subsequent F-actin polymerization. MK5 expression stimulates cell migration in transfected HeLa cells. Overexpression of 14-3-3ɛ resulted in the inhibition of MK5-mediated cell migration by approximately 50% (Fig. 4A). The silencing of 14-3-3ɛ by 14-3-3ɛ siRNA transfection resulted in the stimulation of MK5-mediated cell migration. However, overexpression of MK5-T182A, MK5 mutant lacking MK5 kinase activity, fails to induce cell migration. The treatment of 14-3-3ɛ siRNA did not affect the migration of HeLa cells overexpressing MK5-T182A (Fig. 4B).

3.5. 14-3-3ɛ inhibits HSP27 phosphorylation, actin cytoskeleton organization and cell migration in TNFα-treated HeLa cells

MK5 is activated by proinflammatory cytokines such as tumor necrosis factor-α (TNFα) [15]. Immunoblot analysis shows that treatment of TNFα does not change cellular level of HSP27 but elevates the phosphorylation of HSP27. Overexpression of 14-3-3ɛ inhibited HSP27 phosphorylation in TNFα-treated cells, while the silencing of 14-3-3ɛ restored HSP27 phosphorylation (Fig. 5A). Immunofluorescence microscopy was performed to evaluate the effects of 14-3-3ɛ on TNFα-induced F-actin polymerization. Overexpression of 14-3-3ɛ abolished actin stress fiber formation in transfected HeLa cells (Fig. 5B). The effects of 14-3-3ɛ on TNFα-induced cell migration were also evaluated in cells transiently expressing 14-3-3ɛ. TNFα-induced cell migration was notably
Overexpression of 14-3-3 proteins in signal pathways leading to cell adhesion and cell migration. These studies have shown that 14-3-3 proteins directly or indirectly modulate integrin-mediated signaling events that cause actin cytoskeleton remodeling [6, 27–29].

Biochemical explanation for the positive roles of 14-3-3ε on neuronal cell migration comes from an animal model. A mouse model in which 14-3-3ε binds to its target protein NUDEL that controls cytoplasmic dynein function has been presented [30]. Cytoplasmic dynein function has been generally believed to be essential for neuronal cell migration and development. Another mechanism for the regulation of cell migration by 14-3-3 proteins in C. elegans and mammalian cells involves the interaction of 14-3-3 proteins with PAR protein kinases known to be important for the generation of cell polarity and actin cytoskeleton reorganization [31, 32].

Here, we propose a new biochemical pathway governing the inhibition of cell migration by 14-3-3ε. Coimmunoprecipitation assay provides evidence for the intracellular interaction between MK5 and 14-3-3ε in HeLa cells (Fig. 1A). A number of studies have shown that 14-3-3 proteins require phosphorylation of their target proteins for the interaction [4, 5]. However, the data in Fig. 1B showing the robust binding of 14-3-3ε to MK5-T182A mutant imply the dispensable role of MK5 phosphorylation on the intermolecular interaction between MK5 and 14-3-3ε. Therefore, it is unlikely that phosphorylation of MK5 at Thr182 in the catalytic domain facilitates the interaction between 14-3-3ε and MK5. In vitro binding assay suggests that 14-3-3ε binds to the N-terminal catalytic domain of MK5 containing the only regulatory phosphorylation site at Thr182 (Fig. 1C). Phosphorylation of MK5 at Thr182 is required for the activation of MK5 and its downstream signaling [15].

One plausible scenario is that 14-3-3ε binding to the N-terminal region of MK5 masks the phosphorylation site at Thr182 and prevents the activation of MK5. There is evidence suggesting that the binding of 14-3-3 proteins to their counterparts interferes with the enzymatic property of the target binding proteins. For example, 14-3-3γ interacts with calcium/calmodulin-dependent protein kinase kinase (CaMKK) and directly inhibits the enzymatic activity of CaMKK [33]. If phosphorylation of MK5 in the N-terminal region is a prerequisite for the activation of MK5, it is reasonable to speculate that the binding of 14-3-3ε with the N-terminal catalytic domain of MK5 containing Thr182 inhibits enzymatic ability of MK5 as a protein kinase.

Small heat shock protein HSP27 has a chaperonin activity involved with protein folding and is phosphorylated by activated MK5 in response to extracellular signals. We explored the possible role of 14-3-3ε as an inhibitor of MK5 kinase activity. Overexpression of 14-3-3ε inhibited the phosphorylation of HSP27 as well as the autophosphorylation of MK5 (Fig. 2B). Moreover, 14-3-3ε silencing enhanced MK5 kinase activity in cells transfected with 14-3-3ε siRNA (Fig. 2C). In addition to the ATP-independent chaperonin activity [34, 35], HSP27 has been characterized as a barbed-end microfilament capping protein that is inhibited by phosphorylation. Therefore, the phosphorylation of HSP27 has been shown to stimulate F-actin polymerization [21].

In an attempt to determine whether 14-3-3ε interferes with F-actin polymerization through intervening in MK5 downstream signal pathway, we examined the effects of 14-3-3ε on F-actin polymerization. Immunofluorescence analysis of actin stress fiber in HeLa cells coexpressing MK5 and 14-3-3ε supports the negative role of 14-3-3ε on F-actin polymerization (Fig. 3).

14-3-3 proteins are known to regulate the subcellular localization of their binding counterparts presumably by masking or obscuring the nearby targeting sequences like NLS or NES. The activities of nuclear enzymes such as Cdc25 [12], telomerase [36] or histone deacetylase [37] have been reported to be controlled by the mechanism of 14-3-3 protein-dependent subcellular localization. Likewise, 14-3-3 proteins participate in the regulation of subcellular localization of FOXO transcription factors as well as their transcriptional activity through interfering with the nuclear localization function of NLS [38]. In resting cells, MK5 is majorly localized in the nucleus owing to the functional NLS, whereas MK5 anchors in the cytoplasm in activated cells [39]. The NLS and NES responsible for nuclear-cytoplasmic distribution of MK5 are directly overlapping at the C-terminal region of MK5. Therefore, it is unlikely that NES and NLS are masked by 14-3-3ε which interacts with the N-terminal region of MK5.

4. Discussion

Previous studies have described the intervening of 14-3-3 proteins in signal pathways leading to cell adhesion and cell migration. As an inhibitor of MK5 kinase activity. Overexpression of 14-3-3ε inhibited the phosphorylation of MK5 and 14-3-3ε expression. The increase in cell migration after 14-3-3ε siRNA treatment provides additional evidence for the disruption of TNFα-induced cell migration by 14-3-3ε expression (Fig. 5C). In addition, both F-actin polymerization and cell migration were inhibited in TNFα-treated MFG-14-3-3ε HeLa cells, which stably express high level of 14-3-3ε protein (Fig. 6).

Fig. 7. Proposed model for the inhibition of MK5-mediated cell migration by 14-3-3ε. Upon stimulation, mitogen-activated protein kinases (p38 and ERK3/4) activate MK5 in the cytoplasm. 14-3-3ε bound to MK5 inhibits MK5 protein kinase, which phosphorylates HSP27 substrate. 14-3-3ε inhibits cell migration through blocking HSP27 phosphorylation which is required for F-actin polymerization.
MK5. Since 14-3-3ε binds to N-terminal region of MK5 and inhibits MK5 kinase activity, it is also unlikely that 14-3-3ε bound to MK5 inhibits MK5 activation and HSP27 phosphorylation by interfering with the subcellular localization of MK5. The possibility that 14-3-3ε may directly bind with HSP27, inhibit HSP27 phosphorylation and interfere with F-actin polymerization is also ruled out by the result of an independent experiment in which the intracellular interaction between HSP27 and 14-3-3ε was examined. We found that 14-3-3ε does not interact with HSP27 in HeLa cells (data not shown).

Cell migration is a multi-step cellular process initiated by forming protrusive structures like filopodia, lamellipodia and invadopodia/podosomes. Formation of these structures is driven by the control of F-actin polymerization [40]. Thus, the changes in invadopodia/podosomes. Formation of these structures is driven by the control of F-actin polymerization [40]. Thus, the changes in

5. Conclusions

14-3-3ε interacts with MK5 to inhibit its enzymatic activity as a protein kinase. MK5 phosphorylates HSP27. The phosphorylation of HSP27 is required for F-actin polymerization. 14-3-3ε bound to MK5 disrupts F-actin polymerization by inhibiting the phosphorylation of HSP27 resulting in the inhibition of MK5-mediated cell migration.

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