Interaction of ALG-2 with ASK1 influences ASK1 localization and subsequent JNK activation

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Abstract ALG-2 (apoptosis linked gene-2) is an essential protein for the execution of apoptosis whose function is largely unknown. Here, we demonstrate that ALG-2 could interact with the C-terminus (amino acids 941–1375) of the apoptosis signal-regulating kinase 1 (ASK1) in BOSC23 cells as well as in vitro. ASK1 failed to bind to an isotype of ALG-2 found in the liver, ALG-2,1, in which two amino acids (Gly-121 and Phe-122) are deleted. This implies that the interaction is very specific. Cotransfection with ALG-2 resulted in the nuclear presence of ASK1 and inhibited the activation of c-Jun N-terminal kinase (JNK) by ASK1 in BOSC23 cells. This study reports that ALG-2 could regulate the subcellular localization and the JNK activity modulation of ASK1 by direct interaction.
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Key words: Apoptosis linked gene-2; Apoptosis signal-regulating kinase 1; Interaction; c-Jun N-terminal kinase; Apoptosis; Localization

1. Introduction

Apoptosis is a physiological process of cell death that is essential for the animal development and adult homeostasis [1,2]. Fas is a member of the tumor necrosis factor (TNF) receptor family and induces apoptosis by binding to Fas ligand [3]. Fas can activate two independent signaling pathways. One well-characterized pathway involves the adapter protein FADD, which recruits procaspase-8 and activates the caspase cascade leading to apoptosis [1]. The second pathway is mediated by Daxx, which interacts with apoptosis signal-regulating kinase 1 (ASK1) activating c-Jun N-terminal kinase (JNK) and p38 MAPK (mitogen-activated protein kinase) by phosphorylation [4].

Conflicting data exist regarding the correlation between JNK activation and Fas-mediated apoptosis. There are reports that JNK activation influences multiple proteins controlling apoptosis such as c-Jun, p53 and Bax [5–7]. However, it has also been reported that JNK activation is not required for the execution of Fas-mediated cell killing [8,9].

ASK1 is a kinase that acts upstream of the JNK pathway when stimulated by diverse stimuli such as TNF, Fas, H_2O_2, oxidative stress, DNA damage and polyglutamine repeats [10–15]. The association of thioredoxin with ASK1 promotes ubiquitination/degradation of ASK1 leading to inhibition of ASK1-induced apoptosis [16]. Overexpression of ASK1 induces apoptosis through a mitochondria-dependent caspase activation pathway and a dominant-negative mutant of ASK1 inhibited apoptosis induced by TNF and Fas ligation [11,12,17,18].

Several cellular factors such as Daxx, TRAF2 (TNF receptor-associated factor 2), CDC25A, 14-3-3, thioredoxin and protein phosphatase 5 (PP5), have been reported to bind with ASK1 [19–23]. Daxx and TRAF2 activate the kinase activity of ASK1, but CDC25A, 14-3-3, thioredoxin and PP5 inhibit it [23]. ASK1 is localized in the cytoplasm and Daxx in the nucleus [24,25]. However, Daxx translocates from the nucleus to the cytoplasm after Fas ligation [26,29,30]. ASK1 binds to TRAF2 and this binding is essential for JNK activation [15,19]. CDC25A binds to the C-terminus of ASK1 and inhibits the kinase activity of ASK1. 14-3-3 can inhibit the proapoptotic function of ASK1 through binding to Ser-967 of ASK1, which is phosphorylated by an unknown survival signaling kinase [21]. Thioredoxin in its reduced form binds to the NH_2-terminal part of ASK1 and blocks activation of ASK1 by TNF [13,20,28]. ASK1 also possesses a caspase-independent apoptotic function that is independent of its kinase activity [29].

ALG-2 (apoptosis-linked gene-2) is a protein whose absence causes a failure in apoptosis induced by Fas, T cell receptors and glucocorticoids [30]. However, ALG-2-deficient mice do not seem to block apoptosis induced by various apoptotic signals [31]. ALG-2 is a calpain small subunit subfamily of Ca^{2+} binding proteins and contains a five EF-hand Ca^{2+} binding domain [32,33]. ALG-2 seems to act downstream of caspase activation because ALG-2 depletion does not affect caspase activation [35]. Since ALG-2 depletion blocks apoptosis, it implies that ALG-2 dissociates activation of caspases from the apoptotic event. However, association of ALG-2 with Fas has been reported in Jurkat cells implying that ALG-2 might work upstream of the signaling pathway or at the signal amplification phase [36]. Interaction of human embryonic ectoderm development protein was reported, implying that ALG-2 might be involved in the anterior–posterior patterning process [37]. Subcellular localization of ALG-2 does not seem to be simple. ALG-2 exists in the cytoplasm of L66 fibroblast cells and in the membrane and in the nucleus of Jurkat cells [36,38,39]. Thus, ALG-2 might work in different subcellular spaces depending on the cell type or the physiological conditions.

ALG-2,1, an isotype of ALG-2 missing two internal amino acids (Gly-121 and Phe-122), was found in the mouse liver

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[40]. ALG-2,1 forms heterodimers with ALG-2,5, a predominant ALG-2 isotype containing Gly-121 and Phe-122 (ALG-2 means ALG-2,5 isotype in this study). The heterodimerization of ALG-2 with peflin plays a role in the stabilization of peflin [41]. ALG-2 binds to AIP-1 (ALG-2 interacting protein 1) whose yeast homolog is a member of the MAPK pathway and also binds to the N-terminal region of annexin XI [42]. However, ALG-2,1 could not bind to AIP-1 [38,40]. Thus, it has been proposed that the two ALG-2 isoforms might have regulatory functions against each other.

The interaction between ALG-2 and ASK1 was examined in this study, based on the fact that ASK1 is recruited to Fas upon Fas activation and that ASK1 is a member of the MAPK pathway. Here, we report that ASK1 could bind to ALG-2 and that this interaction would influence subcellular localization and the JNK activation of ASK1.

2. Materials and methods

2.1. Plasmids

pcDNA3/ASK1-HA was a gift from Dr. K.S. Kwon (Korea Research Institute of Bioscience and Biotechnology, Seoul, South Korea). PGEX-4T-1/ASK1 N648 (amino acids (a.a.) 1–648), pGEX4T-1/ASK1 KK (a.a. 649–940) and pGEX4T-1/ASK1 KC (a.a. 941–1375), respectively, were produced by polymerase chain reaction (PCR) using pcDNA3/ASK1-HA as a template. pFLAG-CMV-5a/ALG-2, pGEX4T-1/ALG-2, pGEX4T-1/ALG-2-23N (a.a. 24–191), and pGEX4T-1/ALG-2,1 constructs were produced by PCR using pcDNA3/ALG-2 [36] as a template.

2.2. Cell lines, culture conditions and transfections

BOSC23 cells were grown at 37°C in 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) (Hyclone, Logan, UT, USA) and antibiotics (Invitrogen, Carlsbad, CA, USA). 1×10^6 BOSC23 cells were transfected with indicated plasmids using the calcium phosphate method.

2.3. In vitro binding assays

GST-ALG-2 was expressed in *Escherichia coli* and purified with glutathione-agarose beads. ASK1 was translated in vitro in the presence of [^{35}S]methionine using the T_NT reticulocyte lysate system (Promega, Madison, WI, USA). The ^{35}S-labeled proteins were incubated at 4°C for 3 h with the GST-ALG-2 immobilized onto the beads in the binding buffer containing 50 mM HEPES (pH 7.6), 50 mM NaCl, 5 mM EDTA, 0.1% Nonidet P-40, and 10% glycerol. The beads were harvested and washed three times with the binding buffer. The ^{35}S-labeled proteins were then eluted from the beads and analyzed by SDS–PAGE and autoradiography.

2.4. Western blotting

Cells were washed once with phosphate-buffered saline (PBS) and harvested from the culture dishes using trypsin. The cell pellets were subsequently lysed in the mammalian lysis buffer (50 mM Tris–HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 1% Nonidet P-40, 0.4 mM phenylmethylsulfonyl fluoride). After lysis, the cell extract was sonicated and centrifuged at 13 000 rpm. Protein (30 μg) from the total cell lysate was separated by electrophoresis on a SDS–PAGE gel and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA). The membrane was probed with mouse anti-FLAG monoclonal antibody (Sigma, St. Louis, MO, USA) at a dilution of 1:2000, rabbit anti-hemagglutinin (HA) antibody (Santa Cruz, Santa Cruz, CA, USA) at a dilution of 1:1000, and rabbit anti-ALG-2 antibody (gift from Dr. J.W. Kim, PaiChai University, Taejon, South

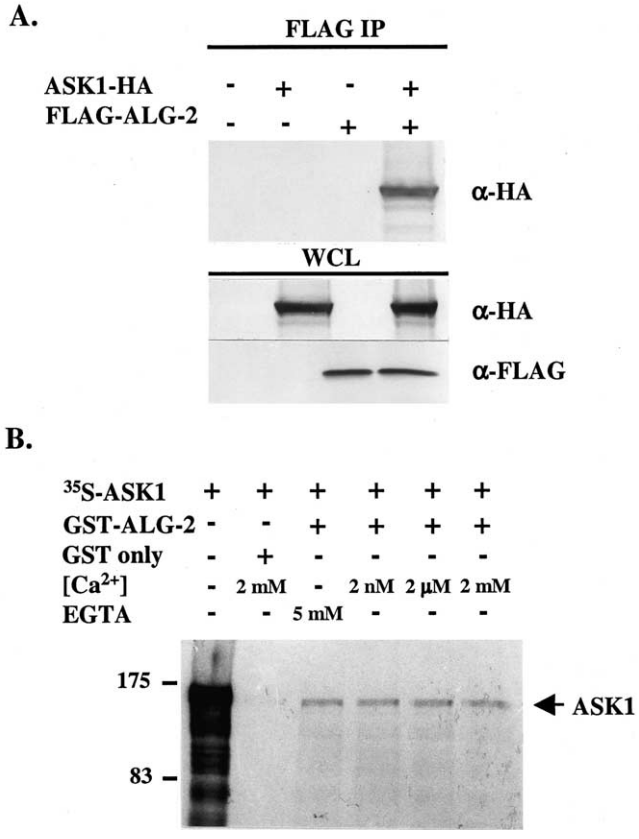

Fig. 1. Interaction between ASK1 and ALG-2 in vivo (A) and in vitro (B). A: BOSC23 cells were transfected with pFLAG-CMV-5a or pFLAG-CMV-5a/ALG-2 and pcDNA3/ASK1-HA. ALG-2 was immunoprecipitated with mouse anti-FLAG antibody and Western blot was carried out with rabbit anti-HA antibody. B: GST-ALG-2 proteins were purified and immobilized on glutathione Sepharose 4B beads. The beads were incubated at 4°C for 3 h with ^{35}S-labeled ASK1 protein in the presence of EGTA or different concentrations of Ca^{2+}. After washing three times, the mixtures were resolved on a 6% SDS–PAGE gel and autoradiographed. Numbers on the left indicate protein molecular weight standards in kDa. WCL, whole cell lysate.

Korea) at a dilution of 1:1000 followed by AP-conjugated goat anti-mouse IgG and anti-rabbit IgG antibodies (Santa Cruz).

2.5. Coimmunoprecipitation analysis

For coimmunoprecipitation experiment, cells were washed in cold PBS, then lysed in the mammalian lysis buffer. Mouse anti-FLAG® M2 affinity gel (Sigma) was added to the cell lysate and incubated for 4 h at 4°C with gentle inversion mixing. After incubation for 4 h, the gel was collected and washed three times with the mammalian lysis buffer. Coimmunoprecipitated proteins were analyzed by SDS–PAGE and Western blot using anti-FLAG and anti-HA antibodies.

2.6. Immunofluorescence analysis

BOSC23 cells were grown on glass coverslips for 24 h in DMEM containing 10% FBS and transfected with the pFLAG-CMV-5a/ALG-2 and pcDNA3/ASK1-HA expression vectors. After transfection, cells were fixed at room temperature for 20 min with 4% paraformaldehyde in PBS and rinsed with 1×PBS containing 10% goat normal serum (Sigma) and incubated with mouse anti-FLAG antibody (at 1:500 dilution) and rabbit anti-HA antibody (at 1:200 dilution) for 1 h. After washing cells three times with 1×PBS, cells were incubated with secondary anti-mouse IgG antibody (at 1:1000 dilution) conjugated with FITC-green (PharMingen, San Diego, CA, USA) and anti-rabbit IgG antibody (at 1:1000 dilution) conjugated with TRITC-red (Sigma) for 1 h. After extensive washing, slides were mounted and confocal laser scanning microscopy was performed with Radiance 2000 confocal microscope (Bio-Rad, Hercules, CA, USA), using excitation wavelengths of 494 nm (FITC-green) and 543 nm (TRITC-red).

2.7. JNK activity assay

BOSC23 cells at 60–70% confluence were transfected and incubated in DMEM containing 10% serum for 24 h after transfection. Subsequently, BOSC23 cells were incubated in serum-free DMEM for an additional 12 h. To measure JNK activity, cells were washed twice in 1×PBS and immediately lysed in the mammalian lysis buffer and sonicated. After sonication, cells were centrifuged at 13 000 rpm and cell debris was removed. 100 μg of cell lysate was incubated with recombinant GST-c-Jun (a.a. 1–79) bound to glutathione-coupled beads. Kinase assay was performed for 30 min at 30°C in a final reaction mixture of 100 μl consisting of 200 μg cell lysate, 15 μM ATP, 10 μCi [γ-^{32}P]ATP and 5 μg of recombinant GST-c-Jun in kinase buffer (20 mM MOPS, 2 mM EGTA, 10 mM $MgCl_2$, 1 mM dithiothreitol, 0.1% Triton X-100, 0.1 mM Na_3VO_4). The reactions were terminated with SDS sample buffer and were subsequently boiled for 3 min before separation on a 10% SDS–PAGE gel. Phosphorylation of GST-c-Jun was detected by autoradiography of the dried gels. Transfection efficiency of cells was judged by Western blotting with anti-ALG-2, anti-HA and anti-JNK1 antibodies (PharMingen).

3. Results

3.1. ALG-2 interacts with ASK1

Since ALG-2 bound to Fas in Jurkat cells [36], we questioned if ALG-2 interacts with ASK1 which is recruited to Fas upon Fas activation. In order to examine the interaction between ALG-2 and ASK1 in mammalian cells, pFLAG-CMV-5a/ALG-2 and pcDNA3/ASK1-HA were transiently transfected in BOSC23 cells. As shown in Fig. 1A, ASK1 was detected by Western blot analysis with rabbit anti-HA antibody after immunoprecipitation with anti-FLAG antibody implying that ASK1 could interact with ALG-2 in mammalian cells.

ALG-2 is a calcium binding protein and showed differential interaction characteristics in the presence of Ca^{2+}, i.e. binding to AIP with Ca^{2+} [38,43] but dissociating from peflin with Ca^{2+} [39]. Thus, the calcium dependence of the binding between ASK1 and ALG-2 was investigated. GST-ALG-2 fusion protein pulled down the in vitro translated ASK1 with nanomolar to millimolar concentrations of Ca^{2+} (Fig. 1B).

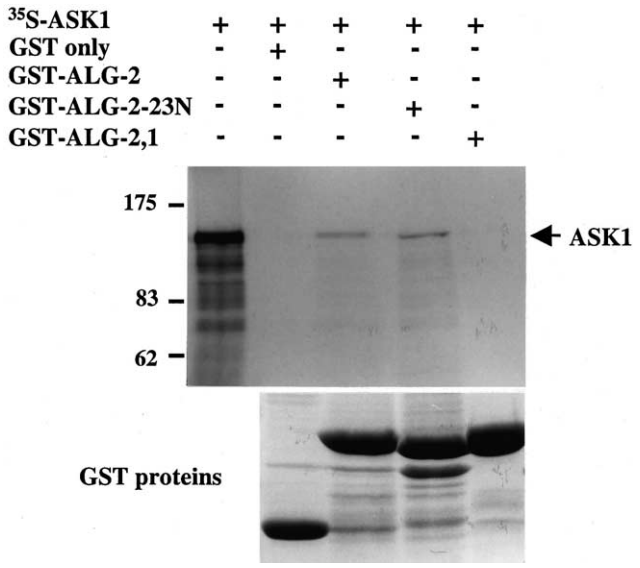

Fig. 2. Specificity of the interaction between ASK1 and ALG-2. GST-ALG-2, GST-ALG-2-23N (a.a. 24–191 of ALG-2) and GST-ALG-2,1 proteins were purified and bound to ^{35}S-labeled ASK1 in the buffer with 2 μM Ca^{2+} (upper panel). The amount of GST fusion protein in the beads was revealed by Coomassie blue staining (lower panel). Numbers on the left indicate protein molecular weight standards in kDa.

ALG-2 bound to ASK1 even in the presence of 5 mM EGTA in the in vitro binding assay. Thus, the interaction between ASK1 and ALG-2 was not influenced by the presence of Ca^{2+}.

ASK1 could bind in vitro to the N-terminal deletion mutant of ALG-2 in which the hydrophobic stretch of N-terminal 23 amino acids is deleted, meaning that the PEF (penta EF)

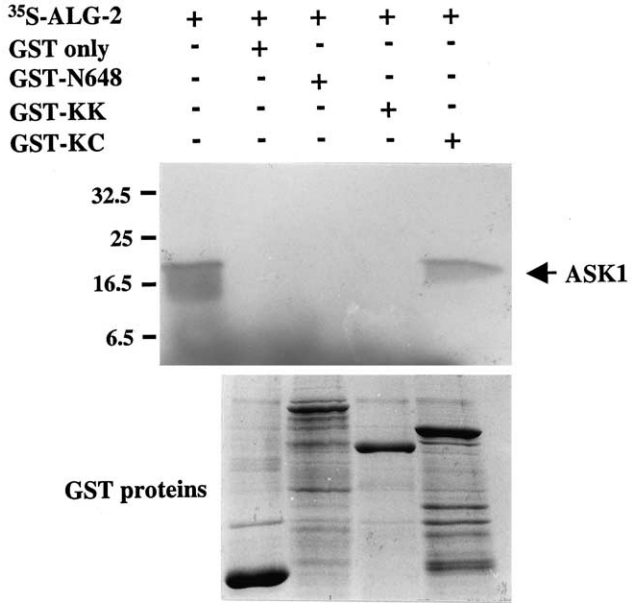

Fig. 3. Binding of ALG-2 to the C-terminus of ASK1. GST-ASK1-N648 (a.a. 1–648), GST-ASK1-KK (a.a. 649–940) and GST-ASK1-KC (a.a. 941–1375) proteins were purified and bound to ^{35}S-labeled ALG-2 (upper panel). The amount of GST fusion protein in the beads was revealed by Coomassie blue staining (lower panel). Numbers on the left indicate protein molecular weight standards in kDa.

domain is sufficient for the binding (Fig. 2). However, GST fusion protein of ALG-2,1 failed to pull down in vitro translated ASK1. This suggests that the binding between ASK1 and ALG-2 is very specific.

In order to map the interacting region of ASK1 to ALG-2, ASK1 deletion mutants were generated as GST fusion proteins. Among various deletion constructs, the C-terminal construct with a.a. 941–1375 of ASK1 (ASK1-KC) was sufficient to bind to ALG-2 in vitro (Fig. 3). Thus, ALG-2 adds itself to the list of proteins binding to the C-terminus of ASK1 such as 14-3-3, CDC25A and TRAF2.

3.2. ALG-2 induces nuclear localization of ASK1

ASK1 has been found in the cytoplasm and has been revealed to transduce Fas-induced apoptosis signal [13]. It has been reported that ASK1 forced Daxx to stay in the cytoplasm through the physical interaction between ASK1 and Daxx in 293 cells [27]. Thus, we questioned if ASK1 influences the subcellular localization of ALG-2. Confocal microscopic analysis was performed with anti-FLAG antibody using goat anti-mouse IgG-FITC as a secondary antibody (for the detection of ALG-2) and anti-HA antibody using goat anti-rabbit IgG-TRITC as a secondary antibody (for the detection of ASK1) in BOSC23 cells. ASK1 was present in the cytoplasm and ALG-2 was predominantly in the nucleus when transfected separately. However, in cells where pFLAG-CMV-5a/ALG-2 and pcDNA3/ASK1-HA were cotransfected, both ASK1 and ALG-2 were predominantly localized in the nucleus (Fig. 4). Thus, ALG-2 seems to force ASK1 to be present in the nucleus by direct interaction with ASK1.

3.3. ALG-2 inhibits JNK activation by ASK1

ASK1 activates JNK when localized in the cytoplasm. Since ASK1 was present in the nucleus when cotransfected with ALG-2, it is probable that the change in the localization of ASK1 might influence the JNK activating function. To answer this question, BOSC23 cells were transfected with pcDNA3/ALG-2 and/or pcDNA3/ASK1-HA. Overexpression of ALG-2 downregulated JNK activation whereas ASK1 overexpression activated JNK as previously reported by others [11,12,17]. Moreover, in the cells cotransfected with ALG-2 and ASK1, JNK activation by ASK1 was inhibited significantly (Fig. 5). This implies that ALG-2 might inhibit the JNK activation of ASK1 by changing the subcellular localization of ASK1.

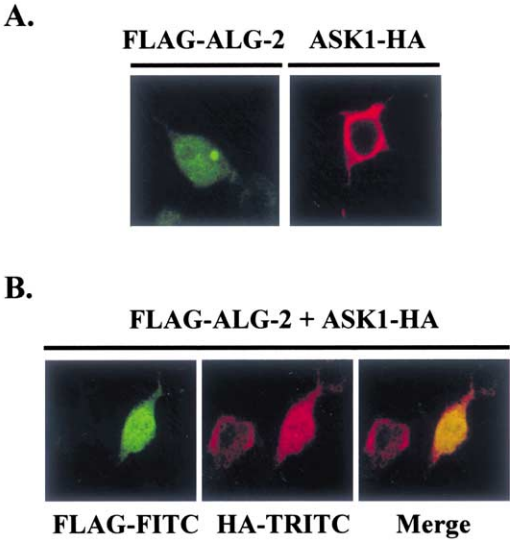

Fig. 4. Subcellular localization of ASK1 in BOSC23 cells. A: BOSC23 cells were transfected with plasmid pFLAG-CMV-5a/ALG-2 and pcDNA3/ASK1-HA. 72 h after transfection, the cells were fixed and processed for immunofluorescence staining. B: Immunofluorescence microphotographs showing the intracellular localization of transfected ALG-2 and ASK1.

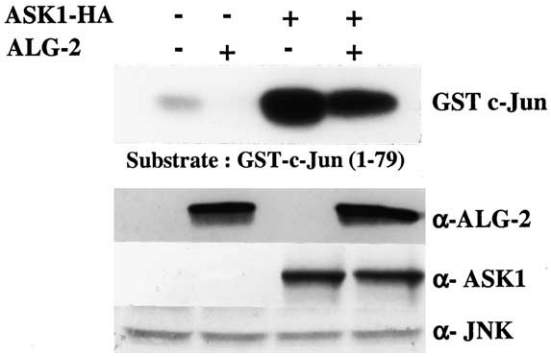

Fig. 5. Effect of ALG-2 upon activation of JNK by ASK1. In vitro kinase assay showing the effect of ALG-2 and ASK1 on JNK activity, BOSC23 cells were transfected pcDNA3/ALG-2 and pcDNA3/ASK1-HA. The cells were lysed and 100 μg of lysate was incubated with 10 μg of GST-c-Jun bound to glutathione beads. The kinase reaction was performed in the presence of 10 μCi of [γ-^{32}P]ATP for 30 min. The proteins were separated by SDS–PAGE and detected by autoradiography. To measure relative levels of protein expression, cell lysates were separated on SDS–PAGE and immunoblotted using indicated antibodies.

4. Discussion

In this study, we demonstrated that ALG-2 could bind to the C-terminus of ASK1 (a.a. 941–1375) and induced the nuclear localization of ASK1 and inhibited JNK activation by ASK1. The interaction was specific because deletion of two internal amino acids of ALG-2 abrogated the interaction with ASK1.

ALG-2 is a Ca^{2+} binding protein containing a PEF motif, and exposes a hydrophobic surface in a Ca^{2+}-dependent manner [32]. This conformational change seemed to be translated into the differential binding characteristics of ALG-2 depending upon the Ca^{2+}. ALG-2 dissociates from peflin and binds to AIP in a Ca^{2+}-dependent manner [32–34]. However, the binding between ALG-2 and ASK1 was not dependent on the presence of Ca^{2+}. This is the first report that ALG-2 interacts in a Ca^{2+}-independent manner.

ALG-2-23N mutant binds to ASK1 meaning that a hydrophobic amino acid stretch is not necessary for the interaction. ALG-2,1, however, does not bind to ASK1. Thus, the interaction between ASK1 and ALG-2 seems to be specific even if it is not dependent upon the presence of Ca^{2+}. Considering that ALG-2,1 and ALG-2,5 are endogenous isotypes, their differential binding characteristics with ASK1 provide an elegant regulatory mechanism of ASK1-mediated signaling. For instance, the stoichiometry of ALG-2 isotypes in cells would be translated to their regulatory capacity upon ASK1.

The C-terminus of ASK1 was mapped for the binding to 14-3-3 and CDC25A. These interactions in the C-terminus of ASK1 caused inhibition of ASK1's kinase activity [20–22].

ALG-2 interacts with the C-terminus of ASK1 and this interaction also inhibits ASK1's downstream JNK activation.

ALG-2 seems to be localized in the cytoplasm and nucleus depending on its cell type and physiological condition [36,39]. In this study, ALG-2 was found predominantly in the nucleus of BOSC23 cells. Nuclear retention of ASK1 by cotransfection with ALG-2 would disconnect ASK1 physically from activating JNK, a cytoplasmic protein. JNK activation is often linked to cell death induced by stress, whereas cell death can be prevented by inhibition of JNK activation [44,45]. Overexpression of ASK1 has been reported to activate the JNK pathway in various cell lines. Nuclear retention of ASK1 might prevent Daxx from being retained in the cytoplasm even though this has not been examined in this study. If so, the nuclear presence of ASK1 would disturb the Daxx-mediated apoptotic pathway. These data present a molecular mechanism for the function of ALG-2, i.e. ALG-2 plays a regulatory role on ASK1 by direct interaction.

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References

[1] Nagata, S. (1997) Cell 88, 355–365.
[2] Vaux, D.L. and Korsmeyer, S.J. (1999) Cell 96, 245–254.
[3] Krammer, P.H. (2000) Nature 407, 789–795.
[4] Yang, X., Khosravi-Far, R., Chang, H.Y. and Baltimore, D. (1997) Cell 89, 1067–1076.
[5] Xia, Z., Dickens, M., Raingeaud, J., Davis, R.J. and Greenberg, M.E. (1995) Science 270, 1326–1331.
[6] Behrens, A., Sibilia, M. and Wagner, E.F. (1999) Nature Genet. 21, 326–329.
[7] Leppa, S. and Bohmann, D. (1999) Oncogene 18, 6158–6162.
[8] Chen, Y. and Lai, M.Z. (2001) J. Biol. Chem. 276, 8350–8357.
[9] Hofmann, T.G., Möller, A., Hehner, S.P., Welsch, D., Dröge, W. and Schmitz, M.L. (2001) Int. J. Cancer 93, 185–191.
[10] Wang, X.S., Diener, K., Jannuzzi, D., Trollinger, D., Tan, T.H., Lichenstein, H., Zukowski, M. and Yao, Z. (1996) J. Biol. Chem. 271, 31607–31611.
[11] Ichijo, H., Nishida, E., Irie, K., ten Dijke, P., Saitoh, M., Moriguchi, T., Takagi, M., Matsumoto, K., Miyazono, K. and Gotoh, Y. (1997) Science 275, 90–94.
[12] Chang, H.Y., Nishitoh, H., Yang, X., Ichijo, H. and Baltimore, D. (1998) Science 281, 1860–1863.
[13] Gotoh, Y. and Cooper, J.A. (1998) J. Biol. Chem. 273, 17477–17482.
[14] Chen, Z., Seimiya, H., Naito, M., Mashima, T., Kizaki, A., Dan, S., Imaizumi, M., Ichijo, H., Miyazono, K. and Tsuruo, T. (1999) Oncogene 18, 173–180.
[15] Nishitoh, H., Matsuzawa, A., Tobiume, K., Saegusa, K., Takeda, K., Inoue, K., Hori, S., Kakizuka, A. and Ichijo, H. (2002) Genes Dev. 16, 1345–1355.
[16] Liu, Y. and Min, W. (2002) Circ. Res. 90, 1259–1266.
[17] Hatai, T., Matsuzawa, A., Inoshita, S., Mochida, Y., Kuroda, T., Sakamaki, K., Kuida, K., Yonehara, S., Ichijo, H. and Takeda, K. (2000) J. Biol. Chem. 275, 26576–26581.
[18] Kanamoto, T., Mota, M., Takeda, K., Rubin, L.L., Miyazono, K., Ichijo, H. and Bazenet, C.E. (2000) Mol. Cell. Biol. 20, 196–204.
[19] Nishitoh, H., Saitoh, M., Mochida, Y., Takeda, K., Nakano, H., Rothe, M., Miyazono, K. and Ichijo, H. (1998) Mol. Cell 2, 389–395.
[20] Saitoh, M., Nishitoh, H., Fujii, M., Takeda, K., Tobiume, K., Sawada, Y., Kawabata, M., Miyazono, K. and Ichijo, H. (1998) EMBO J. 17, 2596–2606.
[21] Zhang, L., Chen, J. and Fu, H. (1999) Proc. Natl. Acad. Sci. USA 96, 8511–8515.
[22] Zou, X., Tsutsui, T., Ray, D., Blomquist, J.F., Ichijo, H., Ucker, D.S. and Kiyokawa, H. (2001) Mol. Cell. Biol. 21, 4818–4828.
[23] Morita, K., Saitoh, M., Tobiume, K., Matsuura, H., Enomoto, S., Nishitoh, H. and Ichijo, H. (2001) EMBO J. 20, 6028–6036.
[24] Pluta, A.F., Earnshaw, W.C. and Goldberg, I.G. (1998) J. Cell Sci. 111, 2029–2041.
[25] Li, H., Leo, C., Zhu, J., Wu, X., O'Neil, J., Park, E.J. and Chen, J.D. (2000) Mol. Cell. Biol. 20, 1784–1796.
[26] Charette, S.J., Lavoie, J.N., Lambert, H. and Landry, J. (2000) Mol. Cell. Biol. 20, 7602–7612.
[27] Ko, Y.G., Kang, Y.S., Park, H., Seol, W., Kim, J., Kim, T., Park, H.S., Choi, E.J. and Kim, S. (2001) J. Biol. Chem. 276, 39103–39106.
[28] Liu, H., Nishitoh, H., Ichijo, H. and Kyriakis, J.M. (2000) Mol. Cell. Biol. 20, 2198–2208.
[29] Charette, S.J., Lambert, H. and Landry, J. (2001) J. Biol. Chem. 276, 36071–36074.
[30] Vito, P., Lacaná, E. and D'Adamio, L. (1996) Science 271, 521–525.
[31] Jang, I.K., Hu, R., Lacaná, E., D'Adamio, L. and Gu, H. (2002) Mol. Cell. Biol. 22, 4094–4100.
[32] Maki, M., Yamaguchi, K., Kitaura, Y., Satoh, H. and Hitomi, K. (1998) J. Biochem. 124, 1170–1177.
[33] Kitaura, Y., Watanabe, M., Satoh, H., Kawai, T., Hitomi, K. and Maki, M. (1999) Biochem. Biophys. Res. Commun. 263, 68–75.
[34] Jia, J., Tarabykina, S., Hansen, C., Berchtold, M. and Cygler, M. (2001) Structure 57, 267–275.
[35] Lacaná, E., Ganjei, J.K., Vito, P. and D'Adamio, L. (1997) J. Immunol. 158, 5129–5235.
[36] Jung, Y.S., Kim, K.S., Kim, K.D., Lim, J.S., Kim, J.W. and Kim, E. (2001) Biochem. Biophys. Res. Commun. 288, 420–426.
[37] Lee, K.H., Kim, E., Lee, J.S., Lee, K.S. and Kim, J.W. (2001) Mol. Cell 12, 298–303.
[38] Vito, P., Pellegrini, L., Guiet, C. and D'Adamio, L. (1999) J. Biol. Chem. 274, 1533–1540.
[39] Kitaura, Y., Matsumoto, S., Satoh, H., Hitomi, K. and Maki, M. (2001) J. Biol. Chem. 276, 14053–14058.
[40] Tarabykina, S., Møller, A.L., Durussel, I., Cox, J. and Berchtold, M.W. (2000) J. Biol. Chem. 275, 10514–10518.
[41] Kitaura, Y., Satoh, H., Takahashi, H., Shibata, H. and Maki, M. (2002) Arch. Biochem. Biophys. 399, 12–18.
[42] Satoh, H., Shibata, H., Nakano, Y., Kitaura, Y. and Maki, M. (2002) Biochem. Biophys. Res. Commun. 291, 1166–1172.
[43] Lo, K.W., Zhang, Q., Li, M. and Zhang, M. (1999) Biochemistry 38, 7498–7508.
[44] Zanke, B.W., Boudreau, K., Rubie, E., Winnett, E., Tibbles, L.A., Zon, L., Kyriakis, J., Liu, F.F. and Woodgett, J.R. (1996) Curr. Biol. 6, 606–613.
[45] Lee, L.F., Li, G., Templeton, D.J. and Ting, J.P. (1998) J. Biol. Chem. 273, 28253–28260.