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Protein kinase A phosphorylates and regulates dimerization of 14-3-3ζ

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Abstract **Recognition of phosphorylated serine/threonine-containing motifs by 14-3-3 depends on the dimerization of 14-3-3. However, the molecular cues that control 14-3-3 dimerization are not well understood. In order to identify proteins that control 14-3-3 dimerization, we analyzed proteins that have effects on 14-3-3 dimerization and report that protein kinase A (PKA) phosphorylates 14-3-3ζ at a specific residue (Ser58). Phosphorylation by PKA leads to modulation of 14-3-3ζ dimerization and affect its interaction with partner proteins. Substitution of Ser58 to Ala completely abolished phosphorylation of 14-3-3ζ by PKA. A phospho-mimic mutant of 14-3-3ζ, Ser58 to Glu substitution, failed to form homodimers, showed reduced interaction with 14-3-3ε and p53, and could not enhance transcriptional activity of p53. Moreover, activation of PKA decreases and inhibition of PKA increases the dimerization of 14-3-3ζ and the functional interaction of 14-3-3ζ with p53. Therefore, our results suggest that PKA is a new member of protein kinases that can phosphorylate and impair the function of 14-3-3.**

Keywords: Protein kinase A; 14-3-3ζ; Dimerization; Phosphorylation

1. Introduction

14-3-3 proteins are small acidic proteins with molecular mass of 28–33 kDa and they usually exist as dimers. 14-3-3 proteins are expressed ubiquitously in eukaryotes and their protein sequences are highly conserved from yeast to mammals [1–3]. Seven isoforms in mammals, encoded by seven distinct genes, more than 10 in plants, and two each in yeast, *Drosophila*, and *Caenorhabditis elegans* have been identified [4]. 14-3-3 proteins from various phyla are functionally conserved, such that yeast homologs are functionally interchangeable with those of plant and mammalian. The 14-3-3 family proteins preferentially bind to phospho-serine/threonine-containing motifs [5,6]. Structural analyses of 14-3-3, crystallized alone or in complex with short synthetic peptides, revealed dimeric structure of 14-3-3 and pointed to the residues involved in dimerization and target binding: specific residues and regions in the amino terminus are critical for dimerization and residues both in the carboxy and amino termini are critical for target bindings [7–9]. Mutational analysis of 14-3-3 proteins supported these findings [10–12].

14-3-3 proteins regulate multiple signaling pathways involved in the control of cell division, growth, and apoptosis [1,13,14]. 14-3-3 binding to conserved peptide motifs of RSXpSXP or RXXXpSXP is required for a range of context dependent effects, including alteration of enzymatic activity, cellular localization, susceptibility to proteases and phosphatases, or the ability to incorporate into protein complexes [1]. For example, 14-3-3 binding is required for the stabilization of active Raf-1 [15,16], Cdc25-mediated cell cycle control [17,18], inhibition of proapoptotic Bad [19], and the function of FoxO [20,21].

Functioning as phospho-Ser/Thr binding protein, 14-3-3 itself appears to be controlled by phosphorylation process [22]. Dimerization and target binding are dependent on the phosphorylation of 14-3-3 [23], and three phosphorylation sites on 14-3-3ζ (Ser58, Ser184 and Thr232) have been identified [1]. Phosphorylation of 14-3-3ζ at Ser184 residue increases its interaction with PKC and this interaction downregulates the enzymatic activity of PKC in vitro [24]. c-Jun NH2-terminal kinase phosphorylated 14-3-3ζ at Ser184 both in vitro and in vivo, and such phosphorylation reduced the affinity of 14-3-3 proteins for Bax and c-Abl [25,26]. In contrast, phosphorylation at Thr232 residue by Casein kinase I dissociates 14-3-3ζ from Raf-1 in HEK293 cells [23]. Although sphingosine-dependent protein kinase-1 (SDK1) and protein kinase B (PKB/Akt) are known to phosphorylate the Ser58 residue of 14-3-3ζ, its significance is not clearly understood [27,28].

Protein kinase A (PKA) plays a key role in the signaling of many G protein-coupled receptors through the activation of adenylate cyclase and production of cAMP. Phosphorylation of nuclear and cytoplasmic substrates by PKA is critical for multiple cell functions, including metabolism, differentiation, synaptic transmission, ion-channel activity, growth and development [29].

From amino acids sequence analysis of 14-3-3ζ, a potential PKA phosphorylation site (RRXpSX) was identified at Ser58. In the present study, we inquire whether PKA phosphorylates 14-3-3ζ at Ser58 and regulates 14-3-3ζ homo- and hetero-dimerization process. Given that 14-3-3ζ can interact with numerous signaling proteins including p53 tumor suppressor,

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it is of interest to see whether any of the interaction is affected by 14-3-3ζ phosphorylation. To test this, we examined the effect of a phospho-mimic mutation of 14-3-3ζ for the interaction with p53 and the ability to enhance p53 transcriptional activity. In contrast to wild type of 14-3-3ζ, the phospho-mimic form appears lose the affinity for p53 and the ability to increase p53 transcriptional activity. In addition, we examined whether modulation of PKA activity affect the function of 14-3-3ζ using specific chemical modulators of PKA activity. Activation of PKA reduces 14-3-3ζ dimerization and its functional interaction with p53. Inhibition of PKA has opposite effects. In conclusion, our results demonstrate that the PKA phosphorylates and controls the dimerization process of 14-3-3ζ resulting in reduced interaction with and activation of p53.

2. Materials and methods

2.1. Cell culture

All culture media and antibiotics were purchased from Invitrogen. 293 cells were maintained in DMEM supplemented with 10% fetal bovine serum and antibiotics-antimycotics at 37 °C, 5% CO_2.

2.2. Plasmids and antibodies

Myc- or HA-tagged 14-3-3ζ, 14-3-3ε and p53 were constructed in a CMV promoter-derived mammalian expression vector (pCS4+). Antibodies against 14-3-3 (Santa Cruz), Myc (9E10, Santa Cruz), HA (12CA5, Roche), PKA (Santa Cruz) and tubulin (Sigma) were used.

2.3. DNA transfection and reporter assay

Transient transfections were performed using the calcium phosphate and Lipofectamine plus methods. For luciferase assays, 293 cells were plated on 24 well plates one day before transfection. pCMV β-gal plasmid was included as an internal control for transfection efficiency. At 12 h post-transfection, cells were treated with Forskolin or H89 (1000-fold stocks in DMSO) for 24 h when necessary. For luciferase assay, cells were lysed at 36 h post-transfection and the luciferase activity was measured using Luciferase Reporter Assay Kit (Promega).

2.4. Immunoprecipitation and immunoblotting

293 cells were lysed in ice-cold lysis buffer (25 mM HEPES [pH 7.5], 150 mM NaCl, 1% NP-40, 0.25% sodium deoxycholate, 10% glycerol, 25 mM NaF, 1 mM EDTA, 1 mM Na_3VO_4, 250 μM PMSF, 10 μg/ml leupeptin and 10 μg/ml aprotinin) and cleared by centrifugation. Supernatants were subjected to immunoprecipitation using appropriate antibodies and protein A or protein G–Sepharose beads. Bound proteins were resolved by SDS–PAGE and transferred to PVDF membranes. Proteins were visualized using appropriate primary antibodies, horseradish peroxidase-coupled secondary antibodies and chemiluminescence Western blotting reagent (Amersham Bioscience).

2.5. In vitro kinase assay

Phosphorylation of 14-3-3ζ by PKA was examined by incubation of active recombinant PKA with 5 μCi of [γ-^{32}P]ATP and anti-Myc immunoprecipitate in 20 μl of kinase buffer (20 mM Tris–Cl (pH 7.4), 10 mM $MgCl_2$, 50 μM ATP) in the presence or absence of a PKA inhibitor peptide (PKI, 10 μM) for 20 min at 37 °C. Reactions were terminated with Laemmli SDS sample buffer. Half of each reaction was used for evaluation of 14-3-3ζ phosphorylation by autoradiography after SDS–PAGE (8% polyacrylamide) and the other half was used for anti-PKA immunoblotting.

3. Results

3.1. PKA phosphorylates 14-3-3ζ in vitro

To determine if PKA is capable of phosphorylating 14-3-3ζ, we performed an in vitro kinase assay. Active recombinant PKA was incubated with immunoprecipitated Myc-tagged 14-3-3ζ and [γ-^{32}P]ATP in the presence or absence of a PKI. PKA phosphorylated 14-3-3ζ and this phosphorylation was inhibited by PKI (Fig. 1A). A search of 14-3-3ζ amino acid sequence revealed a region (RRS^{58}SW) similar to the consensus PKA phosphorylation motif (RRXpSX). To determine if PKA phosphorylates 14-3-3ζ at Ser58, a point mutant with Ser58 to Alanine substitution, 14-3-3ζ (S58A), was generated. The ability of PKA to phosphorylate Myc-tagged 14-3-3ζ and 14-3-3ζ (S58A) was determined by an in vitro kinase assay. PKA phosphorylated 14-3-3ζ but not 14-3-3ζ (S58A), suggesting that the primary PKA phosphorylation site on 14-3-3ζ is Ser58.

3.2. Phosphorylation of 14-3-3ζ at Ser58 inhibits dimerization

Structural studies of 14-3-3ζ showed that Ser58 is located at the dimer interface. To determine if phosphorylation at Ser58 modulates dimerization, dimerization of a phospho-mimic mutant 14-3-3ζ (S58E) was compared to that of 14-3-3ζ and 14-3-3ζ (S58A) by coimmunoprecipitation. Myc-tagged 14-3-3ζ (S58E) or 14-3-3ζ (S58A) is cotransfected with HA-tagged 14-3-3ζ, 14-3-3ζ (S58E) or 14-3-3ζ (S58A) to 293 cells. The binding of HA-tagged 14-3-3ζ proteins to Myc-tagged 14-3-3ζ proteins is examined by anti-HA immunoblotting of anti-Myc immunoprecipitates. 14-3-3ζ (S58E) did not bind to wild type 14-3-3ζ or 14-3-3ζ (S58E) but weakly bound to 14-3-3ζ (S58A) (Fig. 2A). However, 14-3-3ζ (S58A) bound strongly to 14-3-3ζ (S58A) but very weakly, if at all, with 14-3-3ζ (S58E) (Fig. 2B). These results suggest that wild type 14-3-3ζ and 14-3-3ζ (S58A) form dimers, while 14-3-3ζ (S58E) does not.

To determine the effect of 14-3-3ζ phosphorylation on heterodimerization, interaction of Myc-tagged 14-3-3ζ proteins with HA-tagged 14-3-3ζ or 14-3-3ε was examined by coimmunoprecipitation. 14-3-3ζ (S58E) showed significantly reduced ability to heterodimerize with 14-3-3ε compared to wild type 14-3-3ζ and 14-3-3ζ (S58A), while 14-3-3ζ (S58A) interacted strongly with 14-3-3ζ and 14-3-3ε (Fig. 3). These results suggest that heterodimerization of 14-3-3ζ with 14-3-3ε, as well

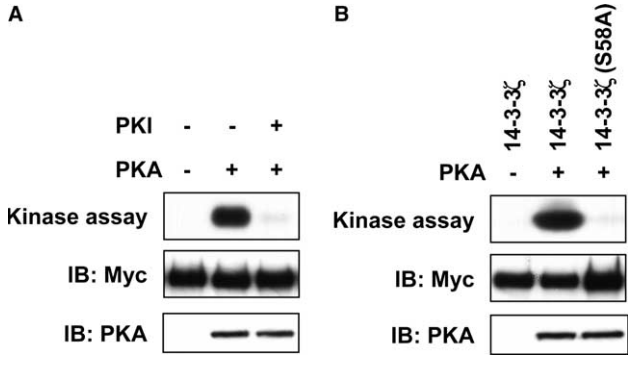

Fig. 1. PKA phosphorylates 14-3-3ζ at Ser58 in vitro. (A) PKA phosphorylates 14-3-3ζ. 293 cells are transfected with Myc-tagged 14-3-3ζ, and anti-Myc immunoprecipitate is incubated with active recombinant PKA and [γ-^{32}P]ATP in the presence or absence of a PKI (10 μM final concentration). Amounts of Myc-tagged 14-3-3ζ and PKA proteins in in vitro kinase reactions are also compared (IB: Myc and IB: PKA). Note that PKA phosphorylates 14-3-3ζ and 14-3-3ζ phosphorylation is inhibited in the presence of PKI. (B) PKA cannot phosphorylate 14-3-3ζ (S58A). 293 cells are transfected with Myc-tagged wild type 14-3-3ζ or 14-3-3ζ (S58A), and anti-Myc immunoprecipitates are incubated with PKA and [γ-^{32}P]ATP.

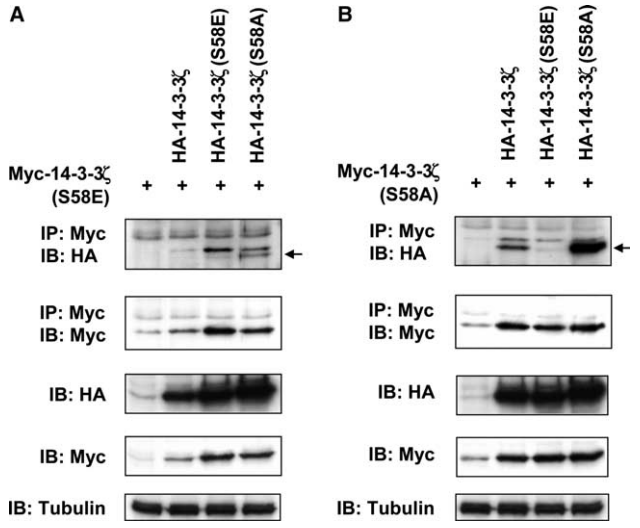

Fig. 2. Phosphorylation of 14-3-3ζ at Ser58 inhibits dimerization. Myc-tagged 14-3-3ζ (S58E) or 14-3-3ζ (S58A) is cotransfected with HA-tagged 14-3-3ζ, 14-3-3ζ (S58E) or 14-3-3ζ (S58A) in 293 cells. Dimerization of Myc-tagged 14-3-3ζ (S58E) or 14-3-3ζ (S58A) with HA-tagged 14-3-3ζ proteins is examined by anti-Myc immunoprecipitation followed by anti-HA immunoblotting (IP: Myc, IB: HA). Arrows indicate the position of HA-tagged 14-3-3ζ proteins. Amounts of Myc-tagged 14-3-3 proteins in anti-Myc immunoprecipitates (IP: Myc, IB: Myc), and amounts of tubulin and epitope-tagged proteins in total lysates are also compared (IB: Tubulin, IB: HA or IB: Myc). (A) A phospho-mimic mutant 14-3-3ζ (S58E) shows reduced homodimerization. (B) A PKA-mediated phosphorylation defective mutant 14-3-3ζ (S58A) shows increased homodimerization.

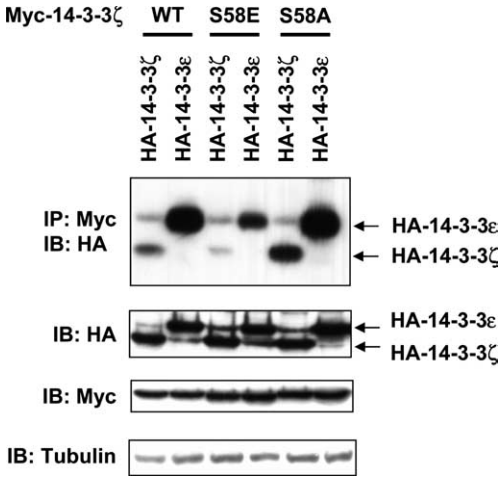

Fig. 3. Phosphorylation of 14-3-3ζ at Ser58 inhibits heterodimerization with 14-3-3ε. Myc-tagged 14-3-3ζ, 14-3-3ζ (S58E) or 14-3-3ζ (S58A) is cotransfected with HA-tagged 14-3-3ζ or 14-3-3ε in 293 cells. Dimerization of Myc-tagged 14-3-3ζ proteins with HA-tagged 14-3-3ζ or 14-3-3ε is examined by anti-Myc immunoprecipitation followed by anti-HA immunoblotting (IP: Myc, IB: HA). Amounts of tubulin and epitope-tagged proteins in total lysates are also compared (IB: Tubulin, IB: HA or IB: Myc).

as homodimerization, can be inhibited by mimicking phosphorylation at Ser58 with Glu substitution.

3.3. PKA inhibits 14-3-3ζ dimerization

We then examined if PKA can regulate dimerization of 14-3-3ζ. Transfected 293 cells were treated with a PKA activator (Forskolin) or a PKA inhibitor (H89), and the effect of PKA activation or inhibition on dimerization of 14-3-3ζ was examined by coimmunoprecipitation. Dimerization of 14-3-3ζ was gradually decreased in the presence of increasing amounts of Forskolin (Fig. 4), whereas the dimerization of 14-3-3ζ was gradually increased in the presence of increasing amounts of H89. These results indicate that PKA inhibits 14-3-3ζ dimerization in vivo.

3.4. Phosphorylation of 14-3-3ζ Ser58 by PKA inhibits 14-3-3ζ interaction with p53

14-3-3 isoforms (σ, ε, τ and ζ) were found to interact with p53 and p53 induces transcription of 14-3-3σ, which in turn positively regulates p53 [30,31]. To investigate the possible role of Ser58 phosphorylation by PKA on the interaction of 14-3-3ζ with p53, interaction of wild type 14-3-3ζ or 14-3-3ζ (S58E) with p53 was examined by coimmunoprecipitation. 14-3-3ζ (S58E) showed significantly decreased interaction with p53 compared to wild type 14-3-3ζ (Fig. 5A). These results suggest that phosphorylation at Ser58 may also regulate 14-3-3ζ interaction with p53.

We then examined if PKA can regulate the interaction of 14-3-3ζ with p53. HA-tagged p53 was cotransfected with wild type or mutant 14-3-3ζ, and transfected cells were treated with Forskolin or H89. The effect of PKA activation or inhibition on the interaction between 14-3-3ζ and p53 was examined. The binding of p53 to wild type 14-3-3ζ or 14-3-3ζ mutants was increased when PKA was inhibited by H89 compared to their binding when PKA was activated by Forskolin or in the absence of any chemical modulator (Fig. 5B). These results suggest that PKA may inhibit the interaction between 14-3-3ζ and p53.

3.5. PKA regulates 14-3-3ζ-enhanced transcriptional activity of p53

14-3-3σ enhances the transcriptional activity of p53. We examined the effect of 14-3-3ζ, 14-3-3ζ (S58E) or 14-3-3ζ (S58A), and the effect of PKA activation or inhibition on the transcriptional activity of p53 using a p53-responsive luciferase reporter, p21 2300-luc. p21 2300-luc contains p53 response elements of *p21* CDKN1A promoter. p21 2300-luc and p53 were

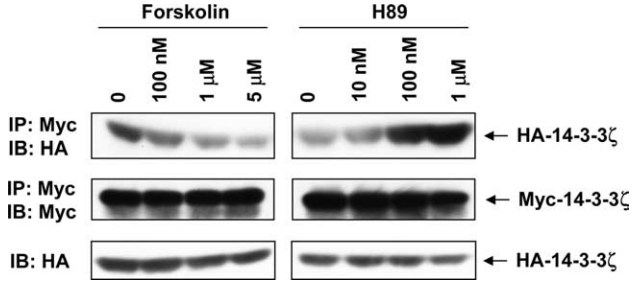

Fig. 4. PKA inhibits 14-3-3ζ dimerization. Myc-tagged 14-3-3ζ and HA-tagged 14-3-3ζ are cotransfected in 293 cells, and transfected cells are treated with increasing amounts of a PKA activator Forskolin (100 nM to 5 μM final concentration) or a PKA inhibitor H89 (10 nM to 1 μM final concentration) or vehicle (DMSO) alone. Dimerization of Myc-tagged 14-3-3ζ with HA-tagged 14-3-3ζ is examined by anti-Myc immunoprecipitation followed by anti-HA immunoblotting (IP: Myc, IB: HA). Amounts of Myc-tagged 14-3-3ζ in anti-Myc immunoprecipitates (IP: Myc, IB: Myc) and HA-tagged 14-3-3ζ in total lysates are also compared (IB: HA).

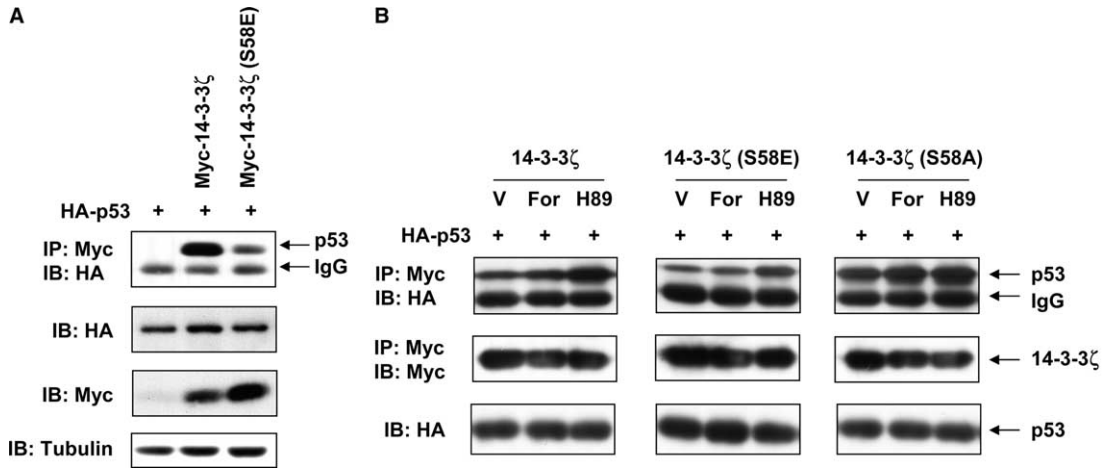

Fig. 5. Phosphorylation of 14-3-3ζ at Ser58 by PKA inhibits 14-3-3ζ interaction with p53. (A) 14-3-3ζ (S58E) show reduced ability to interact with p53. HA-tagged p53 is cotransfected with Myc-tagged 14-3-3ζ or 14-3-3ζ (S58E) in 293 cells. Interaction between 14-3-3ζ proteins and p53 is determined by anti-Myc immunoprecipitation followed by anti-HA immunoblotting (IP: Myc, IB: HA). Amounts of tubulin and epitope-tagged proteins in total lysates are also compared (IB: Tubulin, IB: HA or IB: Myc). (B) PKA inhibits 14-3-3ζ interaction with p53. Myc-tagged 14-3-3ζ and HA-tagged p53 are cotransfected in 293 cells. Transfected cells are treated with a PKA activator Forskolin (For), a PKA inhibitor H89 (H89) or DMSO alone (V). Interaction between 14-3-3ζ proteins and p53 is determined by anti-Myc immunoprecipitation followed by anti-HA immunoblotting (IP: Myc, IB: HA). Amounts of Myc-tagged 14-3-3ζ in anti-Myc immunoprecipitates (IP: Myc, IB: Myc) and HA-tagged p53 in total lysates (IB: HA) are also compared.

cotransfected with 14-3-3ζ, 14-3-3ζ (S58E) or 14-3-3ζ (S58A) in 293 cells. Transfected cells were treated with H89 or DMSO alone, and the transcriptional activity of p53 was determined by luciferase assay. Wild type 14-3-3ζ and 14-3-3ζ (S58A) enhanced the transcriptional activity of p53 while 14-3-3ζ (S58E) did not (Fig. 6). These results suggest that phosphorylation of 14-3-3ζ at Ser58 may control the ability of 14-3-3ζ to enhance the transcriptional activity of p53. The wild type 14-3-3ζ-enhanced p53 transcriptional activity was further increased by inhibiting PKA with H89. These results suggest that PKA inhibits the ability of 14-3-3ζ to enhance the transcriptional activity of p53. Interestingly, H89 also increased p53 transcriptional activity in cells where p53 was cotransfected with 14-3-3ζ (S58E) or 14-3-3ζ (S58A). This increase may occur because inhibition of PKA facilitates dimerization of 14-3-3ζ mutants with endogenous 14-3-3ζ, as the dimerization of 14-3-3ζ (S58E) or 14-3-3ζ (S58A) with wild type 14-3-3ζ was increased by H89, and/or because inhibition of PKA facilitate the dimerization of 14-3-3ζ with 14-3-3ε or other 14-3-3 isoforms, as 14-3-3ζ can dimerize with 14-3-3ε and their interaction is also affected when Ser58 of 14-3-3ζ is mutated.

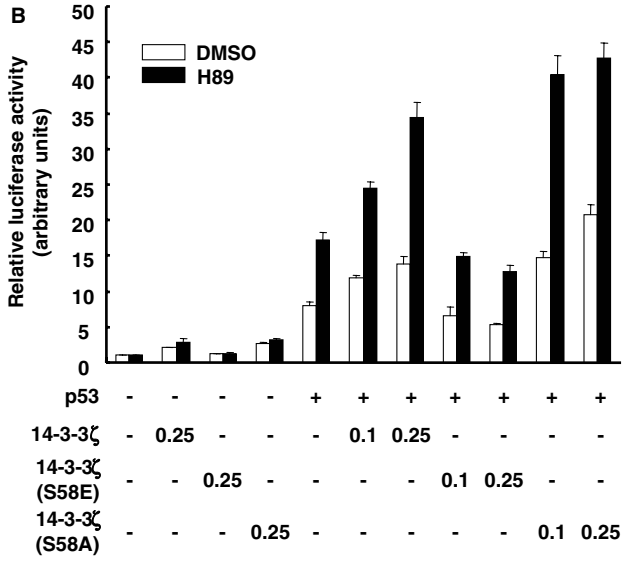

Fig. 6. 14-3-3ζ and inhibition of PKA enhance the transcriptional activity of p53. p21-2300-luc, a p53-responsive luciferase reporter, is cotransfected with indicated combinations of p53, 14-3-3ζ, 14-3-3ζ (S58E) and 14-3-3ζ (S58A) in 293 cells. Transfected cells are treated with a PKA inhibitor (H89, filled bars) or vehicle alone (DMSO, empty bars). Numbers indicate the amount of DNA used for transfection in μg. Relative luciferase activities with S.D. are shown.

4. Discussion

14-3-3 plays essential roles in intracellular signaling by interacting with and regulating the activity of a battery of cell signaling components; including G protein-coupled IL receptors, regulators of G protein signaling proteins, protein kinases (Raf-1, protein kinase C, Akt, PI3K, ASK1, and MEKK1), protein phosphatases (IP5P and PTPH1), and transcription factors (FKHRL1 and GR) [32]. By recognizing and binding to short phosphorylated peptide motifs, 14-3-3ζ controls cell proliferation, apoptosis, vesicle transport, cytoskeletal organization, and gene expression. Intriguingly though, phosphorylation of 14-3-3ζ itself appears to be a critical regulatory mechanism controlling the function of 14-3-3ζ; Aitken et al. reported that phosphorylation at Ser184 of 14-3-3ζ increases interaction with PKC and inhibits PKC activity in vitro [24]. Casein kinase I phosphorylates Thr232, interfering 14-3-3ζ interaction with Raf-1 in HEK293 cells [23]. SDK1 and PKB/Akt phosphorylates Ser58, but the effect of this phosphorylation on 14-3-3ζ function is not clear [27,28]. Here, we analyzed whether PKA phosphorylates 14-3-3ζ and regulates the function of 14-3-3ζ.

The present study demonstrates that PKA phosphorylates 14-3-3ζ at Ser58. Substitution of Ser58 to Ala completely abol-

ishes PKA-mediated phosphorylation of 14-3-3ζ, suggesting that Ser58 is the primary phosphorylation site of 14-3-3ζ by PKA. Ser58 is located at the 14-3-3 dimer interface [11], and this implies that phosphorylation may affect 14-3-3 dimerization [8]. We demonstrate that a phospho-mimic mutant 14-3-3ζ (S58E) loses its ability for homo- and hetero-dimerization compared to wild type. Conversely, a PKA-mediated phosphorylation defective mutant 14-3-3ζ (S58A) shows increased ability for homo- and heterodimerization compared to wild type. In addition, we demonstrate that activation of PKA reduces 14-3-3ζ dimerization while inhibition of PKA increases 14-3-3ζ dimerization.

We also demonstrate that PKA-mediated phosphorylation of 14-3-3ζ at Ser58 inhibits the functional interaction of 14-3-3ζ with p53. 14-3-3ζ (S58E) mutant shows significantly reduced ability to bind p53, and inhibition of PKA increases 14-3-3ζ binding to p53. 14-3-3ζ and 14-3-3ζ (S58A) enhance the transcriptional activity of p53 whereas 14-3-3ζ (S58E) cannot. In addition, inhibition of PKA further increases 14-3-3ζ-enhanced p53 transcriptional activity. Taken together, our results suggest that the phosphorylation of 14-3-3ζ at Ser58 by PKA can regulate 14-3-3ζ dimerization and its interaction with binding partners.

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References

[1] Fu, H., Subramanian, R.R. and Masters, S.C. (2000) 14-3-3 proteins: structure, function, and regulation. Annu. Rev. Pharmacol. Toxicol. 40, 617–647.
[2] Tzivion, G., Shen, Y.H. and Zhu, J. (2001) 14-3-3 proteins; bringing new definitions to scaffolding. Oncogene 20, 6331–6338.
[3] Wang, W. and Shakes, D.C. (1996) Molecular evolution of the 14-3-3 protein family. J. Mol. Evol. 43, 384–398.
[4] Rosenquist, M., Sehnke, P., Ferl, R.J., Sommarin, M. and Larsson, C. (2000) Evolution of the 14-3-3 protein family: does the large number of isoforms in multicellular organisms reflect functional specificity?. J. Mol. Evol. 51, 446–458.
[5] Rittinger, K., Budman, J., Xu, J., Volinia, S., Cantley, L.C., Smerdon, S.J., Gamblin, S.J. and Yaffe, M.B. (1999) Structural analysis of 14-3-3 phosphopeptide complexes identifies a dual role for the nuclear export signal of 14-3-3 in ligand binding. Mol. Cell 4, 153–166.
[6] Yaffe, M.B. and Elia, A.E. (2001) Phosphoserine/threonine-binding domains. Curr. Opin. Cell Biol. 13, 131–138.
[7] Aitken, A., Baxter, H., Dubois, T., Clokie, S., Mackie, S., Mitchell, K., Peden, A. and Zemlickova, E. (2002) Specificity of 14-3-3 isoform dimer interactions and phosphorylation. Biochem. Soc. Trans. 30, 351–360.
[8] Liu, D., Bienkowska, J., Petosa, C., Collier, R.J., Fu, H. and Liddington, R. (1995) Crystal structure of the zeta isoform of the 14-3-3 protein. Nature 376, 191–194.
[9] Xiao, B., Smerdon, S.J., Jones, D.H., Dodson, G.G., Soneji, Y., Aitken, A. and Gamblin, S.J. (1995) Structure of a 14-3-3 protein and implications for coordination of multiple signalling pathways. Nature 376, 188–191.
[10] Luo, Z.J., Zhang, X.F., Rapp, U. and Avruch, J. (1995) Identification of the 14.3.3 zeta domains important for self-association and Raf binding. J. Biol. Chem. 270, 23681–23687.
[11] Tzivion, G., Luo, Z. and Avruch, J. (1998) A dimeric 14-3-3 protein is an essential cofactor for Raf kinase activity. Nature 394, 88–92.
[12] Wang, H., Zhang, L., Liddington, R. and Fu, H. (1998) Mutations in the hydrophobic surface of an amphipathic groove of 14-3-3zeta disrupt its interaction with Raf-1 kinase. J. Biol. Chem. 273, 16297–16304.
[13] Wilker, E. and Yaffe, M.B. (2004) 14-3-3 Proteins – a focus on cancer and human disease. J. Mol. Cell Cardiol. 37, 633–642.
[14] Yaffe, M.B. (2002) How do 14-3-3 proteins work? – gatekeeper phosphorylation and the molecular anvil hypothesis. FEBS Lett. 513, 53–57.
[15] Roy, S., McPherson, R.A., Apolloni, A., Yan, J., Lane, A., Clyde-Smith, J. and Hancock, J.F. (1998) 14-3-3 facilitates Ras-dependent Raf-1 activation in vitro and in vivo. Mol. Cell Biol. 18, 3947–3955.
[16] Thorson, J.A., Yu, L.W., Hsu, A.L., Shih, N.Y., Graves, P.R., Tanner, J.W., Allen, P.M., Piwnica-Worms, H. and Shaw, A.S. (1998) 14-3-3 proteins are required for maintenance of Raf-1 phosphorylation and kinase activity. Mol. Cell Biol. 18, 5229–5238.
[17] Conklin, D.S., Galaktionov, K. and Beach, D. (1995) 14-3-3 proteins associate with cdc25 phosphatases. Proc. Natl. Acad. Sci. USA 92, 7892–7896.
[18] Uchida, S., Kuma, A., Ohtsubo, M., Shimura, M., Hirata, M., Nakagama, H., Matsunaga, T., Ishizaka, Y. and Yamashita, K. (2004) Binding of 14-3-3beta but not 14-3-3sigma controls the cytoplasmic localization of CDC25B: binding site preferences of 14-3-3 subtypes and the subcellular localization of CDC25B. J. Cell Sci. 117, 3011–3020.
[19] Zha, J., Harada, H., Yang, E., Jockel, J. and Korsmeyer, S.J. (1996) Cell 87, 619–628.
[20] Brunet, A., Kanai, F., Stehn, J., Xu, J., Sarbassova, D., Frangioni, J.V., Dalal, S.N., DeCaprio, J.A., Greenberg, M.E. and Yaffe, M.B. (2002) 14-3-3 transits to the nucleus and participates in dynamic nucleocytoplasmic transport. J. Cell Biol. 156, 817–828.
[21] Obsil, T., Ghirlando, R., Anderson, D.E., Hickman, A.B. and Dyda, F. (2003) Two 14-3-3 binding motifs are required for stable association of Forkhead transcription factor FOXO4 with 14-3-3 proteins and inhibition of DNA binding. Biochemistry 42, 15264–15272.
[22] Dubois, T., Howell, S., Amess, B., Kerai, P., Learmonth, M., Madrazo, J., Chaudhri, M., Rittinger, K., Scarabel, M., Soneji, Y. and Aitken, A. (1997) Structure and sites of phosphorylation of 14-3-3 protein: role in coordinating signal transduction pathways. J. Prot. Chem. 16, 513–522.
[23] Dubois, T., Rommel, C., Howell, S., Steinhussen, U., Soneji, Y., Morrice, N., Moelling, K. and Aitken, A. (1997) 14-3-3 is phosphorylated by casein kinase I on residue 233. Phosphorylation at this site in vivo regulates Raf/14-3-3 interaction. J. Biol. Chem. 272, 28882–28888.
[24] Aitken, A., Howell, S., Jones, D., Madrazo, J., Martin, H., Patel, Y. and Robinson, K. (1995) Posttranslationally modified 14-3-3 isoforms and inhibition of protein kinase C. Mol. Cell Biochem. 149–150, 41–49.
[25] Tsuruta, F., Sunayama, J., Mori, Y., Hattori, S., Shimizu, S., Tsujimoto, Y., Yoshioka, K., Masuyama, N. and Gotoh, Y. (2004) JNK promotes Bax translocation to mitochondria through phosphorylation of 14-3-3 proteins. EMBO J. 23, 1889–1899.
[26] Yoshida, K., Yamaguchi, T., Natsume, T., Kufe, D. and Miki, Y. (2005) JNK phosphorylation of 14-3-3 proteins regulates nuclear targeting of c-Abl in the apoptotic response to DNA damage. Nat. Cell Biol. 7, 278–285.
[27] Megidish, T., Cooper, J., Zhang, L., Fu, H. and Hakomori, S. (1998) A novel sphingosine-dependent protein kinase (SDK1) specifically phosphorylates certain isoforms of 14-3-3 protein. J. Biol. Chem. 273, 21834–21845.
[28] Powell, D.W., Rane, M.J., Chen, Q., Singh, S. and McLeish, K.R. (2002) Identification of 14-3-3zeta as a protein kinase B/Akt substrate. J. Biol. Chem. 277, 21639–21642.
[29] Skalhegg, B.S. and Tasken, K. (2000) Specificity in the cAMP/PKA signaling pathway. Differential expression, regulation, and subcellular localization of subunits of PKA. Front. Biosci. 5, D678–D693.
[30] Waterman, M.J., Stavridi, E.S., Waterman, J.L. and Halazonetis, T.D. (1998) ATM-dependent activation of p53 involves dephosphorylation and association with 14-3-3 proteins. Nat. Genet. 19, 175–178.

[31] Yang, H.Y., Wen, Y.Y., Chen, C.H., Lozano, G. and Lee, M.H. (2003) 14-3-3 sigma positively regulates p53 and suppresses tumor growth. Mol. Cell Biol. 23, 7096–7107.

[32] van Hemert, M.J., Steensma, H.Y. and van Heusden, G.P. (2001) 14-3-3 proteins: key regulators of cell division, signalling and apoptosis. Bioessays 23, 936–946.