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Role of Alpha-actinin-4 in the Regulation of Endocytosis

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Abstract Alpha-actinin-4, originally identified as an actin-binding protein, has been associated with cell motility and cancer invasion. However, it forms complexes with a diverse array of partner proteins and is speculated to exert several distinct functions based on the characteristics of these proteins. Immunoprecipitation and mass spectrometric analysis reveal that alpha-actinin-4 forms native complexes with several partner proteins in 22RV1 cells, including β/γ-actin, clathrin heavy chain and nonmuscular myosin heavy chain. Clathrin is a coat protein that covers the internalized membrane pit and plays a central role in early endocytosis. We identified other clathrin-related and unrelated cargo proteins, including dynamin, adaptin-γ, β-NAP and p47A, that interact with alpha-actinin-4. Immunofluorescence microscopy revealed that dynamin and clathrin are co-localized with alpha-actinin-4 at the dorsal sites of membrane rufflings, and the transfection of *ACTN4* cDNA facilitates the transport of transferrin into the peri-nuclear endosomes. In this review, we report that alpha-actinin-4 is involved in the regulation of endocytosis. Endocytosis enforces termination of signalling evoked by the cell surface receptors and regulates recycling of receptors and ligands. The change of alpha-actinin-4 expression levels may therefore induce aberrations in the intracellular trafficking of various cell surface molecules, such as growth factors and receptors.

Key words: actinin-4, endocytosis, clathrin, proteomics

Introduction

Alpha-actinin-4, originally identified as an actin-binding protein, has been associated with cell motility and cancer invasion.[1-4)] However, it forms complexes with a diverse array of partner proteins and is speculated to exert several distinct functions based on the characteristics of these proteins. In this review article, we report that alpha-actinin-4 acts as a regulator of endocytosis.

Partner proteins of alpha-actinin-4

Alpha-actinin-4 interacts with a diverse array of partner proteins (Table 1). Immunoprecipitation of native alpha-actinin-4-containing complex using anti-alpha-actinin-4 antibody identified proteins that interact with alpha-actinin-4.[5)] This is known to prevent the identification of artificial interactions, such as those commonly observed in transfection experiments.[2)6)] Immunoprecipitation of the human prostate cancer 22RV1 cell lysate with anti-alpha-actinin-4 antibody revealed that several proteins selectively co-immunoprecipitated with alpha-actinin-4. The proteins identified by mass spectrometry and a database search[7-10)] and confirmed by Western blot analysis[11)12)] revealed that myosin heavy chain nonmuscle form A (gi:625305),

Table 1 Partner proteins of alpha-actinin-4

Partner	Function
Actin (ACTB)[1]	Scaffolding protein
Alpha chain of type XVII collagen/BP180 (COL17A1)[24,25]	Alpha chain of type XVII collagen.
Beta-catenin (CTNNB1)[6]	Adherens junction protein
PDZ-LIM protein Elfin/CLP-36 (PDLIM1)[26]	Actin stress fiber formation and focal adhesion
Membrane associated guanylate kinase (MAGI-1)[27]	Scaffolding protein at cell-cell junctions
Densin-180 (LRRC7)[28,29]	Transmembrane synaptic adhesion molecule
Zonula occludens-1 (TJP1)[30]	Tight junction protein 1
Androgen receptor (NR3C1)[18]	Transcription factor Regulator of other transcription factors
Intercellular adhesion molecule 1 (ICAM1)[31]	Rhinovirus receptor
Aquaporin-2 (AQP2)[32]	Water channel protein located
Na(+)/H(+) exchanger (NHE)3 kinase A regulatory protein (E3KARP)[33,34]	Sodium/hydrogen exchanger
CART complex (hrs/Actn4/brain expressed ring finger protein (BERP)/myosin V)[16,35]	Organelle transport Neurite outgrowth
Junctional Rab13-binding protein (JRAB)/ molecule interacting with CasL-like 2 (MICALL2)[17]	Endocytic recycling
Endothelial nitric oxide synthase (NOS3)[36]	Nitric oxide synthase
Inducible nitric oxide synthase (NOS2A)[37]	Nitric oxide synthase
DNaseY (DNASE1L3)[38]	DNase family
p65/RelA subunit of NF-kappaB (RELA)[39,40]	NFκB pathways
PDZ and LIM domain 2 (PDLIM2)[41]	NFκB pathways
Histone deacetylase 7 (HDAC7)[42]	Transcriptional regulation Cell cycle progression
HLA-B associated transcript 1 (BAT-1)[43]	Splicing factor
Rac serine/threonine protein kinase (AKT1)[44]	Mediator of growth factor-induced neuronal survival
Glucose transporter 4 (GLUT4)[45]	Insulin-regulated facilitative glucose transporter
OK/SW-CL.16 protein (OK/SW-CL.16)[46]	Signaling proteins in podocytes
RN-tre (USP6NL)[47]	Rab5 GTPase-activating protein
Plasminogen activator inhibitor type 1 (SERPINE1)[48]	Plasminogen activator inhibitor
Nuclear transcription factor Y (NF-Y)[49]	CCAAT-binding factor

clathrin heavy chain 1 (gi:4758012), tubulin-α2/β-1 (gi:135448 and gi:34740335) and cytoskeletal actin-γ1 (gi: 4501887) co-immunoprecipitate with alpha-actinin-4.

Clathrin and endocytosis

Endocytosis is characterized by internalization of molecules from the cell surface into internal membrane compartments. Clathrin is a coat protein that covers the internalized membrane pit and plays a central role in early endocytosis.[13] The internalization of various cargo proteins and lipids from the mammalian cell surface membrane occurs through the clathrin and lipid-raft endocytic pathways, which are closely related with exocytosis, phagocytosis and pinocytosis. Clathrin triskelia assembles into a polygonal lattice at the plasma membrane to form coated pits that bud and pinch off from the

membrane in a dynamin-dependent manner and give rise to clathrin-coated vesicles in clathrin dependent endocytosis. Moreover, clathrin-binding adaptors bind to clathrin directly to initiate this process and bind to cargo proteins, thus mediating their endocytosis. Clathrin-coated vesicles are uncoated following endocytosis and fuse with the early endosome. The early endosome is therefore a key control point for sorting receptors, which can be directed to recycling endosomes back to the cell surface, or directed to the intralumenal vesicle of multivesicular endosomes, including late endosome and lysosome for degradation.

Association of endocytosis-related proteins and alpha-actinin-4

Analysis of the immunoprecipitates identified with anti-alpha-actinin-4 antibody and western blotting using antibodies against other endocytosis-related proteins revealed that Adaptin-δ, β-NAP, p47A and dynamin were co-immunoprecipitated with anti-alpha-actinin-4 antibody. Confocal immunofluorescence microscopy revealed that the clathrin heavy chain and alpha-actinin-4 proteins partially co-localize at the dorsal sites of membrane ruffling, and that dynamin and alpha-actinin-4 co-localize at the membrane ruffling and protrusions. Dynamin assembles around the necks of clathrin-coated pits and assists in pinching of the vesicles from the plasma membrane.[13] Adaptin-δ, β-NAP and p47A were identified as the main components of the adaptor-related protein complex 3,[14] which appears to function independently of clathrin.[15]

Regulation of alpha-actinin-4 for endocytosis

Alpha-actinin-4 interacts with various endocytosis-related proteins.[5] We confirmed the role of alpha-actinin-4 regulation in endocytosis. The expression of HA-ACTN4 in HeLa cells induced the transport of fluorescence-labelled transferrin to peri-nuclear endosomes, confirming the functional involvement of alpha-actinin-4 in endocytosis. Similarly the alpha-actinin-4-Hrs-BERP-myosin V complex was reported to be associated with transferrin receptor recycling.[16] Alpha-actinin-4 is known to associate with JRAB/MICAL-L2, which mediates the endocytic recycling of occludin.[17] Alpha-actinin-4 is also known to interact closely with the androgen receptor, clathrin heavy chain and serine-threonine protein kinase C delta.[18] Endocytosis-regulating GTPase proteins Rab5 has been observed to interact with alpha-actinin-4.[19] *ACTN4* was identified as one of genes up-regulated by Rac1 and Cdc42 by cDNA microarray analysis.[20] Cdc42 is necessary for actin polymerization during compensatory endocytosis.[21] Moreover, alpha-actinin-4 was preferentially concentrated into circular ruffling and macropinocytosis of mouse macrophages.[22] Furthermore, alpha-actinin-4 expression at the apical surface of the intestinal tract epithelium suggests its involvement in the pinocytic absorption of nutrition.[23]

Conclusions

Alpha-actinin-4 interacts with a diverse array of partner proteins, suggesting a multitude of functions based on the specific interactions. We have demonstrated that alpha-actinin-4 interacts with various endocytosis-related proteins.[5] Changes in alpha-actinin-4 expression levels may induce aberrations in intracellular trafficking of various cell surface molecules, such as growth factors and receptors, thereby affecting cell signalling processes.

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References

1) Honda, K., Yamada, T., Endo, R., et al.: Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. *J. Cell Biol.*, **140**: 1383-1393, 1998.
2) Honda, K., Yamada, T., Seike, M., et al.:

Alternative splice variant of actinin-4 in small cell lung cancer. *Oncogene*, **23**：5257-5262, 2004.
3) Honda, K., Yamada, T., Hayashida, Y., et al.: Actinin-4 increases cell motility and promotes lymph node metastasis of colorectal cancer. *Gastroenterology*, **128**：51-62, 2005.
4) Honda, K. and Yamada, T.: Biological role of actinin-4 for cancer invasion and metastasis (in japanese). *Seikagaku*, **79**：643-654, 2007.
5) Hara, T., Honda, K., Shitashige, M., et al.: Mass Spectrometry Analysis of the Native Protein Complex Containing Actinin-4 in Prostate Cancer Cells. *Mol. Cell. Proteomics*, **6**：479-491, 2007.
6) Hayashida, Y., Honda, K., Idogawa, M., et al.: E-cadherin regulates the association between beta-catenin and actinin-4. *Cancer Res.*, **65**：8836-8845, 2005.
7) Rosenfeld, J., Capdevielle, J., Guillemot, J.C., et al.: In-gel digestion of proteins for internal sequence analysis after one- or two-dimensional gel electrophoresis. *Anal. Biochem.*, **203**：173-179, 1992.
8) Seike, M., Kondo, T., Mori, Y., et al.: Proteomic analysis of intestinal epithelial cells expressing stabilized beta-catenin. *Cancer Res.*, **63**：4641-4647, 2003.
9) Idogawa, M., Yamada, T., Honda, K., et al.: Poly (ADP-ribose) polymerase-1 is a component of the oncogenic T-cell factor-4/beta-catenin complex. *Gastroenterology*, **128**：1919-1936, 2005.
10) Shitashige, M., Satow, R., Honda, K., et al.: Regulation of Wnt Signaling by the Nuclear Pore Complex. *Gastroenterology*, **134**：1961-1971, 2008.
11) Yamada, T., Mori, Y., Hayashi, R., et al.: Suppression of intestinal polyposis in Mdr1-deficient ApcMin/+ mice. *Cancer Res.*, **63**：895-901, 2003.
12) Sato, S., Idogawa, M., Honda, K., et al.: Beta-catenin interacts with the FUS proto-oncogene product and regulates pre-mRNA splicing. *Gastroenterology*, **129**：1225-1236, 2005.
13) Le Roy, C. and Wrana, J.L.: Clathrin- and non-clathrin-mediated endocytic regulation of cell signalling. *Nat. Rev. Mol. Cell Biol.*, **6**：112-126, 2005.
14) Simpson, F., Peden, A.A., Christopoulou, L., et al.: Characterization of the adaptor-related protein complex, AP-3. *J. Cell. Biol.*, **137**：835-845, 1997.
15) Robinson, M.S.: Adaptable adaptors for coated vesicles. *Trends Cell Biol.*, **14**：167-174, 2004.
16) Yan, Q., Sun, W., Kujala, P., et al.: CART: An Hrs/actinin-4/BERP/myosin V protein complex required for efficient receptor recycling. *Mol. Biol. Cell*, **16**：2470-2482, 2005.
17) Nakatsuji, H., Nishimura, N., Yamamura, R., et al.: Involvement of actinin-4 in the recruitment of JRAB/MICAL-L2 to cell-cell junctions and the formation of functional tight junctions. *Mol. Cell. Biol.*, **28**：3324-3335, 2008.
18) Jasavala, R., Martinez, H., Thumar, J., et al.: Identification of putative androgen receptor interaction protein modules: cytoskeleton and endosomes modulate androgen receptor signaling in prostate cancer cells. *Mol. Cell. Proteomics*, **6**：252-271, 2007.
19) Bucci, C., Parton, R.G., Mather, I.H., et al.: The small GTPase rab5 functions as a regulatory factor in the early endocytic pathway. *Cell*, **70**：715-728, 1992.
20) Teramoto, H., Malek, R.L., Behbahani, B., et al.: Identification of H-Ras, RhoA, Rac1 and Cdc42 responsive genes. *Oncogene*, **22**：2689-2697, 2003.
21) Sokac, A. M., Co, C., Taunton, J., et al.: Cdc42-dependent actin polymerization during compensatory endocytosis in Xenopus eggs. *Nat. Cell Biol.*, **5**：727-732, 2003.
22) Araki, N., Hatae, T., Yamada, T., et al.: Actinin-4 is preferentially involved in circular ruffling and macropinocytosis in mouse macrophages: analysis by fluorescence ratio imaging. *J. Cell Sci.*, **113** (Pt 18)：3329-3340, 2000.
23) Sugimoto, K., Ichikawa, Y. and Nakamura, I.: Absorption of protein molecules by the small intestine of the bullfrog tadpole, Rana catesbeiana. *J. Exp. Zool.*, **215**：53-62, 1981.
24) Gonzalez, A.M., Otey, C., Edlund, M., et al.: Interactions of a hemidesmosome component and actinin family members.

J. Cell Sci., **114** : 4197-4206, 2001.
25) Li, Z.M., Wen, Y.J., Lai, S.T., et al.: Searching for genes interacting with human PCIA1 gene by using the bacterial two-hybrid system. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi*, **24** : 279-283, 2007.
26) Vallenius, T., Luukko, K. and Makela, T.P.: CLP-36 PDZ-LIM protein associates with nonmuscle alpha-actinin-1 and alpha-actinin-4. *J. Biol. Chem.*, **275** : 11100-11105, 2000.
27) Patrie, K.M., Drescher, A.J., Welihinda, A., et al.: Interaction of two actin-binding proteins, synaptopodin and alpha-actinin-4, with the tight junction protein MAGI-1. *J. Biol. Chem.*, **277** : 30183-30190, 2002.
28) Ahola, H., Heikkila, E., Astrom, E., et al.: A novel protein, densin, expressed by glomerular podocytes. *J. Am. Soc. Nephrol.*, **14** : 1731-1737, 2003.
29) Walikonis, R.S., Oguni, A., Khorosheva, E.M., et al.: Densin-180 forms a ternary complex with the (alpha)-subunit of Ca2+/calmodulin-dependent protein kinase II and (alpha)-actinin. *J. Neurosci.*, **21** : 423-433, 2001.
30) Chen, V.C., Li, X., Perreault, H., et al.: Interaction of zonula occludens-1 (ZO-1) with alpha-actinin-4: application of functional proteomics for identification of PDZ domain-associated proteins. *J. Proteome Res.*, **5** : 2123-2134, 2006.
31) Celli, L., Ryckewaert, J.J., Delachanal, E., et al.: Evidence of a functional role for interaction between ICAM-1 and nonmuscle alpha-actinins in leukocyte diapedesis. *J. Immunol.*, **177** : 4113-4121, 2006.
32) Noda, Y., Horikawa, S., Katayama, Y., et al.: Identification of a multiprotein "motor" complex binding to water channel aquaporin-2. *Biochem. Biophys. Res. Commun.*, **330** : 1041-1047, 2005.
33) Kim, J.H., Lee-Kwon, W., Park, J.B., et al.: Ca(2+)-dependent inhibition of Na+/H+ exchanger 3 (NHE3) requires an NHE3-E3KARP-alpha-actinin-4 complex for oligomerization and endocytosis. *J. Biol. Chem.*, **277** : 23714-23724, 2002.
34) Li, X., Zhang, H., Cheong, A., et al.: Carbachol regulation of rabbit ileal brush border Na+-H+ exchanger 3 (NHE3) occurs through changes in NHE3 trafficking and complex formation and is Src dependent. *J. Physiol.*, **556** : 791-804, 2004.
35) El-Husseini, A.E., Kwasnicka, D., Yamada, T., et al.: BERP, a novel ring finger protein, binds to alpha-actinin-4. *Biochem. Biophys. Res. Commun.*, **267** : 906-911, 2000.
36) Hiroi, Y., Guo, Z., Li, Y., et al.: Dynamic regulation of endothelial NOS mediated by competitive interaction with alpha-actinin-4 and calmodulin. *FASEB J.*, **22** : 1450-1457, 2008.
37) Daniliuc, S., Bitterman, H., Rahat, M.A., et al.: Hypoxia inactivates inducible nitric oxide synthase in mouse macrophages by disrupting its interaction with alpha-actinin-4. *J. Immunol.*, **171** : 3225-3232, 2003.
38) Liu, Q.Y., Lei, J.X., LeBlanc, J., et al.: Regulation of DNaseY activity by actinin-alpha4 during apoptosis. *Cell Death Differ.*, **11** : 645-654, 2004.
39) Babakov, V.N., Bobkov, D.E., Petukhova, O.A., et al.: Alpha-actinin-4 and p65/RelA subunit of NF-kappaB transcription factor are co-localized and migrate together into the nucleus in EGF-stimulated A431 cell. *Tsitologiia*, **46** : 1064-1072, 2004.
40) Babakov, V.N., Petukhova, O.A., Turoverova, L.V., et al.: RelA/NF-kappaB transcription factor associates with alpha-actinin-4. *Exp. Cell Res.*, **314** : 1030-1038, 2008.
41) Torrado, M., Senatorov, V.V., Trivedi, R., et al.: Pdlim2, a novel PDZ-LIM domain protein, interacts with alpha-actinins and filamin A. *Invest. Ophthalmol. Vis. Sci.*, **45** : 3955-3963, 2004.
42) Chakraborty, S., Reineke, E.L., Lam, M., et al.: Alpha-actinin-4 potentiates myocyte enhancer factor-2 transcription activity by antagonizing histone deacetylase 7. *J. Biol. Chem.*, **281** : 35070-35080, 2006.
43) Goffart, S., Franko, A., Clemen, C.S., et al.: Alpha-actinin-4 and BAT1 interaction with the Cytochrome c promoter upon skeletal muscle differentiation. *Curr. Genet.*, **49** : 125-135, 2006.
44) Ding, Z., Liang, J., Lu, Y., et al.: A

retrovirus-based protein complementation assay screen reveals functional AKT1-binding partners. *Proc. Natl. Acad. Sci. U. S. A.*, **103**：15014-15019, 2006.

45) Foster, L.J., Rudich, A., Talior, I., et al.: Insulin-dependent interactions of proteins with GLUT4 revealed through stable isotope labeling by amino acids in cell culture (SILAC). *J. Proteome Res.*, **5**：64-75, 2006.

46) Togashi, M., Okuyama, S., Wakui, H., et al.: Interaction of alpha-actinin-4 with class I PxxP motif-containing OK/SW-CL.16 protein. *Nephron Exp. Nephrol.*, **107**：e65-e72, 2007.

47) Lanzetti, L., Palamidessi, A., Areces, L., et al.: Rab5 is a signalling GTPase involved in actin remodelling by receptor tyrosine kinases. *Nature*, **429**：309-314, 2004.

48) Magdolen, U., Schroeck, F., Creutzburg, S., et al.: Non-muscle alpha-actinin-4 interacts with plasminogen activator inhibitor type-1 (PAI-1). *Biol. Chem.*, **385**：801-808, 2004.

49) Poch, M.T., Al-Kassim, L., Smolinski, S.M., et al.: Two distinct classes of CCAAT box elements that bind nuclear factor-Y/alpha-actinin-4: potential role in human CYP1A1 regulation. *Toxicol. Appl. Pharmacol.*, **199**：239-250, 2004.