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Coactosin accelerates cell dynamism by promoting actin polymerization

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ABSTRACT

During development, cells dynamically move or extend their processes, which are achieved by actin dynamics. In the present study, we paid attention to Coactosin, an actin binding protein, and studied its role in actin dynamics. Coactosin was associated with actin and Capping protein in neural crest cells and N1E-115 neuroblastoma cells. Accumulation of Coactosin to cellular processes and its association with actin filaments prompted us to reveal the effect of Coactosin on cell migration. Coactosin overexpression induced cellular processes in cultured neural crest cells. In contrast, knock-down of Coactosin resulted in disruption of actin polymerization and of neural crest cell migration. Importantly, Coactosin was recruited to lamellipodia and filopodia in response to Rac signaling, and mutated Coactosin that cannot bind to F-actin did not react to Rac signaling, nor support neural crest cell migration. It was also shown that deprivation of Rac signaling from neural crest cells by dominant negative Rac1 (DN-Rac1) interfered with neural crest cell migration, and that co-transfection of DN-Rac1 and Coactosin restored neural crest cell migration. From these results we have concluded that Coactosin functions downstream of Rac signaling and that it is involved in neurite extension and neural crest cell migration by actively participating in actin polymerization.

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Introduction

Particular types of cells such as neural crest cells migrate long way from their origin to differentiate into many kinds of cell types (Le Douarin, 2008). Extension of the leading processes of the migrating cells relies on actin polymerization/depolymerization for its dynamism. ADP-actin is lost from the pointed edge of filamentous actin (F-actin), and ATP-actin is added to the barbed end of F-actin at the front of the leading process (Gungabissoon and Bamburg, 2003). This actin treadmilling allows the leading process to extend so that it can drive cell movement. Similar mechanism is also served as developing neurons, which extend axons and dendrites to make neuronal circuits.

For actin treadmilling, actin depolymerizing factor homology (ADF-H) family plays a central role (Lappalainen et al., 1998; Yang et al., 1998). Among ADF-H family proteins, Cofilin accelerates treadmilling of actin filaments by removing actin monomer from the pointed end (Yang et al., 1998; Bamburg et al., 1999). Twinfilin forms a 1:1 complex with ADP-actin monomers to inhibit nucleotide exchange on actin monomers, further prevents assembly of the monomer into filaments (Palmgren et al., 2002). Conversely, Drebrin binds only to F-actin but not to G-actin (Lappalainen et al., 1998). In the developing axon, Drebrin is localized in filopodia of growth cones (Sasaki et al., 1996), and attenuates actin dynamics by inhibiting activity of the binding of Cofilin to F-actin. Coactosin is also a member of ADF-H family, which binds to F-actin (Goroncy et al., 2009). It was suggested that Coactosin inhibits actin depolymerization by counteracting activity of Capping proteins, which cap the barbed end to prevent actin polymerization (Röhrig et al., 1995). Recently, we have cloned chick Coactosin, and reported that Coactosin is expressed in cells that migrate or extend cellular processes, such as neural crest cells and young neurons in chick embryos (Hou et al., 2009).

We raised a question if Coactosin plays an active role in morphogenesis by accelerating actin dynamics. In order to reveal the role of Coactosin, we focused on neural crest cells, which arise at the dorsal part of the neural tube and migrate long way to differentiate into pigment cells, neurons and supporting cells of the dorsal root ganglion, sympathetic and parasympathetic cells etc. (Le Douarin, 2008). Function of Coactosin in young neurons was also analyzed with N1E-115 neroblastoma cells, which differentiates into neurons in serum deprived medium.

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Here, we show that Coactosin binds with actin and Capping protein. Knock-down of Coactosin resulted in disruption of actin polymerization, and of neural crest cell migration, which suggests that Coactosin is actively involved in cellular dynamism by promoting actin polymerization. We further show that Coactosin functions downstream of Rac signaling to mediate lamellipodia and filopodia formation. Mutated Coactosin that cannot bind to F-actin could not mediate Rac signaling to make lamellipodia, nor support neural crest cell migration. These results suggest that Coactosin is actively involved in actin polymerization, and that plays a crucial role in actin dynamism downstream of the Rac signaling.

Materials and methods

Chick embryos and fixation

Fertile chicken eggs from a local supplier (Yamagishi, Japan) were incubated at 38 °C in a humid atmosphere until embryos reach desired stages.

In situ hybridization and immunohistochemistry

Embryos and cultured cells for histological processes were fixed in 4% paraformaldehyde in PBS at 4 °C for overnight. For visualization of Rac1 by immunohistochemistry, explants were fixed in 10% trichloroacetic acid as previously described by (Yonemura et al., 2004).

Whole-mount *in situ* hybridization was performed as described by (Bally-Cuif et al., 1995) or by (Stern, 1998). *In situ* hybridization for sections was carried out as described by (Ishii et al., 1997).

Primary antibodies used for Immunohistochemistry were: polyclonal anti-Coactosin antibody, which was raised in rabbits using bacterially expressed peptide, NH2-DHKELDEDYIKNELK-COOH, by Sawady Technology (Tokyo) as described previously (Hou et al., 2009), mouse monoclonal antibodies, HNK-1 (anti-CD57, which specifically stains neural crest cells, Zymed), 3F2.3 (anti-β2 subunit of non-muscle capping protein, Developmental Studies Hybridoma Bank (DSHB)), 0.T.127 (Anti-Rac1, Abcam), and M2F6, (Anti-Drebrin E. a kind gift of Prof. Tomoaki Shirao), rat monoclonal antibody 3F10 (Anti-HA (Hemoagglutinin), Roche Applied Science), rabbit monoclonal anti-GFP (Invitrogen) was used.

Secondary antibodies used were anti-mouse Alexa-594 (Invitrogen), anti-mouse Alexa-488, anti-rat Alexa-488 (Invitrogen), and anti-rabbit Alexa-594 (Invitrogen) antibodies.

Visualization of F-actin

Actin filaments were visualized by Rhodamine or Oregon Green 488 conjugated Phalloidin (Invitrogen).

In ovo electroporation

In ovo electroporation was performed at stage 10 as previously described (Funahashi et al., 1999; Odani et al., 2008) using expression vectors; HA tagged Coactosin in pMiwII (Suemori et al., 1990; Wakamatsu et al., 1997; Araki and Nakamura, 1999) (pMiwII-Coactosin-HA), HA tagged mutant Coactosin, in which lysine75 (actin binding site) was changed to alanine by Mutagenesis Kit (Stratagene), in pMiwII (pMiwII-CoactosinK75A-HA), a dominant negative form of Rac1 (N17-Rac1) and constitutive active Rac1 (V12-Rac1) in pEF-BOS-HAx3 vector. GFP fusion was made for the following expression vectors; Coactosin in pEGFP-C1 (Clontech) (pEGFP-Coactosin), mutant Coactosin in pEGFP-C1 (pEGFP-CoactosinK75A) and β-actin expression vector (Clontech) (pAcGFP1-Actin).

In some cases stock solution of DiI (C-7000, Molecular Probe) (0.5% in 100% ethanol) was diluted 10 times in 0.3 M sucrose solutions, and was added to the same volume of plasmid solution at electroporation to label neural crest cells (Omi et al., 2002).

siRNA construction

siRNA was designed referring to Katahira and Nakamura (2003), and the sequences were 5′-AGCTAATTACGATGCACAGAC-3′ for *Coactosin* siRNA and 5′-AGCAAATTACCATCCAGAGAC-3′ for control siRNA. pSuper-GFP (Oligoengine) that contains H1 promoter and a nine-base hairpin loop sequence (5′-TTCAAGAGA-3′) was used to make GFP-coupled siRNA. We also prepared pSuper-siRNA expression vector by deleting GFP construct. We confirmed that the *Coactosin* siRNA does not cross react with other chick ADF-H family members by BLAST search.

Cell culture

HEK 293 cells and neural crest cells were cultured at 38 °C in 10% FBS (Fetal Bovine Serum) containing DMEM (Dlubecco's Modified Eagle's Medium) in an atmosphere of 5% CO_2. N1E-115 cells were maintained in DMEM (NISSUI) containing 10% fetal bovine serum and 1% penicillin, 1% streptomycin, in a humidified incubator with 5% CO_2 at 37 °C as undifferentiated, and were induced to differentiate to neuronal cells by moving to differentiation medium, DMEM with 2% FBS and 1.25% Dimethyl sulfoxide (DMSO).

Transfection of pMiwII-Coactosin-HA and pMiwII-Capping protein β2-Myc to HEK 293 cells, and transfection of pEGFP–Coactosin, pMiwII-Capping protein β2-Myc, pEGFP–CoactosinK75A and/or V12-Rac1 to N1E-115 cells was carried out by Lipofectamine™ 2000 (Invitrogen).

Neural crest cells were cultured as described by Newgreen and Thiery (1980). Briefly, chick embryos were electroporated at stage 10. At 12 h after electroporation, neural tubes were taken out, digested in 1.5 mg/ml Dispase (GODO SHUSEI) for 30 min, washed in Leibovitz-15 media (GIBCO), and cut into small pieces. The tissue pieces were then cultured on 35 mm plastic dish or coverslips coated with fibronectin (BD Biosciences).

After neural crest cells were pre-cultured for 6 h, time lapse images were collected for 12 h by scanning laser microscope (FV300 on IX-81, OLYMPUS). Single-track z-sections (10–20 μm in thickness) at 10 min intervals were collected to produce a 3D time-lapse movie.

Quantification

For statistical analysis of number of filopodia, software of Image-Pro (Nippon Roper) was used. We set the field for the image capture in consideration of cell migration for recording period. Images were captured every 10 min, and all the cells in the field were counted. Image-Pro automatically counts cell number according to the diameter and contours of the object. If 2 or 3 cells were counted as one, modification was made by the author for the final number of cells. Filopodia were abstracted according to the manufacture's protocol, and those that have longer than 4-pixel-length were counted. Then mean number of filopodia per cell and standard error were calculated.

For statistical analysis for migration distance of cultured neural crest cells, lines were made every 20 μm in parallel with the edge of the neural tube (start point) to the cell of farthest migration, and the number of cells between 2 lines was counted. Statistical analysis was performed with Prism 5.0 (MDF) and determined the mean and standard error of percentage.

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