Role of actin binding protein Coactosin in cell migration and axonal extension

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論文題目	Role of actin binding protein Coactosin in cell migration and axonal extension（アクチン結合タンパクCoactosinの神経堤細胞移動・軸索伸長における役割）
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論文内容の要旨

Coactosin is a 17kDa actin binding protein that belongs to ADF/cofilin homology family. Cofilin, which belongs to the family, severs actin filaments and promotes actin dynamics by accelerating the treadmilling of actin filaments, a process in which monomers are removed from the pointed end of filaments and are added to the barbed end. On the other hand, it was reported that Coactosin inhibits barbed-end capping of actin filament, and is involved in actin polymerization in vitro.

Preliminary examination by in situ hybridization showed that Coactosin was expressed in cephalic and trunk neural crest cells (NCCs), and their derivatives such as cranial ganglia and dorsal root ganglia. Coactosin was also expressed in the cells that are forming mesonephric duct, and endodermal cells. Since these cells are highly motile cells, I decided to examine role of Coactosin in cell dynamics. For the study, I elaborated techniques. First, I improved the sagittal slice explant technique, which enables us to observe neural crest cell migration nearly intact condition. Next, I developed conditional siRNA technique by modifying the system originally developed by Shinagawa and Ishii (2003). They developed a plasmid, pDECAP-RNAi expression vector, which contains the CMV promoter, a ribozyme cassette to cut off the m^7G cap structure, and a MAZ site for Pol II pausing. The transcripts of long double strand RNA without cap and poly A tail stay in the nucleus, and processed into siRNA by Dicer, which is present in the nucleus. A ribozyme cassette and a MAZ site were put into BI vector that contains tet-on system. This system enables us to knock-down the target gene at desired stage by administrating doxycyclin, a tetracycline derivative.

Immunocytochemistry with anti-Coactosin antibody showed that Coactosin is localized in the cytoplasm, and associated with actin stress fibers in cultured neural crest cells. Coactosin is also expressed in the axon of oculomotor nerve and trigeminal nerve. In the growth cone of the oculomotor nerve axons, both Coactosin mRNA and protein were localized.

Knockdown of Coactosin by electroporating shRNA expression vector resulted in impairment of neural crest cell migration in vivo and in vitro. In cells that were knocked down of Coactosin, arrangement of actin cytoskeleton was impaired; anchoring of neural crest cells at the focal adhesion site by actin filament was destroyed. The result indicates that migration defects in Coactosin-knocked down cells may be caused by impairment of filopodial contact to the substrate or between neighboring NCCs. Incorporation of G-actin into F-actin was interfered in Coactosin-knocked down cells. Time-lapse analyses in sagittal slice explant confirmed observations that knockdown of Coactosin impaired migration of neural crest cells.

To exclude the possibility that above results are resulted from neural tube closure, conditional knock-down of Coactosin was carried out, that is, Coactosin was knocked down after they had left

neural tube by Tet-on siRNA system. By this it was confirmed that neural crest cell migration was impaired by Coactosin-knock down.

Coactosin protein and mRNA was localized in the oculomotor nerve. I wondered if Coactosin is involved in extension of the axon. Knockdown of Coactosin by Coactosin-siRNA resulted in impairment of oculomoter nerve elongation. To further investigate the function of Coactosin, N1E115 cell line was used. The cells differentiate into neuron in the absence of serum, and we can observe neurite extension. Coactosin was localized at the tip of filopodia of the neuritis.

Next, relation of Coactosin with Rho, Rac system was studied. Expression of dominant-positive Rac1 or RhoA caused Coactosin to rapidly accumulate in lamellipodia or filopodia. Coactosin was partially co-localized with Rac1 and RhoA. Overall, these findings suggest that in vertebrate development, regulation of Coactosin is critical for neural crest cell migration and cell shape maintenance.

Publication list:

1. Directory related papers

Xubin Hou, Tatsuya Katahira, Jun Kimura and Harukazu Nakamura (2009)

Expression of Chick Coactosin in cells in morphogenetic movement.

Develop. Growth Differ. Volume 51 Issue 9, Pages 833 - 840

2. Others

Odani, N., Hou, X., Nakamura, H.

In ovo electroporation as a strong tool to pursue molecular mechanisms of neural development in chick. Electroporation and Sonoporation in Developmental Biology. Springer JP, Tokyo

論文審査結果の要旨

侯旭濱君はアクチン結合タンパク Coactosin に着目し、その機能解析を行った。Coactosin は ADF (actin depolymering factor) domain を持っており、アクチンと結合すること、アクチンの安定化に働く事は分かっていたが、その機能はよく分かっていなかった。侯君はまず Coactosin の mRNA が、動眼神経などの神経軸索の成長円錐、神経堤細胞、中腎管の先端などの動きのある細胞に発現していることを明らかにした。また、Coactosin はアクチンおよびキャッピングプロテインと結合することを示した。

そこで、神経堤細胞の移動、動眼神経の軸索伸長における Coactosin の役割を調べた。培養神経堤細胞で、Coactosin はアクチンと共局在しており、特にストレスファイバーと関連があることを示した。shRNA によるノックダウンにより、神経堤細胞の移動、動眼神経の軸索伸長が阻害された。また、GFP-G-actin 発現ベクター、Coactosin shRNA 発現ベクターを発現させることにより、G-アクチンが F-アクチンに取り込まれるためには Coactosin の存在が必要であることが示された。

細胞移動、軸索伸長においては Rho, Rac のシグナルの下に糸状仮足、あるいは葉状仮足が作られるが、培養 N1E1115 細胞に Coactosin をトランスフェクトし、活性型 Rac で処理すると、糸状突起が活発に作られ、Coactosin が糸状突起に集積した。アクチン結合部位に変異を入れアクチンと結合できない Coactosin をトランスフェクトした場合は、Rac のシグナルがあっても糸状突起の形成は促進されず、また Coactosin が突起に集積することもなかった。このことは、Coactosin が Rac 等のシグナルの下流でアクチンと結合し糸状突起の形成に働き、細胞移動、突起伸展に大きな役割を果たしていることを示唆している。

侯君はこれらの研究を遂行するにあたり、いろいろな工夫を凝らしている。神経堤細胞の移動の観察には体節などを残したまま培養できる semi vivo の培養法を開発した。その方法により、生体に近い条件での共焦点顕微鏡によるタイムラプス画像の解析を可能にした。また、Coactosin ノックダウンにより、神経堤細胞の上皮間充織転換が阻害されるのか、あるいは移動そのものが阻害されるかについては、全く新しい siRNA ノックダウン法を開発し、世界で始めて siRNA の発現の調節を可能にした。この方法により、Coactosin ノックダウンにより阻害されるのは神経堤細胞の上皮間充織転換ではなく、移動そのものであることを示した。

侯旭濱君は自立して研究活動を行うに必要な高度の研究能力と学識を有することを示しており、侯旭濱君提出の論文は，博士（生命科学）の博士論文として合格と認める。