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A conserved sequence in caveolin-1 is both necessary and sufficient for caveolin polarity and cell directional migration

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ABSTRACT

Caveolin-1 (Cav-1) plays an important role in the organization of signaling molecules involved in a variety of signaling pathways, including those mediating cell motility. Here we show that amino acids K47–K57 of Cav-1 are a highly conserved sequence in Cav-1 and Cav-3 proteins, and that expression of either K47–K57 deletion Cav-1 mutant or wild-type Cav-2 that lacks this sequence exhibits a non-polarized distribution pattern. Expression of K47–K57 in Cav-2 leads to Cav-2 polarity, suggesting that expression of K47–K57 is sufficient to direct caveolin polarity. Importantly, we show that expression of this sequence is both necessary and sufficient to promote cell directional migration. Thus, our results support the conclusion that Cav-1 polarity is critical for cell directional migration.

1. Introduction

Directional migration is an essential process for tissue organization, organogenesis, homeostasis, wound healing, and tumor metastasis [1]. A key feature of a migrating celi is the acquisition of structural and functional asymmetry, i.e., cell polarization [2]. The front of a migrating cell generates protrusive force, which is associated with the extension of a lamellipodium or pseudopod through localized polymerization of F-actin. This is coupled to an actomyosin contraction at the cell posterior. Convincing evidence has indicated that the members of the Rho family of small GTPases, including Cdc42, Rac, and Rho, play important roles in the dynamic reorganization of the actin cytoskeleton, regulation of the assembly and disassembly of focal adhesions and cell movement. Cdc42 and Rac are responsible for the formation and organization of cortical actin networks and the protrusion of filopodia and lamellipodia [3], whereas RhoA controls the retraction of cell tail [4]. Recent studies have identified the upstream signaling pathways that control the activation of Rho family GTPases [5,6], and additional important signaling molecules, including phosphatidylinositol-3,4,5-trisphosphate, that direct lamellipod protrusion [7]. Although critical for cell polarity, it is not clear what regulates the polarized activation of signaling molecules in a migrating cell.

Caveolins are major structural proteins of the specialized microdomains in the plasma membranes, named caveolae. Three different genes have been identified in mammalian cells. Caveolin-1 (Cav-1) and caveolin-2 (Cav-2) are co-expressed in many cell types, while caveolin-3 is muscle-specific [8]. Cav-1 interacts with a number of signaling proteins including Src family kinases, phosphoinositide 3-kinase, integrins, Gα subunits, H-Ras, protein kinase C, endothelial nitric oxide synthase, and epidermal growth factor receptor [9–12]. Each caveolin-interacting signaling protein binds the same membrane-proximal region of Cav-1, termed the caveolin scaffolding domain (residues 82–101) [13,14]. In general, interaction with Cav-1 via the caveolin scaffolding domain leads to inactivation of target proteins. Thus, Cav-1 may function generally as an endogenous negative regulator of many signaling proteins. Given this view, one would predict that a relocation of Cav-1 to one part of a cell (i.e., caveolin polarity) without a substantial change in the expression level would reinforce an inhibitory effect at that part of the cell, e.g., the cell rear, but release its inhibitory activity on the other side, i.e., the leading edge. Indeed, we and others have recently demonstrated that Cav-1 is polarized to the rear of migrating cells [15–17], and that Cav-1 polarity appears to be mediated by aa 46–55 at the N-terminus of Cav-1 [15]. Furthermore, the results from our lab and others have showed that

Abbreviations: Cav-1, caveolin-1; CPD, caveolin polarization domain; Cav-2, caveolin-2; aa, amino acids; MEFs, mouse embryonic fibroblasts; WT, wild-type; KO, knockout; GAPDH, glyceraldehyse-3-phosphate dehydrogenase

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disruption of Cav-1 polarity either by target knock-down or genetic depletion of the protein severely impeded cell polarity and directional migration [15,16,18].

To gain insight into the molecular determinant of how Cav-1 polarity was controlled, we designed a number of double aa deletion mutants of Cav-1 within aa 45–60 and examined their effects on Cav-1 polarity. Here, we show that a conserved sequence, i.e., aa K47–K57, in Cav-1 and Cav-3 proteins was identified, and that all the double deletion mutants within this sequence block Cav-1 polarity. In addition, we show that expression of this sequence is sufficient to direct caveolin polarity and promote cell directional migration.

2. Materials and methods

2.1. Reagents

GFP polyclonal antibody and Cav-1 polyclonal antibody (N-20) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Mouse monoclonal antibody against Cav-2 was from BD Transduction Laboratories (San Jose, CA). pEGFP-N1 vector was purchased from BD Biosciences (San Diego, CA). BJ5183-AD-1 electroporation competent cells and pAdTrack-CMV vector were generous gifts from Dr. Bert Vogelstein of The Johns Hopkins Medical Institutions. Cell culture media, Lipofectamine™2000 reagent were purchased from Invitrogen (Carlsbad, CA).

2.2. Plasmids construction

The full-length cDNA encoding murine Cav-1, Cav-2 and their mutants was fused in-frame to the N-terminus of GFP. Mutations in Cav-1 and Cav-2 were generated by PCR from the cDNA of murine Cav-1 or Cav-2 (see Fig. 1). EcoRI and BamHI restriction sites were added to 5′ and 3′ ends of murine Cav-1 or Cav-2 cDNA by PCR using the TripleMaster® PCR System (Brinkmann Instruments Inc.). The PCR products were subcloned into the EcoRI and BamHI sites of the pEGFP-N1 eukaryotic expression vector. The orientation and sequence of the cDNA encoding Cav-1, Cav-2 and their mutants were verified by sequencing.

2.3. Cell culture

Primary mouse embryonic fibroblasts (MEFs) were obtained from either wild-type (WT) or Cav-1$^{-/-}$ Day 13.5 mouse embryos as previously described [15]. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 0.1 mM MEM non-essential amino acids (aa) solution, 2 mM glutamine, 100 units/ml penicillin, and 100 units/ml streptomycin in a 37 °C, 5% CO_2 incubator. Early passages (passage <5) of primary MEFs were used for all experiments.

2.4. Confocal microcopy and image analysis

Cells expressing GFP-tagged normal or mutant forms of caveolins were plated on 0.2% gelatin coated glass coverslips to allow migration, fixed with 2% paraformaldehyde in PBS for 20 min at 22 °C, and then mounted with Fluoromount-G (SouthernBiotech Inc.). Fluorescence images of the cells were acquired on an upright Carl Zeiss LSM 510 confocal microscope equipped with C-Apochromat 40×/1.2 W water-immersion objective using the 488 nm line of an argon laser. Cell borders were outlined from DIC images by white dash lines. Images of polarized cells expressing GFP-tagged proteins were randomly recorded. Green fluorescence intensity in six regions (three at the cell front and three at the cell rear) of each cell was measured using Image J software (http://www.rsb.info.nih.gov/ij/) as previously described [15]. Cav-1 depolarization was assumed if a ratio of rear to front fluorescence intensity was equal to or less than the cells expressing GFP. A partial polarization was assumed if a ratio of rear to front fluorescence intensity of a Cav-1 mutant was significantly higher than the cells expressing GFP, but less than WT Cav-1.

2.5. Chemotaxis assay

Chemotaxis analysis was conducted in Dunn chamber as we have described previously [15]. Cells were seeded on a glass coverslip coated with 0.2% gelatin and starved for 4 h prior to the assay. To set up gradient experiments, both concentric wells of the chamber were filled with starvation medium (DMEM with 0.25% FBS) and the coverslip seeded with cells was inverted onto the chamber in an offset position leaving a narrow slit at one edge for refilling the outer well. The medium of outer well was drained and replaced with DMEM supplemented with 10% FBS. Differential interference contrast (DIC) images of cells were captured at 2 min intervals for a total of 6 h using a Zeiss LSM 510 laser scanning confocal system with 10× objective. The number of migrating cells was determined as we have described previously [15].

2.6. Multiple sequence alignment

The alignment of the sequences of caveolin proteins was obtained using the program CLUSTAL version 2.0.5 (http://www.clustal.org). The sequences are those of cattle Cav-1 (GeneBank accession number, NP_776429), sheep Cav-1 (Q6B3Y2), pig Cav-1 (NP_999603), mouse Cav-1 (NP_031642), rat Cav-1 (AAR16308), rabbit Cav-1 (Q09YN6), human Cav-1 (NP_001744), cattle Cav-3 (Q2KI43), pig Cav-3 (NP_001032226), human Cav-3 (NP_203123), mouse Cav-3 (NP_031643), rat Cav-3 (NP_062028), cattle Cav-2 (NP_001007809), sheep Cav-2 (Q09YJ1), pig Cav-2 (Q2QLE2), rabbit Cav-2 (Q09YN7), mouse Cav-2 (NP_058596), rat Cav-2 (NP_571989), and human Cav-2 (AAB88492).

2.7. Western blot analysis

Cell lysates were separated by SDS–PAGE on a 12% gel, electrotransferred to a nitrocellulose membrane, and immunoblotted with antibody against Cav-1, Cav-2, or glyceraldehyde-3-phosphate dehydrogenase (GAPDH). Bands were visualized by Super-Signal West Pico Chemiluminescent Substrate (Pierce Chemical Co.).

2.8. Adenovirus production, amplification, and purification

Normal and mutant cDNA of Cav-1 or Cav-2 fused in frame with DNA sequence encoding FLAG tag were cloned into pAdTrack-CMV, and the resultant constructs were digested with PmeI and electro-transformed into BJ5183-AD-1 competent cells for recombination. The purified recombinant adenoviral constructs were digested with PacI, and transfected into AD293 cells to generate adenovirus. After 7–10 days, the primary adenovirus was generated. AD293 cells reaching 90% confluency were infected with the primary adenovirus. The cells were then incubated at 37 °C for 48 h. Following incubation, the cells were collected and suspended in 10 mM Tris–HCl (pH 7.9) buffer. Three freeze/thaw cycles were performed at −20 °C (until completely frozen)/37 °C (until fully thawed), and then supernatant was collected for purification. The adenovirus was purified by sequential cesium chloride gradient centrifugation. After purification, the virus was desalted and stored at −80 °C.