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

Xing-Hui Sun\textsuperscript{a}, Zi-Yang Liu\textsuperscript{a}, Hong Chen\textsuperscript{c}, Andrew R. Beardsley\textsuperscript{a}, Qi Qi\textsuperscript{a}, Jun Liu\textsuperscript{a,b,*}

\textsuperscript{a}Mary Babb Randolph Cancer Center, West Virginia University, Morgantown, WV 26506, USA
\textsuperscript{b}Department of Physiology and Pharmacology, West Virginia University, Morgantown, WV 26506, USA
\textsuperscript{c}Department of Radiology, Medical College of Wisconsin, Milwaukee, WI 53226, USA

\textbf{A R T I C L E  I N F O}

Article history:
Received 8 July 2009
Revised 12 September 2009
Accepted 19 October 2009
Available online 23 October 2009

Edited by Beat Imhof

Keywords:
Directional migration
Caveolin
Cell polarity

\textbf{A B S T R A C T}

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.

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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 cell 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.

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, G\textsubscript{\alpha} 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...
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
compotent 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% CO2 incubator. Early passages (passage <5) of primary
MEFs were used for all experiments.

2.4. Confocal microscopy and image analysis

Cells expressing GFP-tagged normal or mutant forms of caveo-
lins 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-Apochro-
matic 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.rsbi.org).

nih.gov/ijj) 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 cov-
erslip 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 end
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 polypeptides was ob-
tained using the program CLUSTAL version 2.0.5 (http://wwwCLUS-
tal.org). The sequences are those of cattle Cav-1 (GeneBank
accession number, NP_776429), sheep Cav-1 (Q683Y2), 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
(Q2K43), 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 (Q09Y11), pig Cav-2 (Q2QLE2), rab-
bbit 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, electro-
transferred to a nitrocellulose membrane, and immunoblotted
with antibody against Cav-1, Cav-2, or glyceraldehyde-3-phosphate
dehydrogenase (GAPDH). Bands were visualized by SuperSignal
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 pAd-
Track-CMV, and the resultant constructs were digested with
Pmel and electro-transformed into BJ5183-AD-1 competent cells
for recombination. The purified recombinant adeno viral con-
structs 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 in-
cubated 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 purifi-
cation, the virus was desalted and stored at −80 °C.
3. Results

3.1. aa K47–K57 are required for Cav-1 polarity

In order to determine which aa within the sequence of aa 46–55 mediated Cav-1 polarity and to gain insight into the molecular determinant of how Cav-1 polarity was controlled, we generated a series of double aa deletion mutants from aa 45 to 60 of Cav-1 (Fig. 1) and expressed the mutants as GFP-fusion proteins in Cav-1 knockout (KO) cells. We compared the polarity of the double deletion mutants with full-length Cav-1-GFP, which was relocated to the cell rear in a migrating cell (a in Fig. 2A). Deletion of either aa 45–46 or 59–60 did not affect Cav-1 relocation to the cell rear (b and i in Fig. 2A). When aa 47–48 were deleted, some signals of the mutant were detected at cell front, although the mutant was concentrated at the cell rear (c in Fig. 2A), suggesting partial polarization of the mutant (see next paragraph). In contrast, double deletion of either aa 49–50, 51–52, 53–54, 55–56 or 57–58 completely blocked Cav-1 polarity (d–h in Fig. 2A).

To statistically analyze polarized Cav-1-GFP signals in migrating cells, fluorescence intensity of each mutant at the cell front and cell rear of migrating cells was detected and a ratio of rear to front fluorescence intensity was determined as we have described previously [15], and compared either with full-length Cav-1-GFP (polarized protein) or GFP (non-polarized protein). The fluorescence intensity ratios of the deletion mutants, Cav45Δ46-GFP and Cav59Δ60-GFP, were very close to that of full-length Cav-1, but 2.5- and 2.2-fold higher than that of GFP, respectively (P < 0.01) (Fig. 2B). By comparison, fluorescence intensity ratios of Cav-1 mutants including double deletions of aa 49–50 (Cav49Δ50-GFP), 51–52 (Cav51Δ52-GFP), 53–54 (Cav53Δ54-GFP), 55–56 (Cav55Δ56-GFP), and 57–58 (Cav57Δ58-GFP) were very close to that of GFP, but 2.8-, 3.0-, 2.5-, 2.7-, and 2.5-fold lower than that of full-length Cav-1, respectively (P < 0.01) (Fig. 2B), indicating a loss of polarity.

Deletion of aa 47–48 resulted in a decrease in the fluorescence intensity ratio by more than 30% compared to full-length Cav-1 (P < 0.05), but an increase by more than 1.8-fold compared to GFP (P < 0.01) (Fig. 2B), indicating a partial polarization of the mutant. The effect of aa deletion on Cav-1 polarity was not caused by protein expressions, since the expression levels of the Cav-1 mutants were comparable (Fig. 2D).

Also, we examined whether the number of Cav-1 polarized cells was affected by mutation of these aa by comparing the ratio of rear to front fluorescence intensity of each mutant with that of GFP. We and others have demonstrated previously that target knockdown or genetic depletion of Cav-1 impedes cell polarization, suggesting that Cav-1 polarity appears to play an important role in cell polarization and directional movement [15,16,18]. We predicted that ectopic expression of full-length Cav-1 in Cav-1 KO cells would restore cell polarization and that expression of Cav-1 depolarization mutants would impede cell polarization and, subsequently, the polarity of the Cav-1 mutants. Thus, in this experiment, we focused only on the polarized subpopulation of the cells. Polarization of a Cav-1 mutant was assumed if fluorescence intensity ratio of the mutant was higher than 2 standard deviations of the mean of GFP. Fig. 2C shows that 77.8 ± 8.8% of morphologically polarized

### Table 1: Schematic representation of wild-type and mutant caveolin-1 and caveolin-2

<table>
<thead>
<tr>
<th>Caveolin-1</th>
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</tr>
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<tbody>
<tr>
<td>CAV1-178GFP</td>
<td>Yes</td>
</tr>
<tr>
<td>Cav45Δ46GFP</td>
<td>Yes</td>
</tr>
<tr>
<td>Cav47Δ48GFP</td>
<td>Partial</td>
</tr>
<tr>
<td>Cav49Δ50GFP</td>
<td>No</td>
</tr>
<tr>
<td>Cav51Δ52GFP</td>
<td>No</td>
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</tr>
<tr>
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<td>No</td>
</tr>
<tr>
<td>Cav59Δ60GFP</td>
<td>No</td>
</tr>
<tr>
<td>ΔCPD-Cav1-GFP</td>
<td>No</td>
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</tbody>
</table>

### Table 2: Schematic representation of wild-type and mutant caveolin-1 and caveolin-2

<table>
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<th>Polarization</th>
</tr>
</thead>
<tbody>
<tr>
<td>wtCav2-GFP</td>
<td>No</td>
</tr>
<tr>
<td>CPD-Cav2-GFP</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic representation of wild-type and mutant caveolin-1 and caveolin-2. Caveolins are divided into three domains. The N-terminus and C-terminus are separated by a hydrophobic transmembrane domain (TM). All full-length and mutant Cav-1 and -2 were fused with GFP. The location of residues deletion in each construct is indicated by an open box. The replacement of amino acids 30–44 of Cav-2 with caveolin polarization domain is indicated by a black box. The ability of the constructs to polarize in migrating Cav-1 KO cells is indicated in the column to the right of each construct.
Fig. 2. Double amino acids deletion reveals minimal amino acid sequence required for Cav-1 rear polarization. (A) Cav-1 KO cells transfected with the constructs encoding double aa deletion mutants were seeded on 0.2% gelatin coated cover-slips and incubated to allow migration. Then, the cells were fixed and examined by confocal fluorescence microscopy. Representative images of the cells expressing the constructs are shown (a–c and i, cell borders are outlined by dot lines). Note that GFP-tagged full-length Cav-1 (a) and Cav-1 deletion mutants including Cav45Δ46-GFP (b) and Cav59Δ60-GFP (i) polarize well at cell rear (arrows in a, b, i), whereas deletion of aa 47–48 partially blocks polarization of the protein (c). In contrast, double deletions among aa 49–58 (d–h) prevent polarization of these Cav-1 mutants. Scale bar, 20 μm. (B) Images of polarized cells expressing the Cav-1 mutants (n ≥ 35 for each construct) as in (A) were randomly recorded. Fluorescence intensity of GFP-tagged proteins at cell front and cell rear was measured as described in Section 2, and a ratio of rear to front fluorescence intensity was determined. Data are the means ± S.E.M. from three independent experiments. *P < 0.01 compared to GFP; **P < 0.01 compared to full-length Cav-1 (Cav1–178-GFP); #P < 0.05 compared to full-length Cav-1. (C) The number of Cav-1 polarized cells was determined by comparing the ratio of rear to front fluorescence intensity in each mutant with that of GFP. Polarization of a Cav-1 mutant was assumed if fluorescence intensity ratio of the mutant was higher than 2 standard deviations of the mean of GFP. Data are the means ± S.E.M. from three independent experiments. (D) Cell lysates from a parallel set of the cells transfected with the Cav-1 mutants as in (A) were subjected to SDS–PAGE, and the levels of Cav-1 expression were detected using antibody for GFP. GAPDH was blotted to show equal protein loading.
cells expressing full-length Cav-1 exhibit Cav-1 rear polarization. The percentages of Cav-1 rear polarized cells expressing either Ca\textsubscript{4S,4A}-GFP or Ca\textsubscript{5S,4A}-GFP are 80.0 \pm 2.3% or 75.1 \pm 1.8%, respectively, which are similar to that expressing full-length Cav-1. In contrast, the percentages of Cav-1 rear polarized cells expressing either Ca\textsubscript{4A,4S}-GFP, Ca\textsubscript{5A,4S}-GFP, Ca\textsubscript{5A,4A}-GFP, or Ca\textsubscript{5A,4A}-GFP are dramatically reduced, with the values ranging from 2.8 \pm 4.8% (Ca\textsubscript{4S,4S}-GFP) to 12.6 \pm 10.8% (Ca\textsubscript{5S,4A}-GFP). These results let us conclude that aa 47–58 at the N-terminus of Cav-1 are essential for the protein polarization.

3.2. Multiple sequences alignment analysis reveals a novel conserved sequence among mammalian caveolins

In addition to caveolin scaffold domain and membrane insertion domain, previous studies have identified the most conserved sequence of caveolins between C. elegans and humans, i.e., aa F68–P75 of Cav-1 (asterisks, Fig. 3), which is known as the “caveolin signature sequence/domain” [19,20]. Notably, another highly conserved region within the N-terminus of Cav-1 proteins, aa K47–K57 (gray box), is 100% identical to Cav-3, but 27% identical and 45% similar to Cav-2 proteins. The analysis result suggests that this sequence may mediate caveolin function shared by Cav-1 and -3, but not Cav-2. Interestingly, our present study shows that mutation of aa within 47–58 blocks Cav-1 polarity (Fig. 2), suggesting that this sequence is critical for Cav-1 dynamics. Consistent with this, WT Cav-2 that does not possess this domain, exhibited non-polarized distribution pattern in migrating cells (see Fig. 4). Based on these results, we propose that the newly identified conserved sequence, aa K47–K57, may represent a new domain that is essential for caveolin polarity, and thus, termed caveolin polarization domain (CPD).

3.3. Expression of CPD is sufficient to induce Cav-2 polarity

The second member of the caveolin gene family, i.e., Cav-2, was first identified by Scherer et al. [19] and has been shown to co-express and co-localize with Cav-1 in many cell types. The precise role of Cav-2 remains largely unknown, although recent evidence has suggested that Cav-2 may modulate Cav-1-dependent caveolae formation [21].

To further investigate the role of the CPD in mediating Cav-1 polarity, we transiently transfected Cav-1 KO cells with the CPD-deletion mutant of Cav-1, ΔCPD-Cav-1-GFP, and observed that, in deed, deletion of the CPD resulted in a loss of polarity (b in Fig. 4B). The fluorescence intensity ratio of the deletion mutant was very close to that of GFP, but 2.7-fold lower than that of full-length Cav-1 (P < 0.01) (Fig. 4C). Because Cav-2 lacks the CPD (see Fig. 3), we reasoned that Cav-2 would not undergo polarization in a migrating cell. To test this, WT Cav-2 was transiently expressed in Cav-1 null cells, and the polarity of Cav-2 was determined as described above. Fig. 4B shows that WT Cav-2 exhibits non-polarized distribution pattern (c). The fluorescence intensity ratio of WT Cav-2 is same as that of GFP (Fig. 4C). This result is consistent with our hypothesis and provides a tool to test whether expression of the CPD would be sufficient to induce Cav-2 polarity. For this purpose, a sequence of 15 aa that contains the CPD was engineered into Cav-2 (Fig. 4A) and expressed in Cav-1 KO cells. Intriguingly, expression of the CPD substituent Cav-2, CPD-Cav-2-GFP, results in polarization of the protein at the cell rear (d in Fig. 4B). The fluorescence intensity ratio of the CPD substituent is now 2.6-fold higher than that of GFP (P < 0.01) (Fig. 4C), which is consistent with polarized distribution of the mutant. Consistent with our observations described above, the number of cells exhibiting polarized distribution of wtCav-2-GFP is only 10%, which can be restored to full-length Cav-1 level by substitution with the CPD in Cav-2 (Fig. 4D).

Taken together, these results let us to conclude that the CPD is both necessary and sufficient for caveolin polarity.

3.4. CPD is both necessary and sufficient for promoting directional migration

A good body of evidence has indicated that Cav-1 may function, in general, as an endogenous inhibitor of signaling molecules, including those involved in cell motility [22]. Based on recent results from our laboratory and the others demonstrating that Cav-1 was polarized at the cell rear of migrating cells [16,17], we hypothesized that polarized signal activity at the cell leading edge that directs cell protrusion may be controlled by the asymmetrical distribution of Cav-1 in a migrating cell [16]. Consistent with our hypothesis, we and others have showed that target knockdown

![Fig. 3. Multiple sequence alignment of mammalian caveolin-1, -2 and -3 proteins. The amino acids highlighted in the gray box are 100% identical among Cav-1 and Cav-3 proteins. Caveolin signature sequence is indicated by asterisks (**).](image-url)
or genetic depletion of Cav-1 impede cell polarization and directional migration [15,16,18], and that re-expression of full-length Cav-1, but not the aa 1–60 deletion mutant Cav-1, in Cav-1 KO cells restores directional migration [15]. However, whether CPD would play a critical role in cell polarity and directional migration is not known. To test this, Cav-1 KO cells were infected with adenovirus encoding either WT caveolins or caveolin mutants, and cell polarity and directional migration were determined as we described previously [15].

As shown in Fig. 5A, genetic depletion of Cav-1 substantially inhibits cell polarity by 4.7-fold, which is consistent with previous observations [16,18]. Re-expression of full-length Cav-1, but not
the CPD-deletion mutant Cav-1 (ΔCPD-Cav-1), WT Cav-2 (wtCav-2) or GFP, restores cell polarity to WT phenotype. Intriguingly, expression of the CPD substituent Cav-2 (CPD-Cav-2) increases significantly cell polarity by 3-fold over that in Cav-2 expressing cells. These results suggest that the CPD is essential for cell polarity. Based on these results, we expected that mutation of CPD would affect directional migration. As can be seen in Fig. 5B, the directional migration of Cav-1 KO cells toward serum gradient is severely impaired. Only 7.5 ± 0.5% of Cav-1 KO cells exhibit directional movement toward serum, whereas 23 ± 1.5% of WT cells show directional migration (P < 0.01). When Cav-1 KO cells are transduced to express full-length Cav-1, 23.8 ± 1.6% of the cells regain the ability to migrate toward serum. In contrast, only 5.6 ± 2.2% of the cells expressing the CPD-deletion mutant Cav-1 show directional movement (P < 0.01). These results allow us to conclude that the CPD is required for directional migration. Interestingly, expression of WT Cav-2 that lacks the CPD and exhibits non-polarized protein distribution (see Fig. 4B) fails to rescue Cav-1 WT phenotype. In contrast, expression of the CPD substituent Cav-2 restores directional migration (Fig. 5B). These results indicate that expression of the CPD is sufficient to promote directional migration. The levels of exogenously expressed full-length and the CPD-deletion mutant Cav-1 are comparable and equal to the level of endogenous Cav-1 (Fig. 5C). Expression of either wtCav-2 or CPD-Cav-2 results in increases in endogenous Cav-2 level (Fig. 5D). Previous study showed that the degradation of Cav-2 was accelerated in the absence of Cav-1 [23] (see Fig. 5D). Transduced expression of exogenous Cav-2 may competitively inhibit the degradation of endogenous Cav-2. It seems unlikely that the gain-of-function of directional migration by CPD-Cav-2 results from increased level of endogenous Cav-2 expression, because expression of non-polarized wtCav-2 also causes an increase in the endogenous Cav-2 level (Fig. 5D).

4. Discussion

Previous studies have identified several functional elements of caveolin. These include the caveolin scaffolding domain [13], caveolin oligomer domain [24], a putative Golgi apparatus targeting sequence [25], the sequences that influence caveolin trafficking [20,26], the sequences that affect caveola formation [15,27], and the most conserved caveolin signature sequence [19,20]. Our present study uncovered a highly conserved region at the N-terminus of Cav-1 proteins, i.e., aa K47–K57. Our results suggest that this sequence may play an important role in Cav-1 polarity and cell directional migration, although the function of this sequence remains to be fully characterized. These observations are supported by data demonstrating that (1) mutations of any aa within this sequence inhibit Cav-1 polarity; (2) expression of this sequence is sufficient to induce the polarity of WT Cav-2, which does not possess this domain and exhibits a non-polarized distribution in migrating cells; (3) expression of either the CPD-deletion mutant Cav-1 or WT Cav-2 cannot rescue cell directional migration; and (4) expression of the CPD is sufficient to induce directional migration.

The CPD is composed of a cluster of acidic (D, E) and basic (K, R) aa. The sequence motif is consistent with the proposed Cav-1 model suggesting that the hydrophilic domain remains cytoplasmic and accessible for cytosolic protein interactions [28]. The CPD has features in common with prostate apoptosis response-4 (Par-4, also known as PAWR), a tumor suppressor gene, which is involved in mediating protein trafficking and function [29,30]. Thus, the highly charged aa are likely interacted with a cytoplasmic factor(s) that may control Cav-1 localization in a migrating cell.

Directional migration is a complex and integrated process. Migrating cells exhibit highly polarized activities at the leading edge. This CPD is composed of a cluster of acidic (D, E) and basic (K, R) aa. The sequence motif is consistent with the proposed Cav-1 model suggesting that the hydrophilic domain remains cytoplasmic and accessible for cytosolic protein interactions [28]. The CPD has features in common with prostate apoptosis response-4 (Par-4, also known as PAWR), a tumor suppressor gene, which is involved in mediating protein trafficking and function [29,30]. Thus, the highly charged aa are likely interacted with a cytoplasmic factor(s) that may control Cav-1 localization in a migrating cell.
edge and the cell rear, which are spatially segregated. These activities must be coordinated in order to achieve efficient migration. Studies in the past have identified the Rho family of small GTPases, including Cdc42, Rac, and Rho, as one of the key players in the dynamic reorganization of the actin cytoskeleton and directional movement [3,4]. Rac is required at the cell front to regulate actin polymerization and membrane protrusion, and it has been shown that activated Rac is concentrated at the leading edge of migrating cells [31]. Rho is required to regulate the contraction and retraction forces in the cell body and cell rear, and it is expected that Rho activity would be concentrated at cell rear. Although critical for cell migration, it is not clear how the polarized activities are spatially controlled.

It has been proposed that caveolins form a scaffold onto which many signaling molecules are assembled to generate signaling complexes. A good body of evidence has demonstrated that many signaling molecules are assembled to generate signaling activity would be concentrated at cell rear. Although critical for cell migration, it is not clear how the polarized activities are spatially controlled.

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edge and the cell rear, which are spatially segregated. These activities must be coordinated in order to achieve efficient migration. Studies in the past have identified the Rho family of small GTPases, including Cdc42, Rac, and Rho, as one of the key players in the dynamic reorganization of the actin cytoskeleton and directional movement [3,4]. Rac is required at the cell front to regulate actin polymerization and membrane protrusion, and it has been shown that activated Rac is concentrated at the leading edge of migrating cells [31]. Rho is required to regulate the contraction and retraction forces in the cell body and cell rear, and it is expected that Rho activity would be concentrated at cell rear. Although critical for cell migration, it is not clear how the polarized activities are spatially controlled.

It has been proposed that caveolins form a scaffold onto which many signaling molecules are assembled to generate signaling complexes. A good body of evidence has demonstrated that many signaling molecules are assembled to generate signaling activity would be concentrated at cell rear. Although critical for cell migration, it is not clear how the polarized activities are spatially controlled.

References

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