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Dynamin 2 and BAR domain protein pacsin 2 cooperatively regulate formation and maturation of podosomes

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ABSTRACT

Podosomes are actin-rich adhesion structures formed in a variety of cell types, such as monocytic cells or cancer cells, to facilitate attachment to and degradation of the extracellular matrix (ECM). Previous studies showed that dynamin 2, a large GTPase involved in membrane remodeling and actin organization, is required for podosome function. However, precise roles of dynamin 2 at the podosomes remain to be elucidated.

In this study, we identified a BAR (Bin-Amphiphysin-Rvs167) domain protein pacsin 2 as a functional partner of dynamin 2 at podosomes. Dynamin 2 and pacsin 2 interact and co-localize to podosomes in Src-transformed NIH 3T3 (NIH-Src) cells. RNAi of either dynamin 2 or pacsin 2 in NIH-Src cells inhibited podosome formation and maturation, suggesting essential and related roles at podosomes. Consistently, RNAi of pacsin 2 prevented dynamin 2 localization to podosomes, and reciprocal RNAi of dynamin 2 prevented pacsin 2 localization to podosomes. Taking these results together, we conclude that dynamin 2 and pacsin 2 co-operatively regulate organization of podosomes in NIH-Src cells.

1. Introduction

Cell migration is fundamental for diverse physiological and pathological processes including development, immune response and tumor metastasis [1]. The ECM is a physical barrier for cells migrating *in vivo* and its degradation and remodeling regulates how cells to move through tissues. Podosomes and invadopodia are actin-based cell-matrix contacts required for ECM degradation [2]. Podosomes are found in motile cells such as monocytic, endothelial and smooth muscle cells in various subcellular arrangements (dots, clusters, rosettes and belts), whereas invadopodia are mainly formed by cancer cells. Despite the structural differences between podosomes (nonprotrusive) and invadopodia (protrusive), they share the F-actin core and other components such as actin regulators (Arp2/3 complex, cortactin and N-WASP), adaptor proteins (Tks4 and Tks5) and adhesion molecules (integrins and paxillin). Furthermore, ECM degradation by podosomes and invadopodia is similarly regulated by membrane type-1 matrix metalloproteinase (MT1-MMP) which locally activates and releases matrix-lytic enzymes such as MMP2 and MMP9. However, a full understanding of the molecular mechanisms that regulate formation and maturation of podosomes/invadopodia still remains elusive.

Dynamin is a large GTPase required for membrane fission in endocytosis and actin organization [3]. There are three dynamin isoforms in mammalian cells: two tissue-specific isoforms, dynamin 1 and dynamin 3, and a ubiquitous isoform, dynamin 2. All three dynamin isoforms share similar domain structures from N- to C-terminus: GTPase, middle, pleckstrin homology (PH), GTPase effector (GE) and proline-rich (PR) domains. Previous studies showed that dynamin 2 functions at invadopodia [4,5] and podsosomes [6,7] and PR domain is indispensable for its function [5,8].

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Since PR domain is a motif known to bind other proteins via their Src homology 3 (SH3) domain [9], dynamin 2 possibly regulates podosome/invadopodia function via currently unknown SH3 domain-containing protein(s). However, the protein network(s) involved in the dynamin-mediated invadopodia/podosome function remains to be elucidated.

In this study, we identified a BAR domain protein pacsin 2 (protein kinase C and casein kinase substrate in neurons protein 2) as a functional partner of dynamin 2 at the podosomes in NIH-Src cells. Pacsin 2 and dynamin 2 colocalized to podosomes and their interaction was confirmed by co-immunoprecipitation assay. RNAi of either dynamin 2 or pacsin 2 caused defects in formation and/or maturation of podosomes. Furthermore, mutual RNAi of pacsin 2 and dynamin 2 demonstrated that their localization at podosomes were interdependent. Thus, our study demonstrated that BAR domain protein pacsin 2 and dynamin 2 play essential roles in regulating formation and maturation of podosomes.

2. Materials and methods

2.1. Molecular biology

Expression constructs were prepared using Gateway Cloning (Thermo Fisher Scientific) as described previously [10]. In short, Entry clones of target genes in pDONR201 were cloned into pCI based Destination vectors for expressing GFP- or FLAG-tagged proteins in mammalian cells by L-R recombination. To prepare Entry clones for truncation mutants of dynamin 2 (dynamin 2 ΔPRD and dynamin 2 PRD) and pacsin 2 (pacsin 2 ΔSH3), PCR fragments amplified from Entry clones of dynamin 2 or pacsin 2 using primers described in the supplementary information were used for B–P recombination with pDONR201.

2.2. Cell culture, DNA transfection and RNAi

Src transformed NIH-3T3 (NIH-Src) cells [11] and HEK293T cells (ATCC CRL-3216) were grown in D-MEM (High Glucose) with L-Glutamine, Phenol Red and Sodium Pyruvate (043–30085, FUJIFILM Wako chemicals) supplemented with 10% fetal bovine serum (FBS) (12483020, Thermo Fisher Scientific) and Penicillin-Streptomycin (100 unit/mL) (15140122, Thermo Fisher Scientific) at 37 °C in 5% CO_2. Transfection of NIH-Src cells and HEK293T was performed using Lipofectamine LTX with Plus Reagent (15338100, Thermo Fisher Scientific) and used at 48 h after the transfection for immunoblot or microscopic analyses (NIH-Src cells) and co-immunoprecipitation analyses (HEK293T cells). For RNAi, NIH-Src cells were transfected with siGENOME SMART pool siRNA for Mouse Dnm2 (M-044919-01, horizon), siGENOME Non-Targeting siRNA Pools #1 (D-001206-13-05, horizon), Mission siRNA for mouse Pacsin 2 siRNA (SASI_Mm01_00026,847 and SASI_Mm01_00026,848, Merck) or MISSION siRNA Universal Negative Control #1 (SIC-001, Merck) using Lipofectamine RNAiMAX Transfection Reagent (13778150, Thermo Fisher Scientific). For the rescue experiments, cells were transfected with expression constructs 24 h after the RNAi started, with cells harvested at 72 h for microscopic or immunoblot analyses.

2.3. Antibodies and reagents

Primary antibodies used in this study were rabbit polyclonal anti-dynamin 2 (ab3457, Abcam), rabbit polyclonal anti-PACSIN1 (M–46) (SC-30127, Santa Cruz), mouse monoclonal anti-PACSIN2 (SAB1402538, SIGMA), rabbit polyclonal anti-PACSIN2 [12], rabbit polyclonal anti-PACSIN3 (AB37612, Abcam), GFP (D5.1) XP rabbit mAb (2956S, CST) and anti-DDDDK-tag pAb (PM020, MBL). Secondary antibodies and Alexa-conjugated phalloidin for immunofluorescence microscopy were purchased from Thermo Fisher Scientific: Alexa Fluor 350 goat anti-rabbit IgG (H+L) (A21068), Alexa Fluor 488 donkey anti-mouse IgG (H+L) (A21202), Alexa Fluor 488 donkey anti-rabbit IgG (H+L) (A21206), Alexa Fluor 555 donkey anti-mouse IgG (H+L) (A31570), Alexa Fluor 555 donkey anti-rabbit IgG (H+L) (A31572), Alexa Fluor 568 donkey anti-goat IgG (H+L) (A11057), Alexa Fluor 350 Phalloidin (A22281) and Alexa Fluor 555 Phalloidin (A34055). Secondary antibodies for

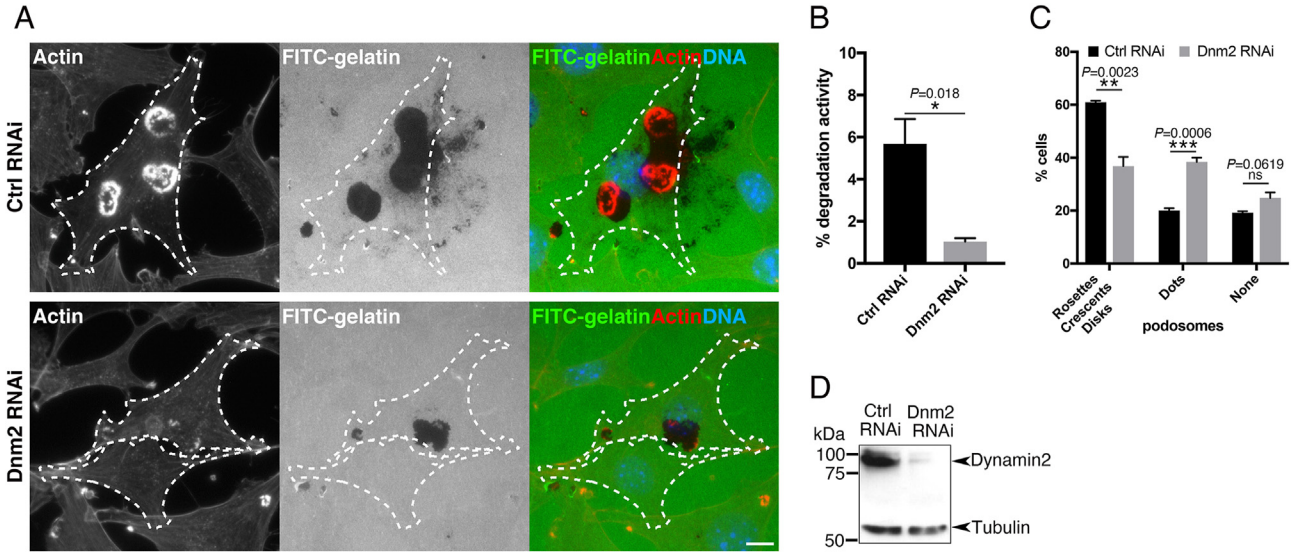

Fig. 1. *Dynamin 2 is required for organization of podosomes in NIH-Src cells.* (A) Localization of actin (red), FITC-gelatin (green) and their merged images with DNA (blue) in control RNAi (Ctrl RNAi) or dynamin 2 RNAi (Dnm2 RNAi) cells. Scale bar is 10 μm. (B) Degradation activities by control RNAi (Ctrl RNAi) or dynamin 2 RNAi (Dnm2 RNAi) cells. Data are means ± SEM (n ≥ 124 cells, N = 3). (C) Quantitation of cells with podosomes in different structures (rosettes, crescents, disks or dots) or without podosomes (None) in control RNAi (Ctrl RNAi) or dynamin 2 RNAi (Dnm2 RNAi) cells. Data are means ± SEM (n ≥ 124 podosomes, N = 3). (D) Immunoblot analysis of control RNAi (Ctrl RNAi) or dynamin 2 RNAi (Dnm2 RNAi) cells using anti-dynamin 2 and anti-tubulin antibodies. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

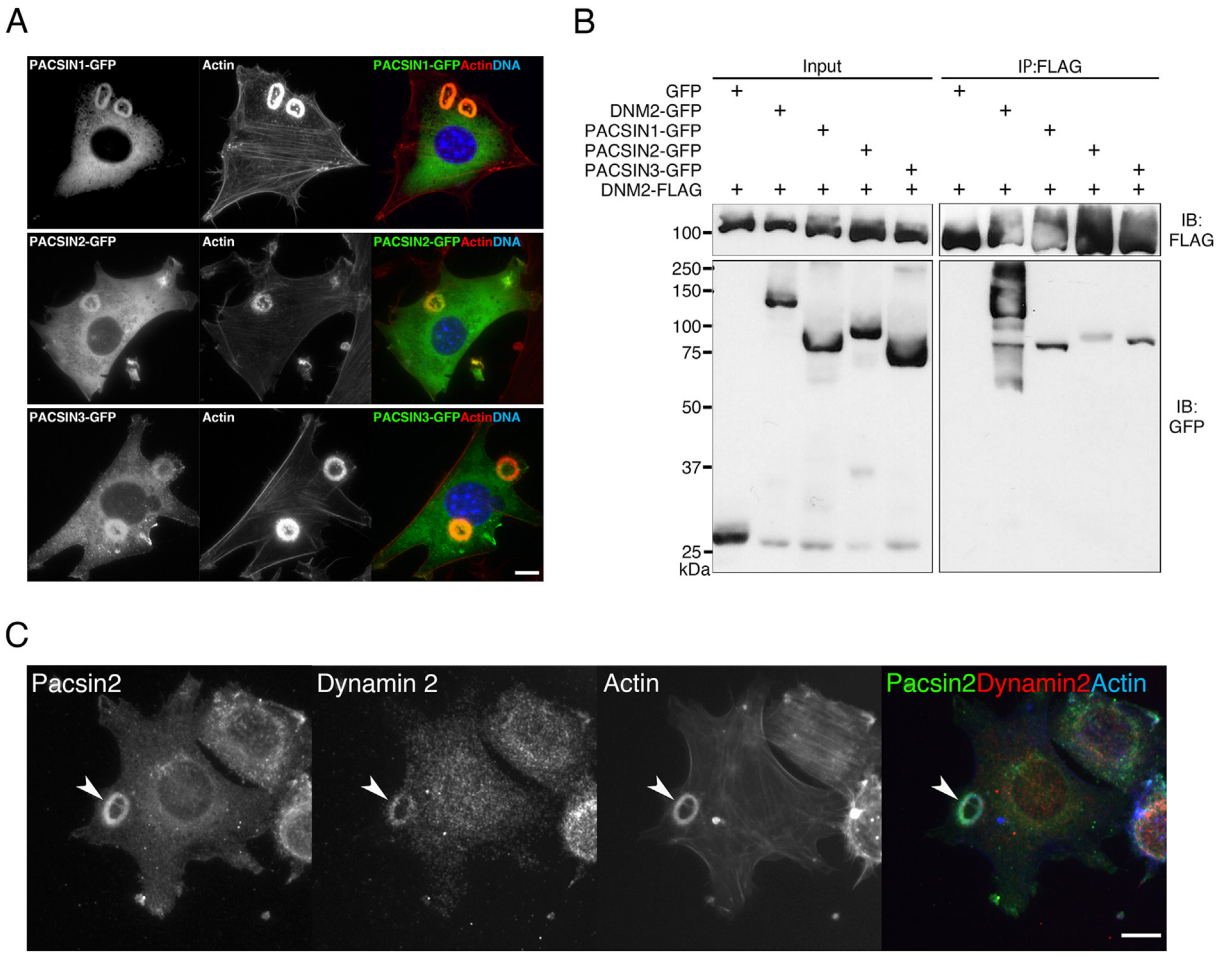

Fig. 2. *Pacsin 2 is associated and co-localized with dynamin 2 at the podosomes.* (A) Localization of GFP-tagged human PACSINs (PACSIN1-GFP, PACSIN2-GFP and PACSIN3-GFP) (green), actin (red) and their merged images with DNA (blue) in NIH-Src cells. (B) PACSIN isoforms interact with dynamin 2. Immunoblots of cell extract (Input) or immunoprecipitates using anti-FLAG antibody (IP:FLAG) from HEK293T cells expressing FLAG-tagged human dynamin 2 (DNM2-FLAG) together with either GFP-tagged human PACSINs (PACSIN1-GFP, PACSIN2-GFP or PACSIN3-GFP), negative control (GFP) or positive control (DNM2-GFP). The blot were labeled with anti-FLAG (IB:FLAG) or anti-GFP (IB:GFP) antibodies. (C) Pacsin 2 and dynamin 2 co-localize to the podosomes in NIH-Src cells. Localization of endogenous pacsin2 (green), dynamin2 (red) and actin (blue) and their merged images on a podosome rosette (arrowhead) are shown. Scale bars are 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

immunoblot analyses were also purchased from Thermo Fisher Scientific: goat anti-rabbit IgG (H+L) secondary antibody, HRP (31460), rabbit anti-mouse IgG (H+L) secondary antibody, HRP (31450). Glutaraldehyde used for fixing gelatin coating coverslips was prepared from Glutaraldehyde 25% EM (G004, TAAB).

2.4. Immunofluorescence microscopy

Immunofluorescence microscopy of NIH-Src cells grown on the gelatin-coated coverslips (prepared as per 2.7 below) was performed as described previously [10] using primary antibodies (1:200 dilution for dynamin2 and pacsin2 antibodies and 1:500 dilution for anti-DDDDK-tag antibody) and secondary antibodies (1:1000 dilution), Alexa Fluor 555 Phalloidin (1:1000 dilution) and Alexa Fluor 350 Phalloidin (1:200 dilution).

2.5. Immunoblot analysis

Immunoblot analyses were performed as described previously [10] using the primary antibodies (1:1000 dilution) and secondary antibodies (1:10,000 dilution) listed above.

2.6. Co-immunoprecipitation assay

Co-immunoprecipitation assay of PACSIN 1, 2, 3 and dynamin 2 using HEK293T cells was performed as described previously [10].

2.7. Preparation of gelatin coated cover slips and matrix degradation assay

Gelatin coated coverslips were prepared as described previously [13] with minor modifications. In short, 1 g gelatin (G2500, Merck) and 0.8 g sucrose (193–09545, Wako) were dissolved in 40 mL PBS to make 25 mg/mL Gelatin solution. HCl-washed clean cover-slips (C012001, Matsunami) were coated with the Gelatin solution and then fixed with 0.5% Glutaraldehyde for 15 min on ice and for 30 min at room temperature. After 3 times washing with 1 × PBS, single gelatin coated coverslips were incubated with 5 mg/mL $NaBH_4$ (192–01472, Wako) for 3 min to quench the aldehyde base in 24-well plate (2-8588-03, AS ONE). After 3 times washing with 1 × PBS, the gelatin coated coverslips were sterilized in 75% ethanol for 30 min, then washed 2 times with autoclaved cold 1 × PBS and incubated in D-MEM medium at 37 °C for 1 h. To induce podosome formation, NIH-Src cells were seeded on the gelatin coated

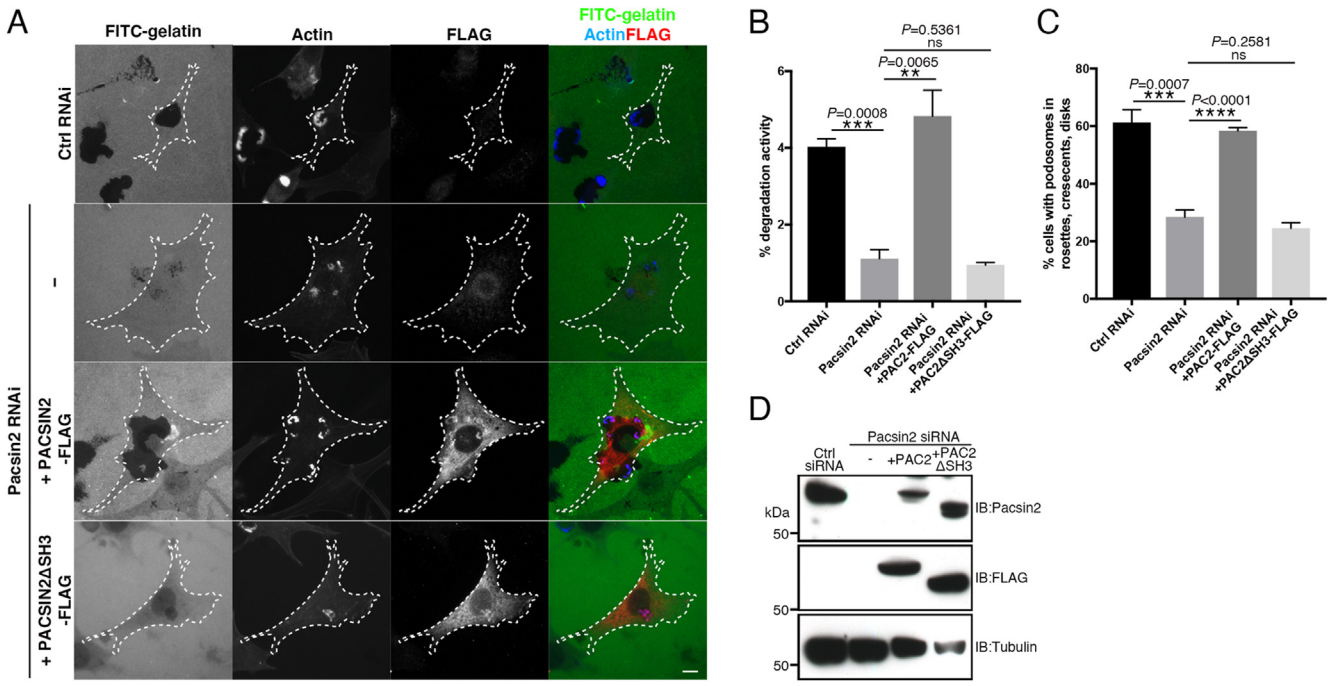

Fig. 3. *Interaction of pacsin 2 and dynamin 2 is required for organization of podosomes in NIH-Src cells.* (A) Immunofluorescence microscopic images of control RNAi (Ctrl RNAi) and pacsin 2 RNAi (Pacsin 2 RNAi) cells without (−) or with expressed FLAG-tagged full length human PACSIN2 (Pacsin 2 RNAi + PACSIN2-FLAG) or SH3 deleted PACSIN2 (Pacsin2 RNAi + PACSIN2ΔSH3-FLAG). FITC-gelatin (green), actin (blue), FLAG-tagged PACSIN2 (red) and their merged images are shown. Scale bar is 10 μm. (B) Quantitation of gelatin degradation activities by cells shown in A. Data are means ± SEM ($n \geq 69$ cells, $N = 3$). (C) Quantitation of cells with podosomes rosettes, crescents and disks based on the images shown in A. Data are means ± SEM ($n \geq 126$ podosomes, $N = 3$). (D) Immunoblot analysis of cells shown in A labeled with anti-pacsin 2 (IB:Pacsin2), anti-DDDDK (IB:FLAG) and anti-tubulin (IB: Tubulin) antibodies. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

coverslips and incubated for 6 h in the DMEM medium before fixation for immunofluorescence microscopy.

For the matrix degradation assay, fluorescein-labeled gelatin was used for coating coverslips. The fluorescein labeled gelatin was prepared following the protocol described previously [14]. In short, 0.5 g gelatin (G2500, Merck), 0.5 mg fluorescein isothiocyanate (FITC) (F7250, Merck), 0.2 g sucrose (193–09545, Wako) and 0.2 mL DMSO (046–21981, Wako) were dissolved in 20 mL borate buffer (40 mM NaCl, 50 mM $Na_2B_4O_7$, pH 9.3) and the gelatin solution was dialyzed against 1 × PBS using dialysis cassette (Pierce, Slide-A-Lyzer, MWCO 3.5 K) for 5 days at 37 °C with twice per day changes of 1 × PBS. The dialyzed FITC-labeled gelatin solution was filtered through a 0.2 μm pore filter (S-2502, Kurabo) and 2 mL aliquots stored at 4 °C with light interception. Proportion of degraded area in comparison to the total cell area were shown as degradation activity as described previously [15].

2.8. Statistical analysis

Statistical data analysis was performed using Prism 7 (GraphPad Software) and Excel (Microsoft). For all quantification provided, the means and SEM are shown. Statistical significance was determined using a two-sided *t*-test and P values as shown in the figures.

3. Results

3.1. Dynamin 2 is required for formation and maturation of podosomes in NIH-Src cells

Previously, dynamin 2 had been localized to the podosomes in osteoclasts [6] and in RSV-transfomed BHK21 cells [7]. Consistently, in NIH-Src cells, dynamin 2 localized to the podosome in various subcellular arrangements [16] from less mature dots to more matured and matrix degradation potent discs, crescents and rosettes (Fig. S1). To elucidate dynamin 2 function at the podosomes, endogenous dynamin 2 in NIH-Src cells was depleted by RNAi and its effect on podosome formation and maturation examined. Using fluorescein-labeled gelatin in a matrix degradation assay, dynamin 2 RNAi cells exhibited significantly reduced degradation activity (1.0%) compared to that of control RNAi cells (5.7%) (Fig. 1A and B). In dynamin 2 RNAi cells, the proportion of podosomes in rosettes, crescents or disks was decreased (36.8%) compared to that in control cells (60.9%) (Fig. 1 A and C). In contrast, the proportion of cells with podosome dots or no podosomes were increased in dynamin 2 RNAi cells (38.4% and 24.8%, respectively) compared to those in control RNAi cells (20.1% and 19.2%, respectively) (Fig. 1A and C). Specific depletion of dynamin 2 by RNAi was confirmed by immunoblot analysis (Fig. 1D). These results implicate dynamin 2 in the formation and/or maturation of podosomes in NIH-Src cells.

We next determined the dynamin 2 domains required for its localization at podosomes. Exogenously expressed GFP-tagged full-length human dynamin 2 localized to podosomes (Fig. S2, DNM2-GFP) just the same as the endogenous dynamin 2 (Fig. S1). In contrast, PR domain-deleted dynamin 2 localized to the podosomes much weaker than the full-length dynamin 2 (Fig. S2, DNM2ΔPRD-GFP). Conversely, sole dynamin 2 PR domain could localize to the podosomes almost to the same level as full-length dynamin 2 (Fig. S2, GFP-DNM2PRD). These results suggest that PR domain of dynamin 2 is required and sufficient for its localization to the podosomes.

3.2. BAR domain protein pacsin 2 is associated and colocalized with dynamin 2 at the podosomes

PR domain of dynamin 2 interacts with the SH3 domain in various proteins including BAR domain proteins involved in

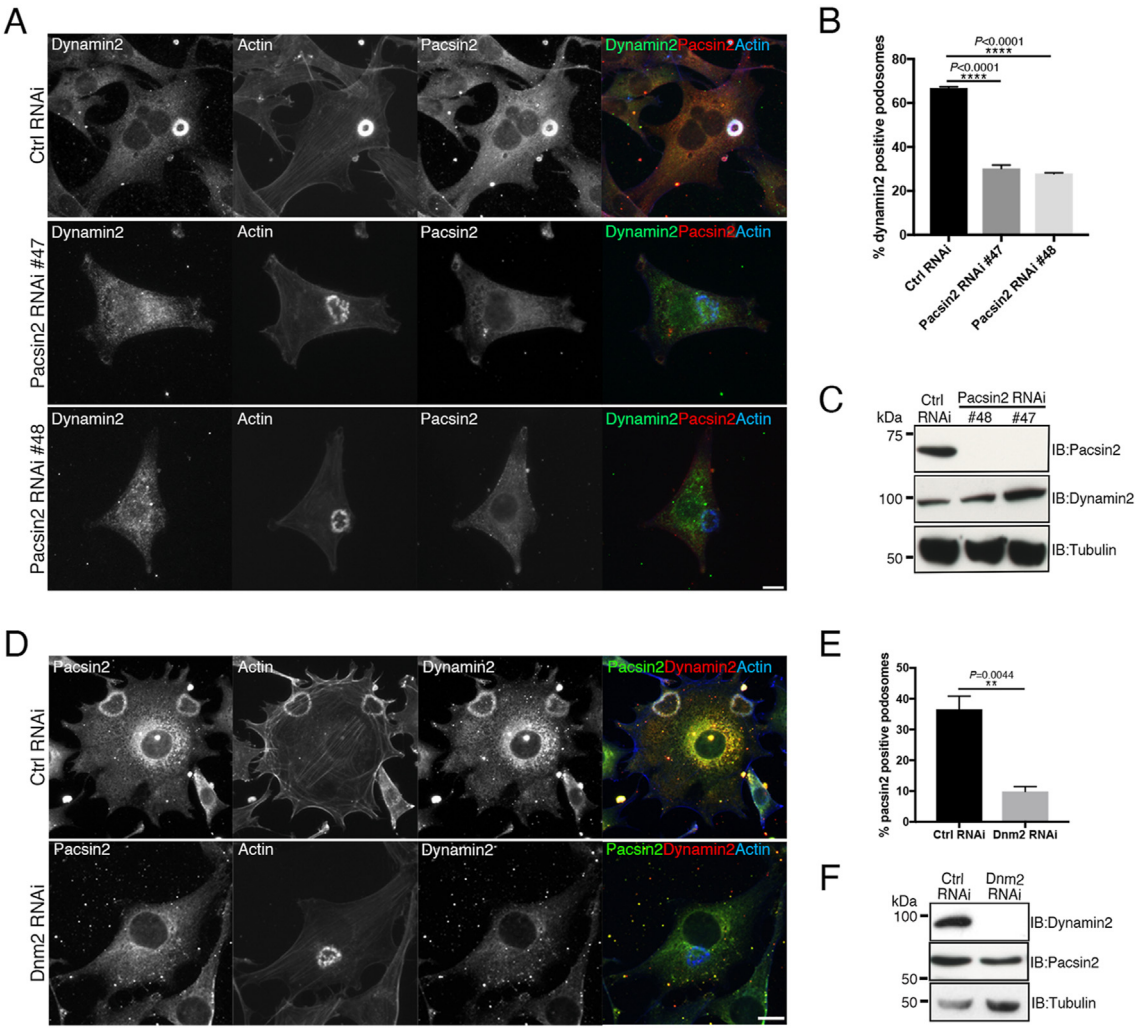

Fig. 4. Interdependent *localization of pacsin 2 and dynamin 2 to the podosomes.* (A) Localization of dynamin 2 (green), actin (blue) and pacsin 2 (red) and their merged images in control RNAi (Ctrl RNAi) and pacsin 2 RNAi (Pacsin 2 RNAi) cells. (B) Proportion of podosomes containing dynamin 2 in control RNAi cells (Ctrl RNAi) and pacsin 2 RNAi cells using two different siRNA (Pacsin2 siRNA#47 and #48). Data are means ± SEM ($n \geq 35$ podosomes, $N = 3$). (C) Immunoblot analysis of control RNAi and pacsin 2 RNAi cells using antibodies against pacsin 2 (IB:Pacsin2), dynamin 2 (IB:Dynamin 2) and tubulin (IB: Tubulin). (D) Localization of pacsin 2 (green), actin (blue) and dynamin 2 (red) and their merged images in control RNAi (Ctrl RNAi) and dynamin 2 RNAi (Dnm 2 RNAi) cells. (E) Proportion of podosomes containing dynamin 2 in control RNAi cells (Ctrl RNAi) and Dnm 2 RNAi cells (Dnm 2 RNAi). Data are means ± SEM ($n \geq 23$ podosomes, $N = 3$). (F) Immunoblot analysis of control RNAi and dynamin 2 RNAi cells using antibodies against dynamin 2 (IB:Dynamin 2), pacsin 2 (IB:Pacsin2) and tubulin (IB: Tubulin). Scale bars are 10 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

dynamin-mediated membrane fission [17]. To identify potential BAR domain proteins associating with dynamin 2 at the podosomes, various BAR domain proteins were screened for their podosome localization. As a result, exogenously expressed GFP-tagged BAR domain proteins (nine N-BAR, seven F-BAR and one I-BAR domain proteins) localized to the podosomes in NIH-Src cells (Fig. 2A and Fig. S3). In contrast, GFP alone failed to localize to the podosomes (Fig. S3). Among these BAR domain proteins examined, PACSIN1, 2 and 3 appeared to both localize to the podosomes (Fig. 2, A) and interact with dynamin 2 by co-immunoprecipitation assay (Fig. 2B). However, immunoblot analysis and immunofluorescence microscopy showed that pacsin 2 was the major pacsin isoform expressed in NIH-Src cells (Figs. S4A and B). Finally, simultaneous immunostaining of endogenous pacsin 2 and dynamin 2 showed that they colocalized to the podosomes in NIH-Src cells (Fig. 2C). These results suggest that pacsin 2 functions at the podosomes together with dynamin 2.

3.3. Interaction between pacsin 2 and dynamin 2 is required for podosome formation in NIH-Src cells

To elucidate pacsin 2 function at the podosomes, endogenous pacsin 2 in NIH-Src cells was depleted by RNAi and its effects on podosome function examined. Resembling dynamin 2 RNAi cells, pacsin 2 RNAi cells exhibited suppressed gelatin degradation activity (Fig. 3A and B; Figs. S5A and B). Furthermore, in pacsin 2 RNAi cells, the proportion of cells with podosomes in rosettes, crescents and disks were decreased, and cells with podosome dots or no podosomes were significantly increased compared to control RNAi cells (Fig. 3A and C; Figs. S5A and C). These phenotypes in pacsin 2 RNAi cells were reconfirmed by using two different siRNA for pacsin 2 (Fig. S5), eliminating possible off-target effects in the RNAi experiments. These results suggest that pacsin 2 is required for formation and maturation of podosomes.

To confirm the podosome formation defects and deficiencies in ECM degradation observed in pacsin 2 RNAi cells are direct effects,

we rescued these phenotypes by expression of FLAG-tagged human PACSIN 2 (Fig. 3A, B and C). In contrast, FLAG-tagged PACSIN 2 ΔSH3, which localizes to the podosomes but was defective in dynamin 2-binding (Fig. S6), failed to rescue the pacsin 2 RNAi phenotype (Fig. 3A, B and C). Expression profiles of the endogenous and exogenous pacsin 2 in the RNAi and rescue experiment was confirmed by immunoblot analyses (Fig. 3D). These data suggest that the pacsin 2-dynamin 2 interaction is required for formation and/or maturation of the podosomes.

3.4. Interdependent localization of pacsin 2 and dynamin 2 to the podosomes

To clarify the requirement of the pacsin 2-dynamin 2 interaction in podosome function, the localization of these proteins was examined after mutual RNAi. In control RNAi cells, 66.8% of the podosomes contained dynamin 2 (Fig. 4A and B), whereas the proportion of the dynamin 2-containing podosomes was decreased in pacsin 2 RNAi cells (30.3% and 27.9%) (Fig. 4A and B). Similarly, in control RNAi cells, 36.5% of the podosomes contained pacsin 2, while the proportion of the pacsin 2-containing podosomes was decreased in dynamin 2 RNAi cells (9.9%) (Fig. 4D and E). Immunoblot analyses showed that RNAi of neither pacsin 2 nor dynamin 2 affected their mutual expression level (Fig. 4C and F). These results suggest that interaction between pacsin 2 and dynamin 2 is crucial for their localization to the podosomes.

4. Discussion

Previous studies showed that dynamin 2 localizes to the podosomes in RSV-transformed cells and osteoclasts [6,7,18] and at the invadopodia in bladder cancer [5] and melanoma [4] cells to regulate actin dynamics during podosomes/invadopodia formation. Dynamin 2 was also implicated in trafficking of MT1-MMP, a master regulator of ECM degradation machinery, at the podosome/invadopodia [19,20]. In this study, we demonstrated that proportion of cells with podosome dots or no podosomes was increased in dynamin 2 RNAi cells (Fig. 1), suggesting that dynamin 2 is involved in formation/maturation of the podosomes in NIH-Src cells.

In our localization-based screening, a BAR domain protein pacsin 2 was identified as a functional partner of dynamin 2 at the podosomes (Fig. 2, Fig. S3 and Fig. S4). Pacsin 2 RNAi cells showed suppressed ECM degradation activity with defective formation/maturation of the podosomes (Fig. 3 and Fig. S5). Pacsin 2 is a member of F-BAR domain protein subfamily involved in intracellular trafficking such as clathrin-mediated endocytosis [21], caveolae-mediated endocytosis [12] and vesicle budding from the trans-Golgi network [22]. Since endocytic and secretion pathways are crucial for intracellular transport of podosome components and lytic enzymes, pacsin 2 may regulate podosome formation and function by controlling these intracellular trafficking pathways. Pacsin 2 is also implicated in actin regulation either in an indirect [23,24] or in direct manner [25]. Thus, pacsin 2 may also contribute to formation of the actin-based architectures at the podosomes.

The PR domain of dynamin 2 was both required and sufficient for its localization to the podosomes (Fig. S2), while pacsin 2 SH3 was dispensable for its localization (Fig. S6). These results suggest that pacsin 2 has a role to recruit dynamin 2 to the podosomes. Consistently, in pacsin 2 RNAi cells, dynamin 2 localization to the podosomes was significantly reduced (Fig. 4A and B). Interestingly, dynamin 2 RNAi cells also showed suppressed localization of pacsin 2 at the podosomes (Fig. 4D and E), suggesting that dynamin 2-pacsin 2 interaction is required for recruitment and/or maintenance of these molecules at the podosomes and may support the structural integrity of the podosomes. Further analyses using live cell imaging and higher resolution microscopy will reveal spatial and temporal arrangements of pacsin 2 and dynamin 2 during podosome formation and maturation in a more precise manner.

BAR domain proteins have been implicated in different processes during podosome formation. For instance, I-BAR domain protein IRSp53 links small GTPase to actin cytoskeletal regulator VASP in podosome formation in NIH-Src cells [26]. Another study demonstrated that F-BAR domain proteins FBP17 and PSTPIP2 antagonistically regulate actin polymerization during podosome formation in macrophages [27]. N-BAR domain protein BIN2 (Bridging Integrator 2) localizes and regulates the podosomes in leucocytes [28]. Interestingly, a recent study showed that N-BAR domain protein BIN1 is recruited to podosomes via membrane invagination, which in turn recruits dynamin 2 to promote integrin endocytosis [29]. Our screening identified multiple BAR domain proteins localizing to podosomes (Fig. S3). Comprehensive analyses of these BAR domain proteins may reveal multiple functions of these conserved membrane remodeling proteins at the podosomes in ECM degradation and cell motility.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrc.2021.07.041.

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