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Palladin Regulates Cell and Extracellular Matrix Interaction Through Maintaining Normal Actin Cytoskeleton Architecture and Stabilizing Beta1-Integrin

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Abstract Cell and extracellular matrix (ECM) interaction plays an important role in development and normal cellular function. Cell adhesion and cell spreading on ECM are two basic cellular behaviors related to cell-ECM interaction. Here we show that palladin, a novel actin cytoskeleton-associated protein, is actively involved in the regulation of cell-ECM interaction. It was found that palladin-deficient mouse embryonic fibroblasts (MEFs) display decreased cell adhesion and compromised cell spreading on various ECMs. Disorganized actin cytoskeleton architecture characterized by faint stress fibers, less lamellipodia and focal adhesions can account for the weakened cell-ECM interaction in *palladin*$^{-/-}$ MEFs. Furthermore, decreased polymerized filament actin and increased globular actin can be observed in *palladin*$^{-/-}$ MEFs, strongly suggesting that palladin is essential for the formation or stabilization of polymerized filament actin. Elevated phospho-cofilin level and proper responses in cofilin phosphorylation to either Rho signal agonist or antagonist in *palladin*$^{-/-}$ MEFs indicate that disrupted stress fibers in *palladin*$^{-/-}$ MEFs is not associated with cofilin phosphorylation. More interestingly, the protein level of ECM receptor β1-integrin is dramatically decreased in MEFs lacking palladin. Down-regulation of β1-integrin protein can be restored by proteasome inhibitor MG-132 treatment. All these data implicate that palladin is essential for cell-ECM interaction through maintaining normal actin cytoskeleton architecture and stabilizing β1-integrin protein. J. Cell. Biochem. 100: 1288–1300, 2007.

Key words: palladin; cell adhesion; cell spreading; Rho GTPase; β1-integrin

Palladin is an actin cytoskeleton associated protein discovered almost simultaneously by two groups. One group from University of North Carolina found that a novel protein is co-localized with α-actinin at the dense regions and focal adhesions of stress fibers. This novel protein was then named as palladin [Parast and Otey, 2000]. Further studies from the same

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group demonstrated that palladin is an important regulator in neuron development [Boukhelifa et al., 2001, 2003; Hwang et al., 2001]. The other group from Shanghai Institute of Haematology identified a series of all-trans retinoic acid (ATRA)-regulated genes in acute promyelocytic leukaemia cell line NB4 treated with ATRA [Liu et al., 2000], including *RIG-K* (Retinoic acid Induced Gene-K), which is highly homologous to mouse palladin. It was originally proposed that RIG-K might function as an important mediator in ATRA-induced myeloid cell differentiation. Gene targeting experiment has shown that disruption of palladin in mice can lead to cranial neural tube closure defect, fetal liver herniation and embryonic lethality [Luo et al., 2005], implicating a crucial role of palladin in mouse embryogenesis. Abnormal actin cytoskeleton dynamics plays a critical role in cranial neural tube closure defect and fetal liver herniation [Copp et al., 2003; Shimizu et al., 2005], however the mechanism by which palladin regulates actin cytoskeleton dynamics remains unclear.

Mouse *palladin* is located on chromosome 8. It spans about 400 Kb of genomic DNA and contains 24 exons. *Palladin* has at least three different transcripts due to alternative splicing. Among them, the longest one encodes a 150 kDa protein whose structure is very similar to myopalladin [Bang et al., 2001]. The middle transcript encodes a 92 kDa protein structurally similar to myotilin [Salmikangas et al., 2003]. Both myopalladin and myotilin are expressed only in muscle cells, while palladin is ubiquitously expressed [Bang et al., 2001; Salmikangas et al., 2003]. To date, no protein product is predicted from the smallest transcript. As an actin cytoskeleton-associated protein, it has been shown that palladin can interact with α-actinin, vasodilator-stimulated phosphoprotein (VASP) and ezrin [Mykkanen et al., 2001; Boukhelifa et al., 2004; Ronty et al., 2004], respectively. Palladin can recruit α-actinin to specific subcellular regions [Ronty et al., 2004]. It may also regulate actin polymerization through the interaction with VASP, since VASP is actively involved in actin polymerization [Bailly, 2004; Boukhelifa et al., 2004]. Ezrin is a component of the cortical cytoskeleton and a tumor metastasis-related protein [Hunter, 2004]. Interaction between palladin and ezrin may be associated with the microfilament localization of ezrin [Mykkanen et al., 2001]. The interactions of palladin with these partner proteins implicate a potential role of palladin in the regulation of actin cytoskeleton and fundamental cellular function.

Cell-extracellular matrix (ECM) interactions are crucial for cell survival and normal cellular function, such as cell adhesion, spreading and migration. Actin cytoskeleton can interact with ECM through integrin family members on the cell membrane. The physical link between integrin adhesion receptors and actin cytoskeleton mediates bidirectional transmission of force and biochemical signals across the plasma membrane [Calderwood et al., 2000; Blystone, 2004]. ECM or other outside signals first activate integrins, transduce signals through adaptor proteins (such as paxilin) to downstream tyrosine kinases (such as focal adhesion kinase), these tyrosine kinases then regulate actin cytoskeleton and cell spreading process mainly through Rho family small GTPase [Degani et al., 2002; Hagel et al., 2002; Zeng et al., 2003]. Cell adhering to ECM is also integrin-dependent. Integrin activation is an important event that governs cell adhesion [Calderwood, 2004]. Intracellular signaling or outside ECM binding can cause integrin conformational change and lead to alteration of integrin-ECM binding affinity. Besides integrin activation, integrin expression and procession are also important for cell adhesion [Salicioni et al., 2004]. To better understand mechanisms by which palladin regulates cell-ECM interaction, we studied cell adhesion and cell spreading properties of palladin-deficient MEFs derived from our previously constructed palladin knockout mice [Luo et al., 2005]. We found that $palladin^{-/-}$ MEFs show weakened cell-ECM interaction, furthermore the disorganized actin cytoskeleton architecture and decreased β1-integrin protein expression in $palladin^{-/-}$ MEFs may account for this weakened cell-ECM interaction. Thus we demonstrate an important role of palladin in the regulation of fundamental cellular functions.

MATERIALS AND METHODS

Preparation and Culture of MEFs

Generation of palladin mutant mice has been described previously [Luo et al., 2005]. To obtain $palladin^{-/-}$ and sibling control wt MEFs, $palladin^{+/-}$ mice were intercrossed. E13.5

embryos were collected and primary embryonic fibroblasts were isolated separately from each embryo according to standard protocols [Hogan et al., 1994]. Genotyping was carried out by PCR on genomic DNA extracted from the head of each embryo as described previously [Luo et al., 2005]. MEFs isolated from embryos with the same genotype were pooled and cultured in DMEM containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 μg/ml streptomycin in a 5% CO_2 humidified incubator at 37°C. After one subculture, aliquots of MEFs from sibling embryos at same passage were frozen for further use.

Reagents and Antibodies

The following antibodies were used in this study: α-actinin (AT6/172, Upstate, MA), vinculin (hVIN-1, Sigma), β1-integrin (18, BD PharMingen), β-actin (AC-74, Sigma), cortactin (Santa Cruz, CA), phospho-cofilin (Ser3) (Santa Cruz, CA), β-catenin (14, BD PharMingen), α-tubulin (B-5-1-2, Sigma) and Cy3-conjugated secondary antibody (Sigma). Polyclonal antibody against palladin was produced as described previously [Luo et al., 2005]. Fibronectin, collagen type I, collagen type IV, lysophosphatidic acid (LPA), Y-27632 and fluorescein isothiocyanate (FITC)-phalloidin were purchased from Sigma. Texas red-DNase I was from Invitrogen, CA and MG-132 from CALBIOCHEM, CA.

Cell Adhesion Assay

Cell adhesion assay was performed on 96-well tissue culture plates as described previously [Xu et al., 1998]. Briefly, 96-well plates were coated with ECMs (fibronectin, collagen type I or collagen type IV) at indicated concentrations. Primary MEFs were serum starved for 16 h, then trypsinized and suspended in serum free DMEM. Cells (5×10^4) were seeded to each well and allowed to adhere at 37°C for indicated time. The attached cells were fixed for 10 min at room temperature by adding 100 μl/well of freshly diluted 1% glutaradehyde in PBS. The fixed cells were stained with filtered crystal violete (0.1% in ddH_2O) for 25 min at room temperature. Dried plate was subjected to either photographing or reading absorbance at OD 595 nm after solubilization with 0.5% Triton X-100.

Cell Spreading Assay

Cell spreading assay was performed according to literature [Enserink et al., 2004]. Coverslips were coated with fibronectin (10 μg/ml), collagen type I (5 μg/ml) or collagen type IV (5 μg/ml) in PBS at 4°C overnight. Cells (1.5×10^5 per well in case of six-well plate) were directly transferred to ECM-coated coverslips, and subjected to adhere at 37°C for 10 min on fibronectin-coated coverslips, and 25 min on collagen type I or collagen type IV-coated coverslips. After removing culture medium, cells were fixed immediately at room temperature for 10 min by adding 4% polyformaldehyde (PFA) directly to coverslips in six-well plate, permeabilized with 0.1% Triton X-100 in PBS for 2 min at room temperature. Then cells were stained with FITC conjugated phalloidin in PBS for 20 min at room temperature. Coverlips were mounted in immunofluorescence mounting solution (DAKO) and visualized at 200× magnification using a cooled CCD mounted on an inverted fluorescence microscope (Olympus).

Immunofluorescence

Sterile and clean coverslips were coated with 10 μg/ml fibronectin in PBS at 4°C overnight. Confluent primary MEFs were trypsinized and 1.2×10^5 cells were seeded to each well of six-well tissue culture plates. Twenty-four hours after plating, cells were fixed in 4% PFA for 10 min at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 2 min at room temperature, blocked in 1% BSA in PBS at room temperature for 45 min, then incubated with following antibodies: α-actinin (1/50), vinculin (1/200), α-tubulin (1/800), cortactin (1/100), palladin (1/300) at room temperature for 2 h, respectively. Coverslips were incubated with Cy3 conjugated anti-mouse or anti-rabbit secondary antibody (1/100) at room temperature for 45 min. Finally, coverslips were washed with 0.1% Triton X-100 in PBS three times, and mounted with immunofluorescence mounting solution (DAKO).

F-Actin Sedimentation Assay

Actin sedimentation assay was performed as described previously [Jahraus et al., 2001]. Confluent MEFs were trypsinized and suspended in 1 ml lysis buffer (10 mM Hepes, pH 7.6, 100 mM KCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF). Cells were further broken with a sonicator. Nuclei were removed by centrifugation (3,000*g*, 30 min) and 800 μl supernatant (low-speed extract) was subjected to a high-speed centrifugation step (400,000*g*,

1 h). High-speed pellets were dissolved in 800 μl of 1% Triton X-100. At these high centrifugal forces all filament-actin (F-actin) in the system is expected to pellet, leaving globular-actin(G-actin) in the supernatant. Protein concentration of low-speed extract was measured. Same amount of protein in low-speed supernatant, corresponded volume of high-speed supernatant and pellet were loaded on a 10% SDS-PAGE. Western blot was performed with anti-actin antibody (A2066, Sigma).

Western Blot

For cofilin phosphorylation assays, primary confluent MEFs were serum starved for 30 min and treated with 200 ng/ml LPA for 20 min in the absence or presence of gradient Y-27632. For β1-integrin processing assay, primary confluent MEFs were treated with 20 or 50 μM proteasome inhibitor MG-132 in complete culture medium (DMEM+10% FBS) for 24 h. Then cells were lyzed with radioimmunoprecipitation assay buffer (RIPA) containing protease and phosphatase inhibitors. Lysates were clarified by centrifugation for 15 min at 4°C. Protein concentration was determined by Lowry assay. Cell lysates were analyzed by SDS-PAGE. Proteins were electroblotted onto PVDF membrane (Bio-Rad) and probed with appropriate primary and peroxidase conjugated secondary antibodies. Signals were detected by enhanced chemiluminescence.

Semi-Quantative RT-PCR

Total cellular RNA was extracted using Trizol reagent. One microgram RNA was reverse-transcribed with AMV reverse transcriptase. Semi-quantative PCR was performed by using the following primers: *Palladin* (5′-TCAACATCCAGGAGCCAGAG-3′ and 5′-TAGAGGCCGTGGTGTGGAGA-3′); *α-actinin4* (5′-AAACCAGATCCTCACCCGAGAT-3′ and 5′-CTCCAATCGGCTAAGAACCCAC-3′); *β1-integrin* (5′-GCTGGTTCTATTTCACCTATTCA-3′ and 5′-TCTCAAGTCTTCTGTCAGzTCCCT-3′);*cortactin*(5′-GCAAGCTGACCGTGTAGACAA-3′ and 5′-ATGGCACCTGGACCACTTC-3′); *vinculin* (5′-ATGCTGGCTTTACTCTGCG-3′ and 5′-CTTCCGGCTAGTGTTCACG-3′); *β-actin* (5′-GCTTCTTTGCAGCTCCTTCGTT-3′ and 5′-GATGCCACAGGATTCCATACCC-3′); *p130CAS* (5′-ACCCACTCCCAGCAAGACTCAG-3′ and 5′-AACTACACTCCCATCAGCCACCTC-3′); *zyxin* (5′-CTGGACGACATGACCAAGAACG-3′ and 5′-CTGACACTGATGGCAAGTGAAGC-3′); *Ril* (5′-ATGACAAGGCTCAAGCACATAGG-3′ and 5′-AAGTGAAGCAGAGGCAAGACAAG-3′); *Hprt* (5′-GCTGGTGAAAAGGACCTCT-3′ and 5′-CACAGGACTAGAACACCTGC-3′).

Filament Actin and Globular Actin Staining

Primary MEFs were allowed to grow on fibronectin-coated coverslips for 24 h, fixed with 4% PFA for 10 min at room temperature and then, permeabilized with 0.1% Triton X-100 for 2 min at room temperature. The cells were incubated with FITC-phalloidin (1 mg/L) and Texas red-DNase I (25 mg/L) for 30 min to stain filament actin and globular actin, respectively. After wash, the coverslips were air dried and mounted with immunofluorescence mounting medium (DAKO) to prevent photobleaching. The slides were examined using a Zeiss LSM 510 confocal microscope. To standardize the fluorescence measurements, the settings were optimized before each experiment and then kept constant throughout.

RESULTS

Palladin-Deficient MEFs Show Decreased Adhesion on Various Kinds of ECMs

Cell adhesion is critically involved in the regulation of morphogenesis during embryonic development in both vertebrates and invertebrates. It is an actively regulated process by both outside and inside signals through actin cytoskeleton. We first analyzed the adhesion properties of *palladin*$^{-/-}$ MEFs on different kinds of ECMs including fibronectin, collagen type I and collagen type IV. We found that palladin-deficient MEFs are defective in cell adhesion on the three ECMs tested. The defect in cell adhesion of *palladin*$^{-/-}$ MEFs is more significant on collagen types I and IV than that on fibronectin (Fig. 1A). To further characterize the adhesion property of *palladin*$^{-/-}$ MEFs, we compared the cell adhesion of mutant and wt MEFs under different ECM concentrations and different adhesion time, respectively. Palladin-deficient MEFs show consistently decreased adhesion to each substratum compared to wt MEFs. As shown by ECM concentration curves, both types of MEFs reach adhesion platform at the same concentration, but palladin mutant MEFs show much lower adhesion under each substratum concentration (Fig. 1B). Adhesion

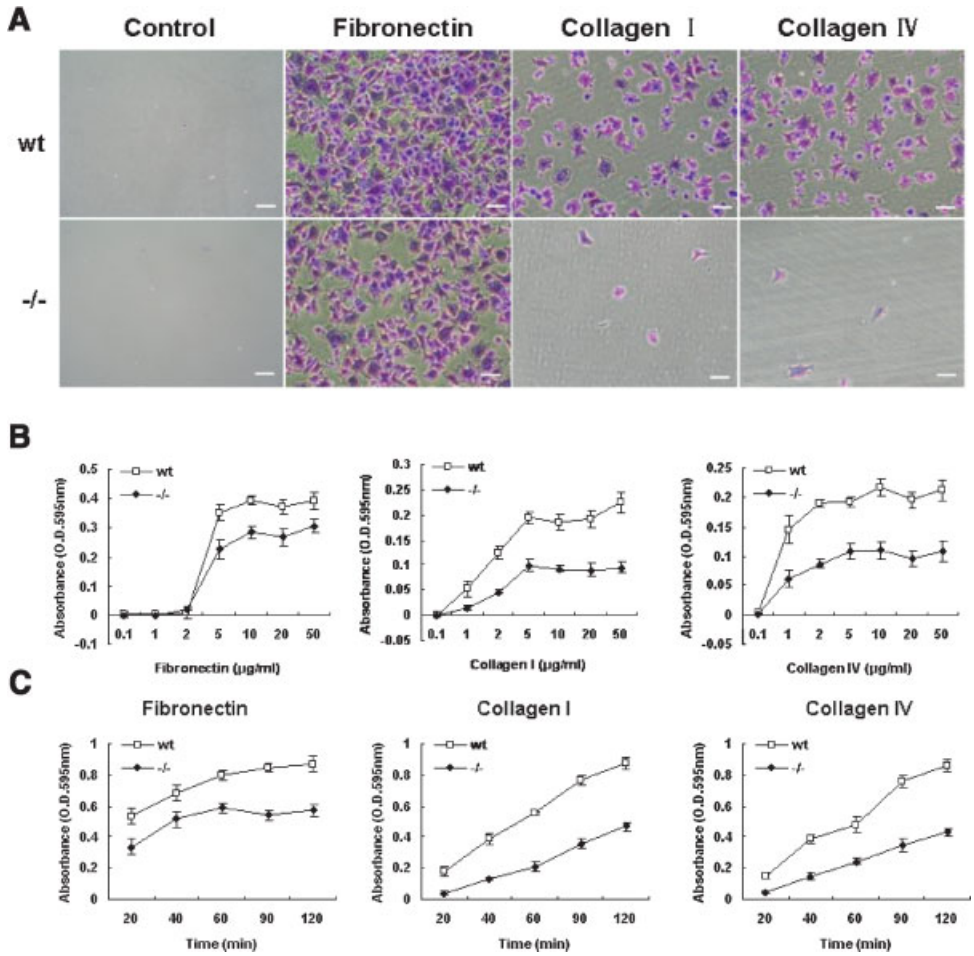

Fig. 1. Decreased adhesion of *palladin*$^{-/-}$ MEFs on different ECMs. **A**: Equal numbers of wt and *palladin*$^{-/-}$ MEFs were allowed to adhere to fibronectin (10 μg/ml), collagen type I (5 μg/ml), collagen type IV (5 μg/ml) coated surfaces for 30 min. Non-ECM coated surface was served as a control. Scale bar, 50 μm. **B**: Cell adhesion properties of MEFs on gradients of purified ECMs. As indicated, MEFs adhere to three different ECMs in a dose-dependent manner, while *palladin*$^{-/-}$ MEFs show impaired adhesion on the surfaces coated with different concentrations of fibronectin, collagen type I and collagen type IV, respectively. **C**: Time courses of cell adhering to fibronectin (10 μg/ml), collagen type I (5 μg/ml) and collagen type IV (5 μg/ml) coated surfaces at the indicated time points were measured, respectively. One representative result of three independent experiments is shown. The results are expressed as mean of quadrupled samples ±SD.

time courses show that MEFs with the two different genotypes have near linear curves, while *palladin*$^{-/-}$ MEFs display much less adherent cells at each adhesion time point in comparison with wt MEFs (Fig. 1C).

Impaired Cell Spreading on Various ECMs in Palladin-Deficient MEFs

Suspended fibroblasts will extend lamellipodia and filopodia after they attach to ECMs. This process is regulated by many kinds of extracellular and intracellular signals. Actin cytoskeleton dynamics plays a pivotal role in cell spreading process. Some actin cytoskeleton regulators or signal transducers are required for cell spreading. Cells lacking either adaptor protein paxillin or protein-tyrosine phosphatase Shp2 exhibit decreased spreading ability [Yu et al., 1998; Hagel et al., 2002]. To address whether palladin is also involved in cell spreading process, we plated serum-starved MEFs on fibronectin, collagen type I or collagen type IV coated coverslips and allowed cells to spread for indicated times. When MEFs were allowed to spread on 10 μg/ml fibronectin for 10 min, or 25 min on 5 μg/ml collagen type I or collagen type IV, nearly half of wt MEFs extended lamellipodia and started spreading, while most *palladin*$^{-/-}$ MEFs were unable to spread (Fig. 2). These data clearly demonstrate that palladin may play a crucial role in cell spreading and adhesion on the ECMs tested.

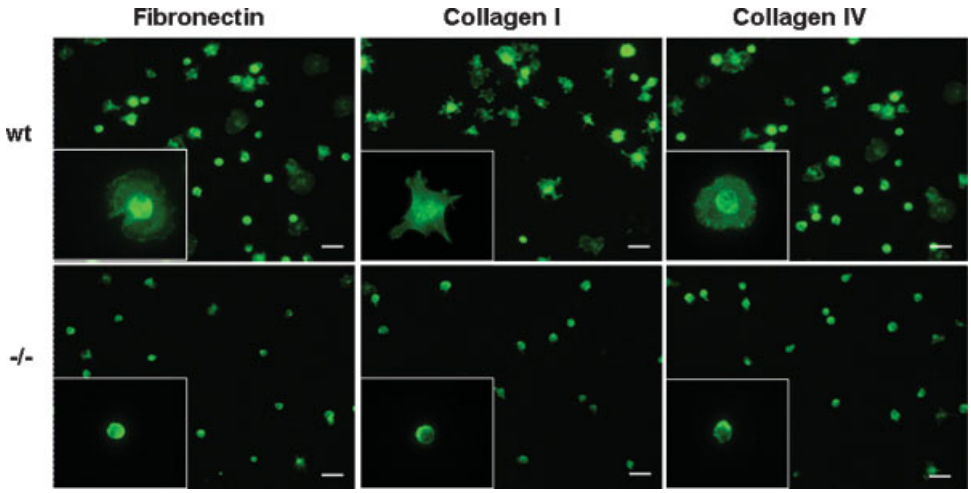

Fig. 2. Defect of cell spreading in *palladin*$^{-/-}$ MEFs. **A**: Serum-starved MEFs were allowed to spread on fibronectin (for 10 min), collagen type I or collagen type IV (for 25 min) coated coverslips in serum-free medium at 37°C, and then fixed and stained with FITC-phalloidin. Nearly half wt MEFs extend their lamellipodia within a certain period of time on the ECM coated surfaces as indicated, while *palladin*$^{-/-}$ MEFs do not spread at the same conditions. Inserts are higher magnification images; scale bar, 50 μm.

Palladin Is Required for the Formation of Normal Actin Cytoskeleton Architecture

Normal actin cytoskeleton is critical for cell adhesion, migration, and morphology maintenance. As previously reported, palladin localizes along stress fibers (Fig. 3A), and stress fibers become extremely faint and disordered in MEFs without palladin (bottom left insert in Fig. 3B). This is similar to the finding of decreased stress fibers in fibroblasts in which palladin expression was inhibited by using an antisense approach [Parast and Otey, 2000], suggesting that palladin plays a role in the formation or stabilization of stress fibers. It is known that β-actin is the main component of actin cytoskeleton in fibroblasts. It may exist in two forms: polymerized filament actin and unpolymerized globular actin. Interestingly, unpolymerized β-actin is increased while polymerized β-actin is decreased in *palladin*$^{-/-}$ MEFs compared to control wt MEFs (Fig. 3C,D). We further compared filament (FITC-phalloidin staining) and globular (Texas red-DNase I staining) actin distributions between wt and *palladin*$^{-/-}$ MEFs. Similar results were observed (Fig. 3E,F). We also performed actin sedimentation assay to detect filament actin and globular actin level in wt and *palladin*$^{-/-}$ MEFs. The results show that *palladin*$^{-/-}$ MEFs have increased globular actin and decreased filament actin level (Fig. 3G).

α-actinin is a well characterized interaction partner of palladin [Parast and otey, 2000; Ronty et al., 2004]. We found that palladin disruption leads to abnormal distribution of α-actinin. In wt MEFs α-actinin is distributed in the dense regions and focal adhesions of stress fibers, but in palladin mutant MEFs α-actinin staining displays a more diffused and cytoplasmic non-cytoskeleton associated distribution (Fig. 4A,B). This data indicates that palladin is required for the proper localization of α-actinin through maintaining normal stress fibers. It is known that α-actinin is a dense region marker on stress fibers. It is required for F-actin crosslinking into stress fibers [Otey and Carpen, 2004]. The attenuated stress fibers in palladin-deficient MEFs may be associated with the dislocalization of α-actinin.

Focal adhesion is important for cells to adhere to ECM. It often terminates at the end of stress fibers, and can also be visualized by immunofluorescent staining of vinculin. The result reveals that centrally distributed focal adhesions are largely missing from the palladin null cells, and only peripheral focal adhesions remain (Fig. 4C,D). This finding is similar to images of palladin knockdown cells that have been published previously [Parast and Otey, 2000], indicating that decreased focal adhesions may be resulted from the disruption of stress fibers due to palladin deficiency.

Membrane cytoskeleton, as indicated by cortactin staining, is also affected after palladin

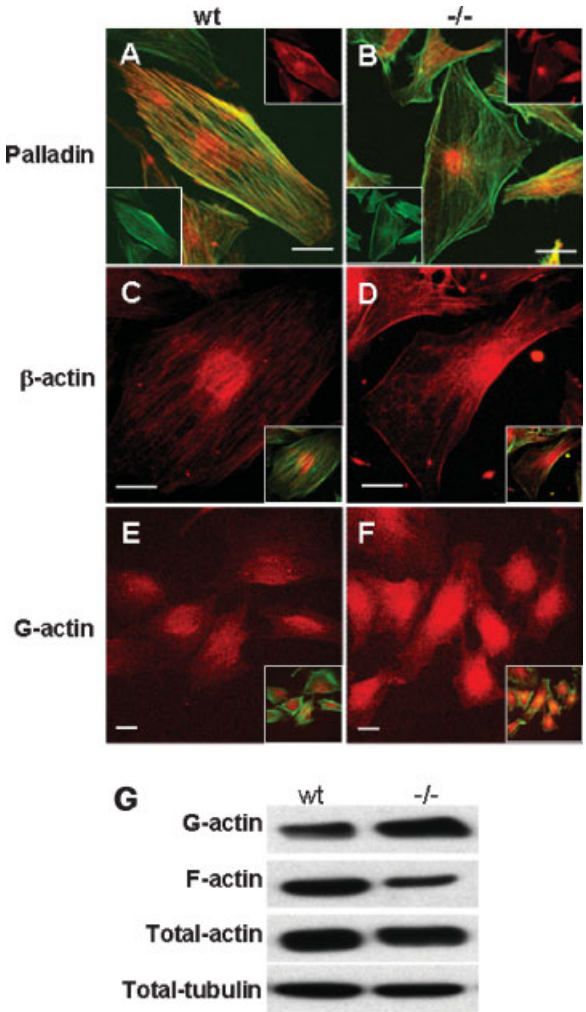

Fig. 3. Decreased filament actin (F-actin) and increased globular actin (G-actin) in *palladin*$^{-/-}$ MEFs. wt (**A**) or *palladin*$^{-/-}$ (**B**) MEFs were stained with a polyclonal antibody against palladin, visualized with a Cy3-conjugated secondary antibody (red, top right insert) and stress fibers were visualized with FITC-phalloidin staining (green, bottom left insert), two color-merged images are shown. Co-localization of palladin and stress fibers in wt MEFs and markedly attenuated stress fibers in the absence of palladin can be observed. Immunofluorescent staining of β-actin in wt (**C**) or *palladin*$^{-/-}$ (**D**) MEFs. Double staining for β-actin (red) and stress fibers (green, inserted) shows overlapping distribution in wt MEFs (C), whereas filament actin as well as stress fibers are almost abolished, as a result, unpolymerized globular β-actin is increased in cells lacking palladin (D). **E**, **F**: MEFs were stained with Texas red-DNase I and FTTC-phalloidin. As shown in F, G-actin is increased in palladin-deficient MEFs with a decrease in F-actin (inserted) compared with wt MEFs (E & inserted). Scale bar, 20 μm. **G**: G-actin is increased with a decrease in F-actin in *palladin*$^{-/-}$ MEFs compared with wt MEFs as measured by actin sedimentation assay. Total actin and tubulin are shown as loading control.

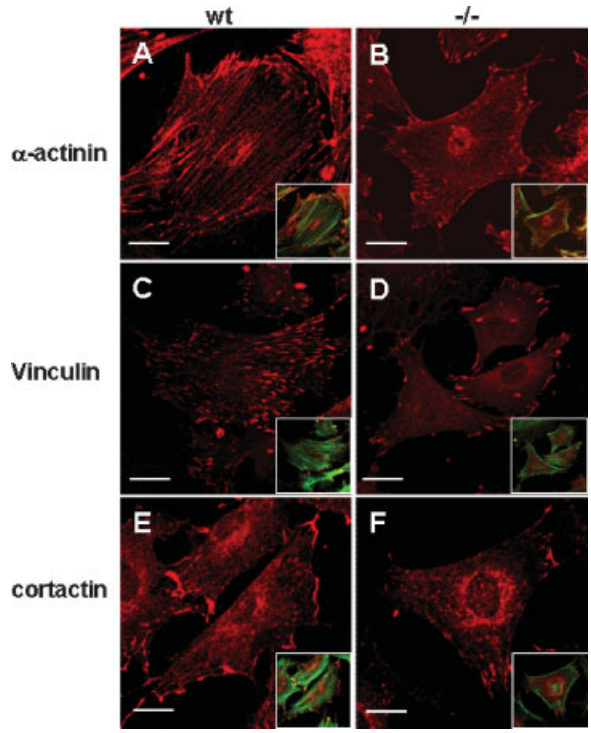

Fig. 4. Abnormal actin cytoskeleton architecture in *palladin*$^{-/-}$ MEFs. Immunofluorescence staining of actin cytoskeleton associated protein α-actinin (**A**, **B**), vinculin (**C**, **D**) and cortactin (**E**, **F**) in wt (A, C, E,) or *palladin*$^{-/-}$ (B, D, F) MEFs. α-actinin is normally co-localized with palladin along F-actin in dense regions and focal adhesions as shown in A, while a remarkable decrease in dense regions and focal adhesions, and more diffused distribution of α-actinin can be observed in MEFs lacking palladin (B). Focal adhesions can also be visualized by immunofluorescent staining of vinculin. Disruption of palladin leads to a dramatic reduction in the number of focal adhesions (D) as compared with wt MEFs (C). Cortactin is a marker of membrane cytoskeleton. In *palladin*$^{-/-}$ MEFs, membrane-localized cortactin is significantly decreased (F) as compared with wt MEFs (E). Stress fiber distributions (green) and co-localization with α-actinin, vinculin and cortactin (red) in wt and *palladin*$^{-/-}$ MEFs are shown in inserts, respectively.

disruption (Fig. 4E,F). Cortactin localizes to the membrane and perinuclear region. It can interact with VASP and plays an important role in actin polymerization when it localizes to the membrane region [Weed and Parsons, 2001]. Cortactin can be regarded as one of the lamellipodia markers. In steady state, *palladin*$^{-/-}$ MEFs showed attenuated lamellipodia formation compared to wt MEFs. This finding may underlie the spreading defects in *palladin*$^{-/-}$ MEFs, since lamellipodia extension is an essential step in cell spreading.

Palladin and the Rho Signal Pathway

Cells can reply to outside actin cytoskeleton regulation signals (such as those induced by LPA, insulin etc.) through Rho family small GTPases. Rho, Rac and Cdc42 are three-well characterized members of Rho family GTPases.

They regulate different aspects of actin cytoskeleton architecture. It has been shown that Rho activation can lead to the formation of stress fibers and associated focal adhesions, and the contractile rings necessary for cytokinesis. Rac regulates the formation of lamellipodia. Cdc42 can induce filopodia formation [Hall, 1998; Burridge and Wennerberg, 2004]. Palladin-deficient MEFs have significantly attenuated stress fibers. This is similar to the phenotype induced by inhibition of Rho signal transduction. But the relationship between the Rho signal pathway and palladin remains unclear.

Previous studies have shown that Rho stabilizes stress fibers through cofilin and myosin light chain (MLC) phosphorylations [Etienne-Manneville and Hall, 2002]. We tested cofilin phosphorylation levels in wt and palladin mutant MEFs under normal culture condition. It was found that cofilin phosophorylation levels were consistently higher in palladin mutant MEFs than wt cells (Fig. 5A). This is quite unexpected since Rho signal can stabilize filament actin through cofilin phosphorylation. We further examined cell responses to Rho signal pathway agonist LPA and inhibitor Y-27632 in palladin mutant MEFs. The results show that

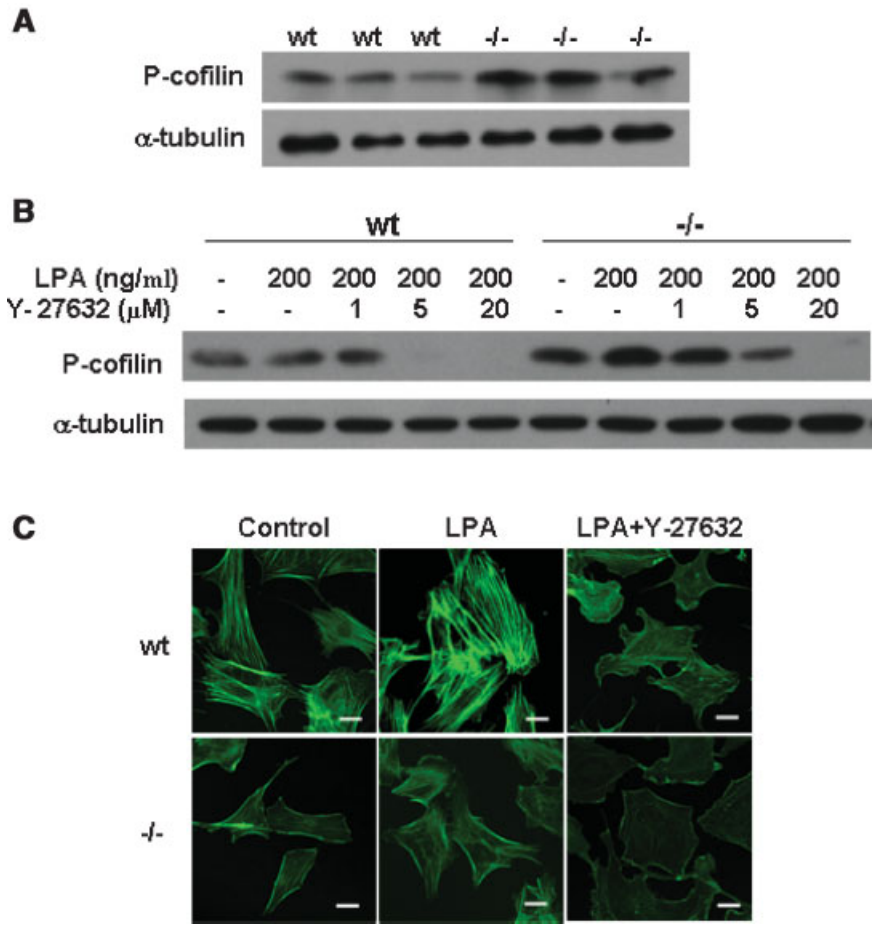

Fig. 5. Palladin and Rho signal pathway. **A**: Protein lysates were prepared from three different batches of wt or *palladin*$^{-/-}$ MEFs and Western blot analysis was performed with an antibody recognizing phospho-Ser3 of cofilin. As indicated in (A), *palladin*$^{-/-}$ MEFs show increased phospho-cofilin levels compared with wt MEFs. α-tubulin was used as loading control. **B**: MEFs were serum-starved and treated with Rho signaling agonist LPA (200 ng/ml) or LPA (200 ng/ml) plus gradient Rho signaling inhibitor Y-27632 (from 1 to 20 μM) for 20 min. Western blot using the same antibody against phospho-Ser3 of cofilin shows a high level of phospho-cofilin in *palladin*$^{-/-}$ MEFs and comparable responses to LPA and LPA plus Y-27632 between wt and *Palladin*$^{-/-}$ MEFs. **C**: MEFs were grown on fibronectin coated coverslips, and then serum-starved and treated with LPA (200 ng/ml) or LPA (200 ng/ml) plus Y-27632 (20 μM) as in (B). Stress fibers are intensified upon LPA treatment and almost abolished in the presence of both LPA and Y-27632 in wt MEFs. While less response in stress fibers formation upon LPA treatment and complete abolishment of stress fibers after Y-27632 treatment can be observed in *palladin*$^{-/-}$ MEFs, suggesting that alteration of stress fibers in *palladin*$^{-/-}$ MEFs may not be associated with cofilin phosphorylation or Rho signal pathway; Scale bar, 20 μm.

palladin-deficient MEFs can respond properly to both reagents as compared with wt MEFs, although MEFs lacking palladin show consistently higher phospho-cofilin levels and attenuated stress fibers under each stimulating condition (Fig. 5B,C). This data indicates that palladin may regulate actin cytoskeleton in a Rho signal pathway independent manner. The elevated cofilin phosophorylation level in palladin-deficient MEFs may reflect a compensatory response to weakened stress fibers.

Decreased Expression of β1-Integrin in Palladin$^{-/-}$ MEFs

Cell adhesion and cell spreading rely on the interaction between cell and ECM, and integrins are critical for this kind of interaction. To date, there are at least 18 α and 9 β subunits identified [Miranti and Brugge, 2002]. Among them, β1-integrin is well known for its involvement in cell adhesion to fibronectin and collagen [Brakebusch et al., 1997]. For this reason, we checked β1-integrin expression in MEFs. We found that expression of β1-integrin in MEFs lacking palladin is significantly decreased at the protein level (Fig. 6B,C), but remains comparable to wt MEFs at mRNA level (Fig. 6A). Since the expression of β1-integrin is critically required for cell adhesion and cell spreading, down-regulation of β1-integrin may underlie cell adhesion and spreading defects in *palladin*$^{-/-}$ MEFs. It also raised a possibility for the potential role of palladin in stabilization of β1-integrin. To demonstrate this possibility, we treated MEFs with a proteasome inhibitor MG-132 to inhibit protein degradation through proteasome. As expected, β1-integrin level in *palladin*$^{-/-}$ MEFs was restored to normal levels after MG-132 treatment (Fig. 6D), suggesting that palladin can protect β1-integrin from degradation through the proteasome pathway.

We also tested the expression of other actin cytoskeleton associated genes at protein and mRNA levels, such as cortactin, α-actinin4, vinculin, β-actin, p130CAS, zyxin, RIL, β-catenin. At the transcriptional level, there were no differences in the expression of all above genes between wt and *palladin*$^{-/-}$ MEFs (Fig. 6A). While at the protein level, aside from β1-integrin, we also found that the expression of cortactin, a membrane actin cytoskeleton associated protein, is slightly decreased in *palladin*$^{-/-}$ MEFs (Fig. 6B,C) while all other proteins showed similar levels in both wt and *palladin*$^{-/-}$ MEFs.

DISCUSSION

Our studies demonstrate unequivocally that palladin plays a critical role in cell and ECM interaction. Cellular behaviors related to this interaction, such as cell adhesion and cell spreading, are compromised by the palladin disruption. The impaired cell adhesion and spreading may result from disorganized actin cytoskeleton and decreased expression of β1-integrin protein.

β1-integrin is translated as an 87 kDa peptide. This premature peptide is further processed and glycosylated into a 130 kDa mature form in the endoplasmic reticulum (ER) and Golgi apparatus [Akiyama and Yamada, 1987]. Down-regulation of β1-integrin can arise from abnormal transcription, glycosylation, and proteiolysis. Our data indicates that cytoskeleton protein palladin is involved in the proteolysis process of β1-integrin. By using a proteasome inhibitor MG-132, we found that the protein level of β1-integrin remains unchanged in wt MEFs but is significantly elevated in *palladin*$^{-/-}$ MEFs. Thus the proteasome lysis of β1-integrin is not significant in wt MEFs, but increased after palladin disruption.

Current studies have shown that many factors control the expression regulation of β1-integrin. Focal adhesion protein talin is an example. In *talin*$^{-/-}$ cells, the maturation of β1-integrin was completely inhibited and no mature β1-integrin was detected. The defect in the protein processing can be caused by both partial glycosylation and proteolytic cleavage of immature peptide [Albiges-Rizo et al., 1995; Martel et al., 2000]. Other proteins such as membrane-bound chaperone calnexin, kinesin associated protein kinectin, immunoglobulin superfamily protein HEMCAM/CD146, RAP [low-density lipoprotein receptor-related protein (LRP) receptor-associated protein], transforming growth factor-beta (TGF-beta), and the activation state of Ras also regulate the maturation of β1-integrin [Ignotz and Massague, 1987; Lenter and Vestweber, 1994; Bellis et al., 1999; Alais et al., 2001; Tran et al., 2002]. Our data implied another type of β1-integrin expression regulation that occurred through the proteasome lysis of the mature form. But how palladin regulates the stability of β1-integrin remains to

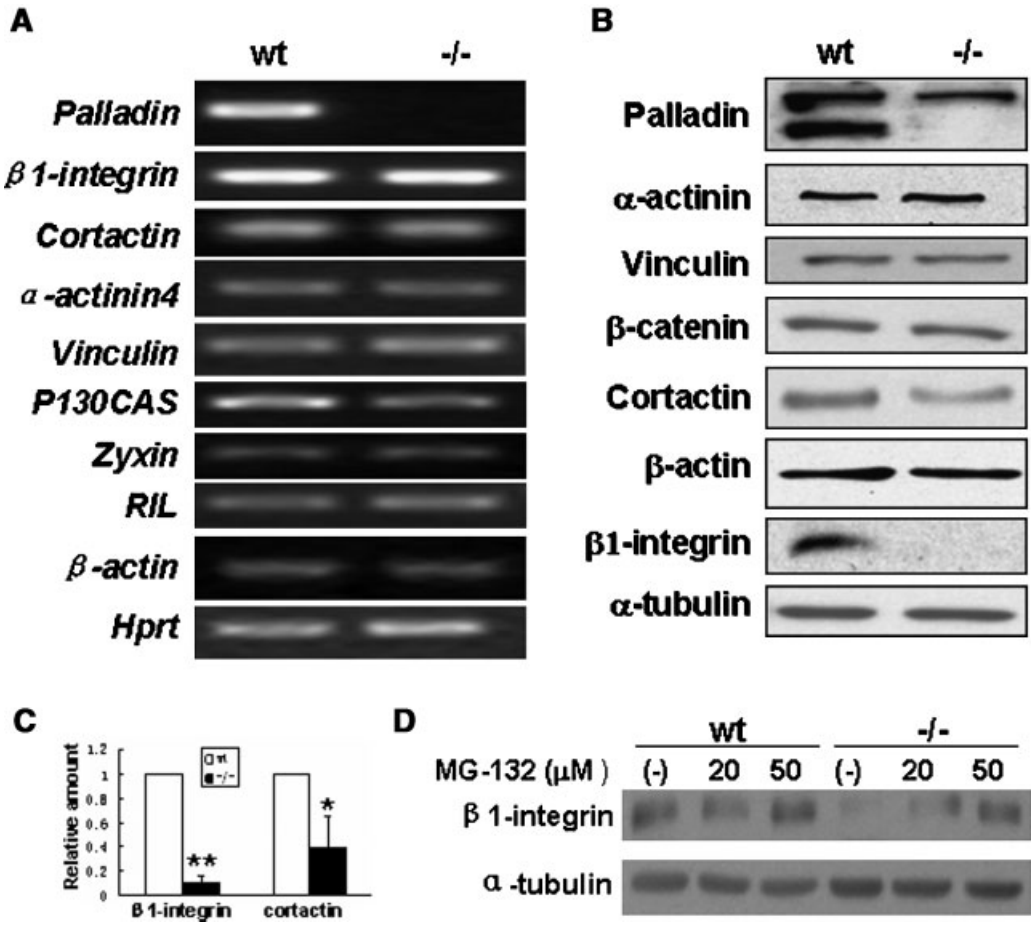

Fig. 6. Decreased expression of β1-integrin in palladin-deficient MEFs. **A**: Expression of actin cytoskeleton related gene *β1-integrin, cortactin, α-actinin4, vinculin, β-actin, p130CAS, zyxin* and *Ril* in wt and *palladin*$^{-/-}$ MEFs assayed by semi-quantitative RT-PCR. *Hprt* is shown as an internal control. No significant differences between wt and *palladin*$^{-/-}$ MEFs were observed in the expression of these genes at mRNA level. **B**: Expression of actin cytoskeleton associated protein palladin, α-actinin, vinculin, β-catenin, cortactin, β-actin, and β1-integrin as assayed by Western blot analysis. Note that β1-integrin is significantly decreased and cortactin is slightly decreased in *palladin*$^{-/-}$ MEFs. All other proteins assayed remain comparable to those in wt MEFs. α-tubulin is shown as loading control. Note the larger size bands detected by the palladin antibody in both wt and *palladin*$^{-/-}$ lanes are non-specific. **C**: Quantification of endogenous β1-integrin and cortactin levels in four different batches of wt and *palladin*$^{-/-}$ MEFs by Western blot and density scan. Expression of β1-integrin and cortactin in *palladin*$^{-/-}$ MEFs decreased to 10 and 40% of those in wt MEFs ($^{*}P < 0.05$; $^{**}P < 0.01$), respectively. **D**: Decreased β1-integrin level can be rescued by proteasome inhibitor MG-132 treatment. Wt or *palladin*$^{-/-}$ MEFs were grown to confluence and treated with 20 or 50 μM MG-132 for 24 h. Whole-cell lysates were subjected to Western blot with a β1-integrin antibody. In wt MEFs, the expression of β1-integrin shows no response to MG-132 treatment. Whereas β1-integrin protein level can be restored, in a dose-dependent manner, to that of wt MEFs, indicating that decreased β1-integrin expression is mainly due to increased degradation of β1-integrin in the absence of palladin.

be determined. Previous study showed that integrin at the cell membrane is rapidly recycled through membrane internalization and endosomes traffic [Caswell and Norman, 2006]. We propose that palladin may inhibit this post-biosynthetic β1-integrin trafficking and consequent β1-integrin degradation through its association with β1-integrin at the cell membrane. Until now, no data indicates a direct interaction between palladin and β1-integrin. But palladin can interact with β1-integrin at least indirectly since palladin's interaction partner α-actinin can interact with the cytoplasmic tail of β1-integrin directly. Another possible reason for the stability regulation of β1-integrin is related to the attenuated stress fibers and decreased focal adhesions in *palladin*$^{-/-}$ MEFs. Attenuated stress fibers may cause "overproduced" β1-integrin which can be degraded through membrane internalization and the proteasome lysis pathway. Whereas, it is also possible that decreased expression of

β1-integrin is a cause, rather than a consequence of the disruption of stress fibers in *palladin*$^{-/-}$ MEFs.

Expression regulation of integrin is critically involved in tumor progression and cell differentiation [Gogali et al., 2004; Guo and Giancotti, 2004; Lafuste et al., 2005]. Palladin may be served as a target for drugs that aim to suppress tumor through the regulation of integrin expression. The expression regulation of palladin mainly occurs at the transcriptional level [Liu et al., 2000; Pohl et al., 2005], it will be of interest to investigate the signals that regulate the expression of palladin at mRNA level.

Actin cytoskeleton is mainly composed of actin and actin associated proteins. It forms a meshwork for nearly all kinds of cytoplasmic biological reactions. Its dynamic regulation is critically involved in most cell behaviors such as cell adhesion, spreading, migration, proliferation, etc. Since palladin is an actin cytoskeleton associated protein, we are interested in the roles palladin played in actin cytoskeleton architecture regulation. We found that actin cytoskeleton architectures such as stress fibers, focal adhesions, lamellipodia were impaired after palladin disruption. The phenotype in the strength of stress fibers is especially significant. Filament actin levels in *palladin*$^{-/-}$ MEFs are significantly decreased, while the total amount of actin remains unchanged in *palladin*$^{-/-}$ cells.

Focal adhesion lies at the rear of stress fibers. It is a dynamic structure [Zimerman et al., 2004] that can disassembly and reform during cell spreading and migration. Focal adhesion number is correlated with the strength of stress fibers. Swiss 3T3 cells had weak stress fibers and peripheral focal adhesion when cultured under serum free conditions. In the presence of LPA (a Rho signal pathway activator), swiss 3T3 cells can form strong stress fibers with increased focal adhesion number [Hall, 1998]. Therefore, decreased focal adhesion number in *palladin*$^{-/-}$ MEFs may be the result of attenuated stress fibers in mutant MEFs.

Palladin$^{-/-}$ cells are more shrinking, and form less lamellipodia under normal culture conditions as indicated by cortactin staining. We also found that the expression of cortactin is downregulated at the protein level in palladin-deficient MEFs. It has been reported that cortactin regulates lamellipodia formation through cortical actin assembly [Zeng et al., 2003]. In addition, the leading edges of lamellipodia are major sites for actin polymerization. The Arp2/3 complex has been implicated in nucleating actin polymerization at these sites [Welch et al., 1997; Mullins et al., 1998]. Thus actin polymerization can also be affected by down-regulated cortactin expression and lamellipodia formation.

Actin polymerization and filament actin stability have been proposed to be targets of actin cytoskeleton regulation enrolled by tumor cells to become metastasic [Rao and Li, 2004]. It is known that filament actin levels can be regulated by (i) decreased monomeric globular actin polymerization rate and (ii) decreased stability of polymeric F-actin. In this study, we found that filament actin levels are significantly decreased and unpolymerized globular actin accumulates in *palladin*$^{-/-}$ MEFs. It is possible that palladin is involved in both polymerization of globular actin and stability of filament actin. Palladin localizes along filament actin. It may stabilize existing filament actin through assembling them into more stable forms—stress fibers. Although there are no F-actin or G-actin binding domains on palladin, we still cannot rule out the possibility that palladin can bind and bundle filament actin directly, since myotilin, a homolog of palladin, can cross link actin filament directly [Salmikangas et al., 2003]. On the other hand, palladin may act through the interaction with VASP or through regulating cortical actin cytoskeleton to monitor the polymerization of globular actin into filament actin.

The dynamic regulation of actin cytoskeleton is mainly exerted by two kinds of proteins: signal transducers and actin cytoskeleton structural proteins. Rho family small GTPases can function as typical signal transducers that regulate actin cytoskeleton. The second kind of actin cytoskeleton regulators are actin cytoskeleton associated structural proteins, such as palladin. Newly identified actin cytoskeleton associated protein RIL and FHL3 may also function as actin cytoskeleton regulators, since these proteins can function directly in actin cytoskeleton dynamics regulation as structural proteins [Coghill et al., 2003; Vallenius et al., 2004]. Rho signaling is well known for its ability to regulate the stability of filament actin through the phosphorylation of cofilin and MLC. At least four kinases responsible for cofilin phosphorylation have been identified: LIM-kinase 1 and 2 and TES-kinase 1 and 2. LIMK2 is specifically phosphorylated and acti-

vated by Rho-associated, coiled-coil-forming protein kinase (ROCK) downstream of Rho [Ozawa et al., 2005]. Unexpectedly we found that cofilin phosphorylation level is elevated in *palladin*$^{-/-}$ MEFs. Upon LPA and Y-27632 treatments, there are comparable responses in cofilin phosphorylation between wt and *palladin*$^{-/-}$ MEFs, which are accompanied by less response in stress fibers formation to LPA treatment and complete disappearance of stress fibers after Y-27632 treatment in *palladin*$^{-/-}$ MEFs. These data suggest that disruption of stress fibers in *palladin*$^{-/-}$ cells may not be associated with changed cofilin phosphorylation. Thus we propose that palladin may function as a structural protein, an "organizer" in stress fibers formation or stabilization. However, the reason why phospho-cofilin level is elevated in *palladin*$^{-/-}$ cells remains unclear. It could be simply a compensatory response to deficiency of palladin or collapsed stress fibers through unknown mechanism.

In conclusion, palladin is crucial for fundamental cellular function and is required for the formation or stabilization of stress fibers and normal expression of β1-integrin.

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REFERENCES

Akiyama SK, Yamada KM. 1987. Biosynthesis and acquisition of biological activity of the fibronectin receptor. J Biol Chem 262:17536–17542.

Alais S, Allioli N, Pujades C, Duband JL, Vainio O, Imhof BA, Dunon D. 2001. HEMCAM/CD146 downregulates cell surface expression of beta1 integrins. J Cell Sci 114: 1847–1859.

Albiges-Rizo C, Frachet P, Block MR. 1995. Down regulation of talin alters cell adhesion and the processing of the alpha 5 beta 1 integrin. J Cell Sci 108:3317–3329.

Bailly M. 2004. Ena/VASP family: New partners, bigger enigma. Dev Cell 7:462–463.

Bang ML, Mudry RE, McElhinny AS, Trombitas K, Geach AJ, Yamasaki R, Sorimachi H, Granzier H, Gregorio CC, Labeit S. 2001. Myopalladin, a novel 145-kilodalton sarcomeric protein with multiple roles in Z-disc and I-band protein assemblies. J Cell Biol 153:413–427.

Bellis SL, Newman E, Friedman EA. 1999. Steps in integrin beta1-chain glycosylation mediated by TGFbeta1 signaling through Ras. J Cell Physiol 181:33–44.

Blystone SD. 2004. Integrating an integrin: A direct route to actin. Biochim Biophys Acta 1692:47–54.

Boukhelifa M, Parast MM, Valtschanoff JG, LaMantia AS, Meeker RB, Otey CA. 2001. A role for the cytoskeleton-associated protein palladin in neurite outgrowth. Mol Biol Cell 12:2721–2729.

Boukhelifa M, Hwang SJ, Valtschanoff JG, Meeker RB, Rustioni A, Otey CA. 2003. A critical role for palladin in astrocyte morphology and response to injury. Mol Cell Neurosci 23:661–668.

Boukhelifa M, Parast MM, Bear JE, Gertler FB, Otey CA. 2004. Palladin is a novel binding partner for Ena/VASP family members. Cell Motil Cytoskeleton 58:17–29.

Brakebusch C, Hirsch E, Potocnik A, Fassler R. 1997. Genetic analysis of beta1 integrin function: Confirmed, new and revised roles for a crucial family of cell adhesion molecules. J Cell Sci 110:2895–2904.

Burridge K, Wennerberg K. 2004. Rho and Rac take center stage. Cell 116:167–179.

Calderwood DA. 2004. Integrin activation. J Cell Sci 117: 657–666.

Calderwood DA, Shattil SJ, Ginsberg MH. 2000. Integrins and actin filaments: Reciprocal regulation of cell adhesion and signaling. J Biol Chem 275:22607–22610.

Caswell PT, Norman JC. 2006. Integrin trafficking and the control of cell migration. Traffic 7:14–21.

Coghill ID, Brown S, Cottle DL, McGrath MJ, Robinson PA, Nandurkar HH, Dyson JM, Mitchell CA. 2003. FHL3 is an actin-binding protein that regulates alpha-actinin-mediated actin bundling: FHL3 localizes to actin stress fibers and enhances cell spreading and stress fiber disassembly. J Biol Chem 278:24139–24152.

Copp AJ, Greene ND, Murdoch JN. 2003. The genetic basis of mammalian neurulation. Nat Rev Genet 4:784–793.

Degani S, Balzac F, Brancaccio M, Guazzone S, Retta SF, Silengo L, Eva A, Tarone G. 2002. The integrin cytoplasmic domain-associated protein ICAP-1 binds and regulates Rho family GTPases during cell spreading. J Cell Biol 156:377–387.

Enserink JM, Price LS, Methi T, Mahic M, Sonnenberg A, Bos JL, Tasken K. 2004. The cAMP-Epac-Rap1 pathway regulates cell spreading and cell adhesion to laminin-5 through the alpha3beta1 integrin but not the alpha6-beta4 integrin. J Biol Chem 279:44889–44896.

Etienne-Manneville S, Hall A. 2002. Rho GTPases in cell biology. Nature 420:629–635.

Gogali A, Charalabopoulos K, Constantopoulos S. 2004. Integrin receptors in primary lung cancer. Exp Oncol 26: 106–110.

Guo W, Giancotti FG. 2004. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 5:816–826.

Hagel M, George EL, Kim A, Tamimi R, Opitz SL, Turner CE, Imamoto A, Thomas SM. 2002. The adaptor protein paxillin is essential for normal development in the mouse and is a critical transducer of fibronectin signaling. Mol Cell Biol 22:901–915.

Hall A. 1998. Rho GTPases and the actin cytoskeleton. Science 279:509–514.

Hogan B, Beddington R, Costantini F, Lacy E. 1994. Manipulating the mouse embryo: A laboratory manual, 2nd edition, Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Hunter KW. 2004. Ezrin, a key component in tumor metastasis. Trends Mol Med 10:201–204.

Hwang SJ, Pagliardini S, Boukhelifa M, Parast MM, Otey CA, Rustioni A, Valtschanoff JG. 2001. Palladin is

expressed preferentially in excitatory terminals in the rat central nervous system. J Comp Neurol 436:211–224.
Ignotz RA, Massague J. 1987. Cell adhesion protein receptors as targets for transforming growth factor-beta action. Cell 51:189–197.
Jahraus A, Egeberg M, Hinner B, Habermann A, Sackman E, Pralle A, Faulstich H, Rybin V, Defacque H, Griffiths G. 2001. ATP-dependent membrane assembly of F-actin facilitates membrane fusion. Mol Biol Cell 12:155–170.
Lafuste P, Sonnet C, Chazaud B, Dreyfus PA, Gherardi RK, Wewer UM, Authier FJ. 2005. ADAM12 and alpha9beta1 integrin are instrumental in human myogenic cell differentiation. Mol Biol Cell 16:861–870.
Lenter M, Vestweber D. 1994. The integrin chains beta 1 and alpha 6 associate with the chaperone calnexin prior to integrin assembly. J Biol Chem 269:12263–12268.
Liu TX, Zhang JW, Tao J, Zhang RB, Zhang QH, Zhao CJ, Tong JH, Lanotte M, Waxman S, Chen SJ, Mao M, Hu GX, Zhu L, Chen Z. 2000. Gene expression networks underlying retinoic acid-induced differentiation of acute promyelocytic leukemia cells. Blood 96:1496–1504.
Luo HJ, Liu XS, Wang F, Huang QH, Shen SH, Wang L, Xu GJ, Sun X, Kong H, Gu MM, Chen SJ, Chen Z, Wang ZG. 2005. Disruption of palladin results in neural tube closure defects in mice. Mol Cell Neurosci 29:507–515.
Martel V, Vignoud L, Dupe S, Frachet P, Block MR, Albiges-Rizo C. 2000. Talin controls the exit of the integrin alpha 5 beta 1 from an early compartment of the secretory pathway. J Cell Sci 113:1951–1961.
Miranti CK, Brugge JS. 2002. Sensing the environment: A historical perspective on integrin signal transduction. Nat Cell Biol 4:E83–E90.
Mullins RD, Heuser JA, Pollard TD. 1998. The interaction of Arp2/3 complex with actin: Nucleation, high affinity pointed end capping, and formation of branching networks of filaments. Proc Natl Acad Sci USA 95:6181–6186.
Mykkanen OM, Gronholm M, Ronty M, Lalowski M, Salmikangas P, Suila H, Carpen O. 2001. Characterization of human palladin, a microfilament-associated protein. Mol Biol Cell 12:3060–3073.
Otey CA, Carpen O. 2004. Alpha-actinin revisited: A fresh look at an old player. Cell Motil Cytoskeleton 58:104–111.
Ozawa T, Araki N, Yunoue S, Tokuo H, Feng L, Patrakitkomjorn S, Hara T, Ichikawa Y, Matsumoto K, Fujii K, Saya H. 2005. The neurofibromatosis type 1 gene product neurofibromin enhances cell motility by regulating actin filament dynamics via the Rho-ROCK-LIMK2-cofilin pathway. J Biol Chem 280:39524–39533.
Parast MM, Otey CA. 2000. Characterization of palladin, a novel protein localized to stress fibers and cell adhesions. J Cell Biol 150:643–656.
Pohl G, Ho CL, Kurman RJ, Bristow R, Wang TL, Shih IeM. 2005. Inactivation of the mitogen-activated protein kinase pathway as a potential target-based therapy in ovarian serous tumors with KRAS or BRAF mutations. Cancer Res 65:1994–2000.
Rao J, Li N. 2004. Microfilament actin remodeling as a potential target for cancer drug development. Curr Cancer Drug Targets 4:345–354.
Ronty M, Taivainen A, Moza M, Otey CA, Carpen O. 2004. Molecular analysis of the interaction between palladin and alpha-actinin. FEBS Lett 566:30–34.
Salicioni AM, Gaultier A, Brownlee C, Cheezum MK, Gonias SL. 2004. Low density lipoprotein receptor-related protein-1 promotes beta1 integrin maturation and transport to the cell surface. J Biol Chem 279:10005–10012.
Salmikangas P, van der Ven PF, Lalowski M, Taivainen A, Zhao F, Suila H, Schroder R, Lappalainen P, Furst DO, Carpen O. 2003. Myotilin, the limb-girdle muscular dystrophy 1A (LGMD1A) protein, cross-links actin filaments and controls sarcomere assembly. Hum Mol Genet 12:189–203.
Shimizu Y, Thumkeo D, Keel J, Ishizaki T, Oshima H, Oshima M, Noda Y, Matsumura F, Taketo MM, Narumiya S. 2005. ROCK-I regulates closure of the eyelids and ventral body wall by inducing assembly of actomyosin bundles. J Cell Biol 168:941–953.
Tran H, Pankov R, Tran SD, Hampton B, Burgess WH, Yamada KM. 2002. Integrin clustering induces kinectin accumulation. J Cell Sci 115:2031–2040.
Vallenius T, Scharm B, Vesikansa A, Luukko K, Schafer R, Makela TP. 2004. The PDZ-LIM protein RIL modulates actin stress fiber turnover and enhances the association of alpha-actinin with F-actin. Exp Cell Res 293:117–128.
Weed SA, Parsons JT. 2001. Cortactin: Coupling membrane dynamics to cortical actin assembly. Oncogene 20:6418–6434.
Welch MD, DePace AH, Verma S, Iwamatsu A, Mitchison TJ. 1997. The human Arp2/3 complex is composed of evolutionarily conserved subunits and is localized to cellular regions of dynamic actin filament assembly. J Cell Biol 138:375–384.
Xu W, Baribault H, Adamson ED. 1998. Vinculin knockout results in heart and brain defects during embryonic development. Development 125:327–337.
Yu DH, Qu CK, Henegariu O, Lu X, Feng GS. 1998. Protein-tyrosine phosphatase Shp-2 regulates cell spreading, migration, and focal adhesion. J Biol Chem 273:21125–21131.
Zeng L, Si X, Yu WP, Le HT, Ng KP, Teng RM, Ryan K, Wang DZ, Ponniah S, Pallen CJ. 2003. PTP alpha regulates integrin-stimulated FAK autophosphorylation and cytoskeletal rearrangement in cell spreading and migration. J Cell Biol 160:137–146.
Zimerman B, Volberg T, Geiger B. 2004. Early molecular events in the assembly of the focal adhesion-stress fiber complex during fibroblast spreading. Cell Motil Cytoskeleton 58:143–159.