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Regulation of cell-matrix adhesion by OLA1, the Obg-like ATPase 1

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ABSTRACT

Attachment of cells to the extracellular matrix induces clustering of membrane receptor integrins which in turn triggers the formation of focal adhesions (FAs). The adaptor/scaffold proteins in FAs provide linkage to actin cytoskeleton, whereas focal adhesion kinase (FAK) and other FA-associated kinases and phosphatases transduce integrin-mediated signaling cascades, promoting actin polymerization and progression of cell spreading. In this study, we explored the role of OLA1, a newly identified member of Obg-like ATPases, in regulating cell adhesion processes. We showed that in multiple human cell lines RNAi-mediated downregulation of OLA1 significantly accelerated cell adhesion and spreading, and conversely overexpression of OLA1 by gene transfection resulted in delayed cell adhesion and spreading. We further found that OLA1-deficient cells had elevated levels of FAK protein and decreased Ser3 phosphorylation of cofilin, an actin-binding protein and key regulator of actin filament dynamics, while OLA1-overexpressing cells exhibited the opposite molecular alterations in FAK and cofilin. These findings suggest that OLA1 plays an important negative role in cell adhesion and spreading, in part through the regulation of FAK expression and cofilin phosphorylation, and manipulation of OLA1 may lead to significant changes in cell adhesion and the associated phenotypes.

1. Introduction

Cell adhesion plays key roles in embryonic development, immune response, tissue repair, and cancer metastasis [1–3]. Upon the initial attachment of cells to ECM components, the cell membrane receptor integrins are induced to form clusters at the attachment sites. In the inner surface of the cell membrane, the clustered integrins serve as a platform for the recruitment of various adaptor and signaling proteins, resulting in the formation of focal adhesions (FAs). The adaptor/scaffold proteins in FAs, including talin, paxillin, and α-actinin [4], provide strong linkages to the actin cytoskeleton, thus connecting the cells firmly to the ECM, whereas the signaling proteins, predominantly the focal adhesion kinase (FAK) and Src, play central roles in transduction of the integrin-mediated signaling. FAK is an ubiquitously expressed non-receptor tyrosine protein kinase [5,6] and has multiple functions within the cell, including contributing to FA maturation and turnover, phosphorylation of other focal adhesion proteins, and regulation of motility and survival [6].

Actin is the primary component of the cytoskeleton, and in the cell adhesion processes the dynamics of actin polymerization and depolymerization are required for the generation of cellular forces that lead to FA maturation, turnover, and cell spreading [7]. Cofilin is an actin-binding protein and plays an essential role in regulating actin filament dynamics by stimulating the severing and depolymerization of actin filaments, and as such providing abundant actin monomers for further actin polymerization and filament assembly. Cofilin's severing activity is inhibited upon phosphorylation at the Ser3 residue by Lim family kinases (LIMKs) [8,9] and testicular protein kinases (TESKs), and restored when cofilin is dephosphorylated by various phosphatases [10,11].

To date nearly 200 different molecules have been described as components of a complex network, collectively referred to as the integrin adhesome, contributing to the structural and signaling functions of FAs [12,13]. However, current knowledge about the adhesome is just the tip of the iceberg; recent proteomics studies suggest that we still have much to learn about its complexity [14]. In the present study we describe the addition of a novel regulatory factor to this network: Obg-like ATPase 1 (OLA1).

Abbreviations: OLA1, Obg-like ATPase 1; FAs, focal adhesions; FAK, focal adhesion kinase; ECM, extracellular matrix; ROCK, Rho-associated kinase; F-actin, filamentous actin; LIMKs, Lim family kinases; TESK, testicular protein kinases; pFAK(Y397), Tyr 397 phosphorylated FAK; pFAK(Y925), Tyr 925 phosphorylated FAK; pCofilin, Ser 3 phosphorylated cofilin; pRac/cdc42, Ser 71 phosphorylated Rac/cdc42; pVASP, Ser 157 phosphorylated VASP.

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OLA1 belongs to the translation factor-related (TRAFAC) class, Obg family, and YchF subfamily of *P*-loop GTPases [15,16]. *P*-loop NTPases are the most abundant nucleotide-binding proteins [17], and involved in the regulation of diverse cellular processes including protein translation, intracellular transport, signal transduction, and cell proliferation [18,19]. The OLA1/YchF proteins are highly conserved from yeast to human [17,20], and unlike other Obg family members they bind and hydrolyze ATP more efficiently than GTP [21]. However, the physiological functions of these unconventional Obg-like ATPases are poorly understood. Recently, our group demonstrated that human OLA1 functions as an intrinsic regulator in cellular stress responses such as oxidative stress [22] and heat shock [23]. In another study, we reported that down regulation of OLA1 causes changes in cell migration and invasiveness in cultured human cancer cells [15].

In this report we demonstrate that down regulation of OLA1 results in significantly accelerated cell adhesion and spreading associated with increased levels of FAK and decreased cofilin Ser3 phosphorylation while overexpression of OLA1 causes delayed cell adhesion associated with the opposite molecular changes in FAK and cofilin. Our findings suggest that OLA regulates the essential cell-matrix adhesion processes by affecting multiple components of the adhesome.

2. Materials and methods

2.1. Cells

MDA-MB-231, WI-38, and HeLa cells were obtained from ATCC and cultured in Dulbecco's Modified Eagle's medium (DMEM, Thermo Scientific) containing 10% fetal bovine serum (FBS, Thermo Scientific), 100 units/ml penicillin, and 100 mg/ml streptomycin at 37 °C with 5% CO_2.

2.2. RNAi of OLA1

Human OLA1 cDNA (NM_013341.3)-specific siRNA (SASI_Hs01_00244684) and the control siRNA (MISSION siRNA Universal Negative Control, #1, SIC001) were acquired from Sigma–Aldrich. Cells seeded in 6-well plates were transiently transfected with 5 μM siRNA with the DharmaFECT1 siRNA Transfection Reagent (Thermo Scientific) according to the manufacturer's instructions. To establish stable OLA1-knockdown cell lines (HeLa) SMARTvector lentiviral shRNA particles (Thermo Scientific) containing a shRNA sequence specific for OLA1 (TGTTCGCTTCCAGATACTT) and the control shRNA sequence were used at the range of 5–20 TU/cell. Cell clones expressing the respective shRNAs were selected with puromycin (5 μg/ml) for 1 month. The knockdown efficiency of the target gene was verified by Western blot analysis.

2.3. Cell adhesion assay

Cells were serum starved overnight before detaching with 0.25% trypsin for 1 min. The cells were re-suspended in fresh medium, and then seeded into 6-well dishes pre-coated with 10 μg/ml human fibronectin or 20 μg/ml laminin at the density of 1×10^5 cells/well. Attached cells were fixed in 1 ml of 4% paraformaldehyde at the indicated times and photographs were taken under a microscope.

2.4. Quantification of cell adhesion

The 96 well plates were pre-coated with fibronectin (10 μg/ml), laminin (20 μg/ml), or BSA (1 mg/ml, negative control) and seeded with 2×10^4 cells/well for 30 min or 60 min. At the end of incubation, cells were fixed with 4% paraformaldehyde and non-adherent cells were washed off with phosphate buffered saline (PBS). The remaining attached cells were stained with crystal violet solution (Sigma–Aldrich, 5 mg/ml in 2% ethanol), and the final color was measured with a plate reader (Fluostar Optima) at 570 nM.

2.5. Overexpression of OLA1

cDNA fragments encoding full-length human OLA1 cDNA (NM_013341.3) were cloned into the pdEYFP-N1gen plasmid with a C-terminal YFP tag as previously described [22,23]. Cells in 6-well plates were transfected with the OLA1-YFP plasmid (4 μg DNA/well) or the pdEYFP-N1gen (YFP control) plasmid using Lipofectamine 2000 (10 μl/well, Invitrogen). The parental plasmids were a kind gift from Stefan Wiemann of European Molecular Biology Laboratory, Heidelberg, Germany [24].

2.6. Immunoblot analysis

Western immunoblot analysis was performed according to our standard procedures as previously described [22,23]. All antibodies used in these studies were purchased from Cell Signaling Technology except anti-OLA1 and anti-β-actin antibodies, which were from Sigma–Aldrich, and anti-rabbit IgG peroxidase linked whole antibody and anti-mouse Ig peroxidase linked whole antibody, from GE Healthcare.

2.7. Statistical analysis

Statistical analysis was performed by a two-tailed Student's *t*-test. Values of $p < 0.05$ were considered to be statistically significant.

3. Results

3.1. Downregulation of OLA1 accelerates cell adhesion and spreading

To investigate the role of OLA1 in cell adhesion and spreading, we manipulated the expression of OLA1 in multiple human cell lines, first by siRNA-mediated gene silencing. Human lung fibroblasts (WI-38) and breast cancer cells (MDA-MB-231) were transiently transfected with OLA1-specific siRNA or non-targeting siRNA, and at time 0 identical numbers of cells were plated on fibronectin-coated plates. Compared with the control transfected cells, OLA1-siRNA cells exhibited a much faster progression of attachment and cell body spreading (Fig. 1A and B). As early as 30 min, notably more OLA1-siRNA cells had attached to the plate and initiated spreading. Here spread cells were defined as cells with flat and extended cell margins lacking phase-brightness, whereas non-spread cells were rounded and phase-bright under microscope. More strikingly, at 60 min, while only a small portion of the control cells had entered the "intermediate stage" of spreading with the rest of the cells remaining small and rounded, almost all OLA1-siRNA cells had achieved maximum cell areas with well-developed membrane protrusions indicating "late-stage" spreading (Fig. 1A and B). After prolonged incubation the difference evened out as the control cells also completed the whole course of adhesion and spreading. After overnight incubation, the two groups of cells showed no apparent variation in either total adherent cells or the morphology of single cells (Fig. 1D). Consistent with our previous report [22], general cell growth was not affected by the RNAi treatment.

In order to rule out the possibility that the observed phenotype was dependent on the transient RNAi strategy, stable cell sub-lines were raised by transduction of lentiviruses expressing shRNA