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Prominin-2 expression increases protrusions, decreases caveolae and inhibits Cdc42 dependent fluid phase endocytosis

Raman Deep Singh *, Andreas S. Schroeder, Luana Scheffer, Eileen L. Holicky, Christine L. Wheatley, David L. Marks *, Richard E. Pagano

Departments of Medicine, Biochemistry and Molecular Biology, Mayo Clinic College of Medicine, Rochester, MN 55905, USA

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ABSTRACT

Background: Membrane protrusions play important roles in biological processes such as cell adhesion, wound healing, migration, and sensing of the external environment. Cell protrusions are a subtype of membrane microdomains composed of cholesterol and sphingolipids, and can be disrupted by cholesterol depletion. Prominins are pentaspan membrane proteins that bind cholesterol and localize to plasma membrane (PM) protrusions. Prominin-1 is of great interest as a marker for stem and cancer cells, while Prominin-2 (Prom2) is reportedly restricted to epithelial cells.
Aim: To characterize the effects of Prom-2 expression on PM microdomain organization.
Methods: Prom2-fluorescent protein was transfected in human skin fibroblasts (HSF) and Chinese hamster ovary (CHO) cells for PM raft and endocytic studies. Caveolae at PM were visualized using transmission electron microscopy. Cdc42 activation was measured and caveolin-1 knockdown was performed using siRNAs.
Results: Prom2 expression in HSF and CHO cells caused extensive Prom2-positive protrusions that co-localized with lipid raft markers. Prom2 expression significantly decreased caveolae at the PM, reduced caveolar endocytosis and increased caveolin-1 phosphorylation. Prom2 expression also inhibited Cdc42-dependent fluid phase endocytosis *via* decreased Cdc42 activation. Effects on endocytosis were reversed by addition of cholesterol. Knockdown of caveolin-1 by siRNA restored Cdc42 dependent fluid phase endocytosis in Prom2-expressing cells.
Conclusions: Prom2 protrusions primarily localize to lipid rafts and recruit cholesterol into protrusions and away from caveolae, leading to increased phosphorylation of caveolin-1, which inhibits Cdc42-dependent endocytosis. This study provides a new insight for the role for prominins in the regulation of PM lipid organization.

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1. Introduction

Membrane protrusions play important roles in biological processes such as cell adhesion, wound healing, migration, and sensing of the external environment [1,2]. Several types of plasma membrane (PM) protrusions exist, such as lamellipodia, sheet-like extensions of the cell supported by branched actin filaments, and filopodia, finger-like projections supported by parallel actin bundles [3,4]. Cell protrusions have been proposed to be a type of membrane microdomain, as they possess elevated levels of cholesterol and glycosphingolipids (GSLs), relative to other regions of the cell membrane [5–7] and protrusion structure can be disrupted by cholesterol depletion [8–10].

Prominin proteins (Prom1 and Prom2) are pentaspan transmembrane proteins that are enriched at PM protrusions in some cell types [5,11,12]. Both prominins have been shown to directly bind cholesterol and associate with membrane microdomains in living cells [13–15]. Prom1 (CD133) has been widely studied as a marker for certain stem cells and cancer stem cells [5,12,16], whereas Prom2 has been shown to be present in some epithelial cells but is otherwise little studied [11,17,18]. The prominins have been proposed to be involved in the organization of membrane protrusions but the specific function of these proteins is presently unknown [5,11].

Our laboratory has been interested in the function and distribution of microdomains on the PM of living cells, and has used various fluorescent probes such as BODIPY-lactosylceramide (Bodipy-LacCer), polyethylene glycol-coupled cholesterol (PEG-Chol), and cholera toxin B subunit (CtxB) to label such domains [19–21]. Since cell protrusions have been reported to be a type of GSL-enriched microdomain [7,22], we over-expressed Prom2 as a marker for pro-

* Corresponding authors. Address: Mayo Clinic, 200 First Street, S.W., Rochester, MN 55905-0001, USA. Fax: +1 507 284 4521.
E-mail addresses: Takhter.Ramandeep@mayo.edu (R.D. Singh), Marks.david@mayo.edu (D.L. Marks).

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trusions and investigated the colocalization of this protein with Bodipy-LacCer and other lipid raft markers. The fluorescent lipid, along with other lipid raft markers, was found to be highly co-localized with Prom2 in protrusions. Over-expression of Prom2 led to significant changes in PM organization and function, including increased protrusions, decreased caveolae at the PM, and decreased caveolar and fluid phase endocytosis. It also resulted in increased caveolin-1 phosphorylation, which inhibited Cdc42-dependent endocytosis due to Cdc42 inactivation. This study provides new insight into possible roles of prominin proteins in regulating PM organization.

2. Materials and methods

2.1. Cell culture

Normal human skin fibroblasts (HSFs; GM-5659, Coriell Institute for Medical research, Camden, NJ) and CHO cells (ATCC, Manassas, VA) were grown as described [20].

2.2. Constructs and transfection experiments

A DNA construct encoding full length human Prom2 was purchased (Thermo Scientific, Waltham MA) and modified (see Suppl.). Transfection of DNA constructs was performed using a Nucleofector II apparatus (Lonza).

2.3. Lipids, fluorescent probes and miscellaneous reagents

Bodipy-D-*e*-LacCer was complexed to defatted BSA as described [23]. Fluorescent AF488 transferrin, AF488-dextran (10kD), and secondary antibodies were from Invitrogen (Eugene, OR). Rhodamine-wheat germ agglutinin (Rh-WGA) was from Vector Laboratories (Burlingame, CA). PEG-Chol (a kind gift from Toshihide Kobayashi, Riken) was labeled with AF488 carboxylic acid (Invitrogen). Caveolin-1 (Cav1) and pY14-Cav1 antibodies were from BD Biosciences (San Jose, CA). A Cdc42 activation kit and HRP-conjugated secondary antibodies were from Millipore (Billerica, MA). All other reagents were from Sigma–Aldrich.

2.4. PM labeling with fluorescent probes

HSFs were transfected with Prom2-GFP or -mKate for 48 h. Transfected HSFs were then washed with ice cold HMEM and incubated with 5 μg/ml Rh-WGA, 2.5 μM Bodipy-LacCer, 2 μg/ml AF488-CtxB or AF594-StxB, or AF488-PEG-Chol for 30 min at 10 °C to label the PM. Samples were then washed, and images were acquired at appropriate wavelengths for the different fluorophores.

2.5. Endocytosis assays

For endocytosis assays, HSFs were transfected for 48 h with Prom2-mKate or -GFP and endocytosis assays were performed as described [20]. In some experiments 5 mM methyl ß-cyclodextrin/cholesterol (MßCD/Chol) complex (Sigma) was added to cells for 30 min at 37 °C prior to endocytosis assays.

2.6. Electron microscopy

Electron microscopy studies were carried out in the Mayo Clinic Electron Microscopy Core Facility (see Suppl.) using FEI Tecnai T12 transmission electron microscope and Hitachi S-4700 cold field-emission electron microscope for transmission and scanning electron microscopy, respectively.

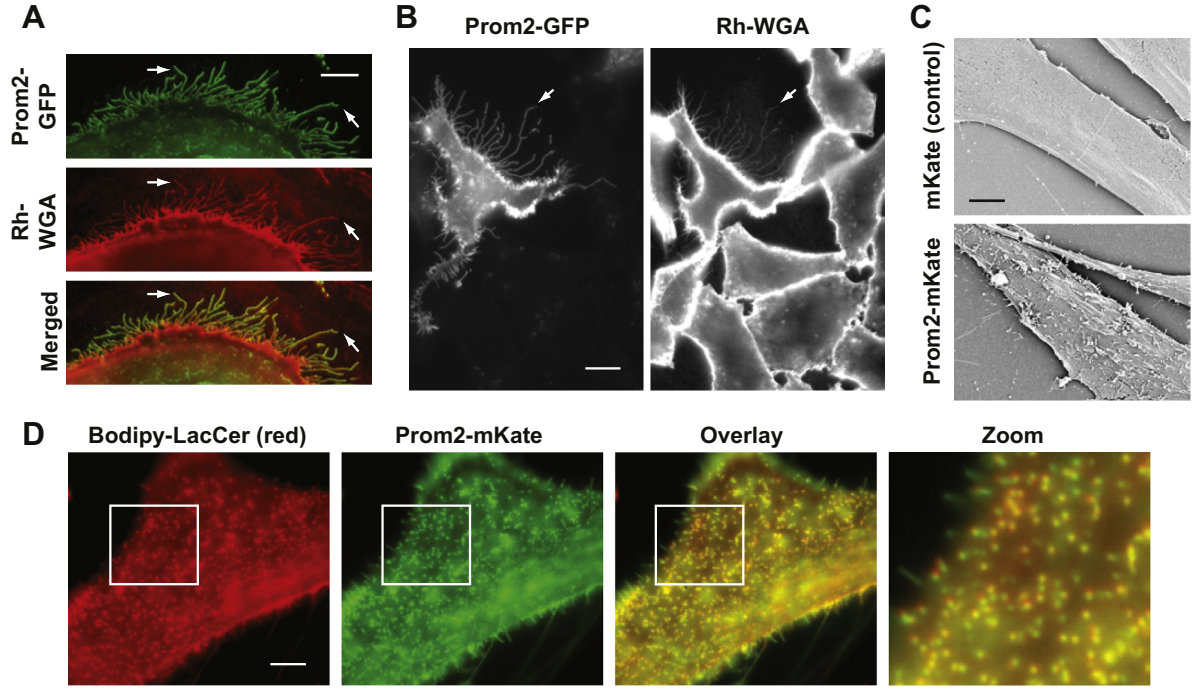

Fig. 1. Prom2 expression induced extensive protrusions that colocalize with a lipid raft marker. HSFs (A) or CHO cells (B) were transfected with Prom2 GFP (48 h) and stained with Rh-WGA to label cell surface carbohydrates. Note the extensive protrusions labeled with both Prom2-GFP and Rh-WGA (e.g., at arrows) Bar, 10 μm. (C) HSFs were transfected with Prom2-mKate or mKate only and SEM was performed on fixed cells. Transfected cells showed extensive protrusions on their surface whereas control cells had very few protrusions. Bar, 5 μm. (D) Cells transfected with Prom2-mKate, were incubated with Bodipy-LacCer and fluorescence images of living cells were acquired for Prom-2-mKate and Bodipy-LacCer and merged. Note the extensive co-localization of Prom2 with Bodipy-LacCer. Imaging at low temperature inhibited endocytosis.

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