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The transmembrane domain of podoplanin is required for its association with lipid rafts and the induction of epithelial-mesenchymal transition

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ABSTRACT

Podoplanin is a transmembrane glycoprotein that is upregulated in cancer and was reported to induce an epithelial-mesenchymal transition (EMT) in MDCK cells. The promotion of EMT was dependent on podoplanin binding to ERM (ezrin, radixin, moesin) proteins through its cytoplasmic (CT) domain, which led to RhoA-associated kinase (ROCK)-dependent ERM phosphorylation. Using detergent-resistant membrane (DRM) assays, as well as transmembrane (TM) interactions and ganglioside GM1 binding, we present evidence supporting the localization of podoplanin in raft platforms important for cell signalling. Podoplanin mutant constructs harbouring a heterologous TM region or lacking the CT tail were unable to associate with DRMs, stimulate ERM phosphorylation and promote EMT or cell migration. Similar effects were observed upon disruption of a GXXXG motif within the TM domain, which is involved in podoplanin self-assembly. In contrast, deletion of the extracellular (EC) domain did not affect podoplanin DRM association. Together, these data suggest that both the CT and TM domains are required for podoplanin localization in raft platforms, and that this association appears to be necessary for podoplanin-mediated EMT and cell migration.

1. Introduction

Podoplanin (PDPN), also called PA2.26 antigen, Aggrus, and T1α, is a small membrane mucin-like type I glycoprotein expressed in mesothelia, osteoblasts, osteocytes, certain types of neurons, and several epithelia (Wicki and Christofori, 2007). It is also expressed in the lymphatic but not blood vessel endothelium, making podoplanin a useful immunohistochemical marker for lymphangiogenesis analysis (Ordonez, 2006). Studies with podoplanin-deficient mice have revealed an important role for this glycoprotein in the morphogenesis of the lung (Ramirez et al., 2003), the formation of the lymphatic vasculature (Schacht et al., 2003) and cardiac development (Mahtab et al., 2008). Nevertheless, the precise biological function of podoplanin in normal tissues remains to be elucidated.

Podoplanin is upregulated in a variety of cancers, and has been recently identified as a candidate cancer stem cell marker in squamous cell carcinomas (Atsumi et al., 2008). The general consensus about the role of podoplanin in cancer is that it promotes tumor cell migration, invasion and metastasis (Wicki and Christofori, 2007).

The human podoplanin precursor polypeptide is 162 amino acids long. It is composed of an O-glycosylated EC domain followed by a hydrophobic TM segment and a CT tail of only nine amino acids (Martin-Villar et al., 2005). The EC region contains three repeats (PLAG domains) that have been shown to induce tumor-platelet aggregate formation and facilitate pulmonary metastasis (Kunita et al., 2007). We have shown that in premalignant keratinocytes and MDCK cells podoplanin induces an EMT that is associated with increased migration/invasion and lymph node metastasis (Scholl et al., 1999, 2000; Martin-Villar et al., 2005, 2006). Podoplanin binds ezrin/moesin members of the ERM protein family through basic residues of its CT domain linking it to the actin cytoskeleton. This interaction is crucial for podoplanin-mediated activation of the small GTPase RhoA and its associated kinase ROCK, thereby promoting an EMT (Martin-Villar et al., 2006). On the other hand, Wicki et al. (2006) reported that podoplanin is able to stimulate collective tumor cell migration/invasion in the absence of EMT by inducing a

Abbreviations: Cav-1, caveolin-1; CD, methyl-β-Cyclodextrin; CT, cytoplasmic; CTB, cholera toxin B-subunit; DRM, detergent-resistant membrane; EC, extracellular; E-CD, E-cadherin; EMT, epithelial-to-mesenchymal transition; ENAC, epithelial sodium channel α subunit; ERM, ezrin, radixin, moesin; PDPN, podoplanin; ROCK, RhoA-associated kinase; TM, transmembrane.

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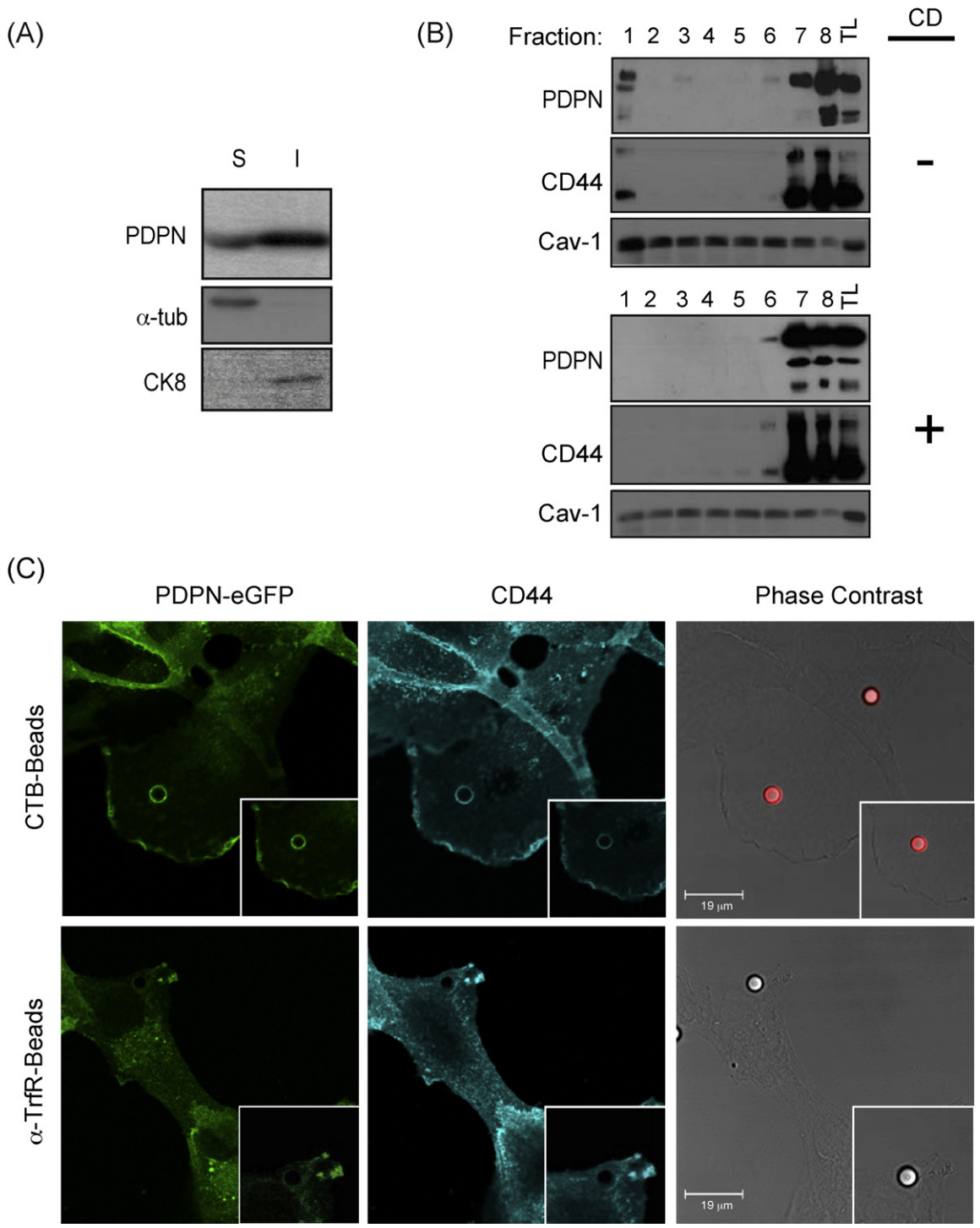

Fig. 1. Podoplanin is associated with the detergent-resistant cholesterol-rich membrane fraction. (A) MDCK cells expressing wild type PDPN fused to eGFP were lysed at 4 °C in 0.2% Triton X-100 and the levels of podoplanin in the soluble (S) and insoluble (I) fractions were determined by Western blotting. α-tubulin and cytokeratin 8 (CK8) were used as controls for soluble and insoluble proteins, respectively. (B) MDCK cells expressing wild-type PDPN fused to eGFP were treated with CD or vehicle and lysed at 4 °C in 0.5% Triton X-100. Cell lysates were subjected to flotation on OptiPrep™ density gradients, and the expression levels of PDPN, CD44 and caveolin-1 (Cav-1) in the gradient fractions determined by Western blotting. Fractions 1–8 represent low (0%) to high (35% Optiprep) density. TL, total lysate. (C) Recruitment of both PDPN and CD44 by CTB-coated beads. MDCK-PDPN-eGFP cells plated on coverslips were incubated with 5-μm polystyrene beads coated with CTB or α-TrfR (negative control) for 15 min. Cells were then processed for immunofluorescence to detect CD44.

rearrangement of the actin cytoskeleton. These studies point to a crucial role of the EC and CT domains in podoplanin-induced cell adhesion and motility, respectively.

Recently, Barth et al. (2010) have shown that podoplanin/T1α is associated with lipid raft micro domains in microvillar protrusions of lung alveolar epithelial cells. Raft constituents are normally present in the detergent-resistant membrane (DRM) fraction after non-ionic detergent solubilization at 4 °C. They are dynamic nanoscale assemblies of sphingolipids, cholesterol and proteins that can be stabilized into larger platforms and are involved in viral infection, membrane trafficking and signal transduction (Simons and Gerl, 2010). In the present work, we have