Podoplanin overexpression in rat podocytes induces morphological changes similar to flattening of foot processes via regulating Rac1 and Cdc42 activity

Hitonari Nosaka[1], Sigurd Krieger[2], Nobuharu Fujiwara[1], Dai Oono[1], Shunya Uchida[3], Dontscho Kerjaschki[2] and Kenichiro Kojima[1]

[1]Division of Nephrology, Ageo Central General Hospital, Saitama, Japan
[2]Clinical Department of Pathology, Medical University of Vienna, Austria
[3]Department of Internal Medicine, Teikyo University School of Medicine, Tokyo, Japan

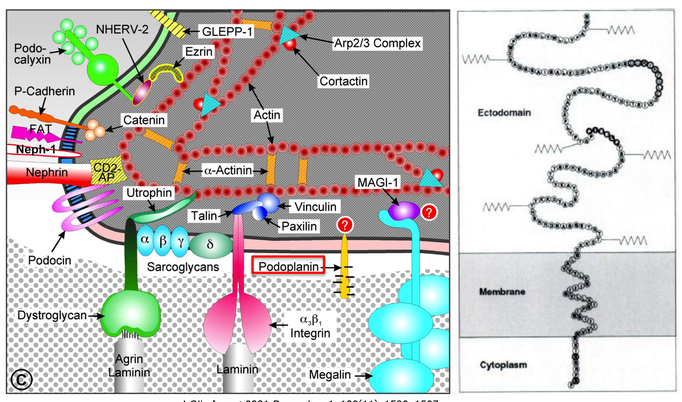
INTRODUCTION

Proteinuria is a common feature in virtually all glomerular diseases. It is generally agreed that podocytes play a major role in determining the permselectivity of the glomerular filtration barrier and their damage is associated with proteinuria.

"Podoplanin" was identified as a podocyte membrane protein, that was transcriptionally downregulated in puromycin aminonucleoside nephrosis.

Interestingly, intravenous injection of antibodies against podoplanin causes very rapid flattening of foot processes and massive albuminuria in rats.

However, the mechanisms of this podocyte injury caused by anti-podoplanin antibodies are poorly understood, and also detailed studies about the role of podoplanin in podocytes are lacking.



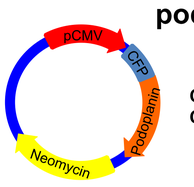
J Clin Invest 2001 December 1; 108(11): 1583–1587.

OBJECTIVES

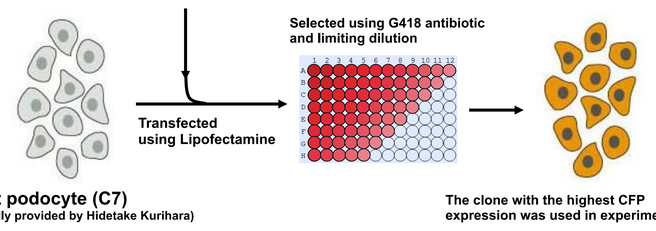
To investigate the role of podoplanin in podocytes, we generated the podoplanin overexpessing rat podocyte and examined the effects on cellular morphology, cytoskeletal architecture and intracellular signaling.

METHODS

1. Generating podoplanin overeppressing rat podocytes



CFP-PDPN expression vector or CFP-control vecter



Rat podocyte (C7)
(kindly provided by Hidetake Kurihara)

The clone with the highest CFP expression was used in experiments.

2. Cellular morphology

3. Rhodamine Phalloidin staining (actin cytoskeletal architecture)

4. Migration assay

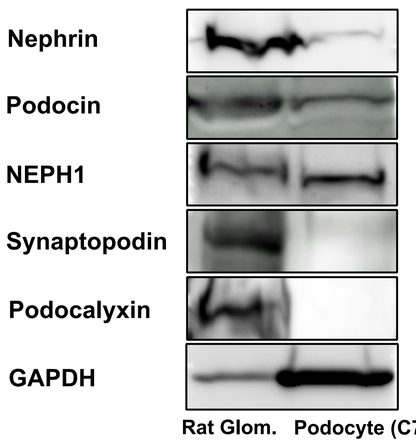
5. Small GTPase activation assay (RhoA, Rac1 and Cdc42)

6. Western Blotting (podocyte marker protein, ERM protein)

RESULTS

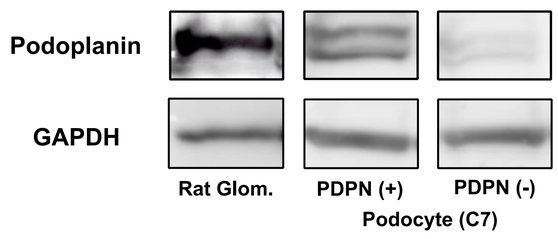
Expression of podocyte marker protein in C7

Nephrin was weakly positive, podocin and NEPH1 were positive, and synaptopodin and podocalyxin were negative.



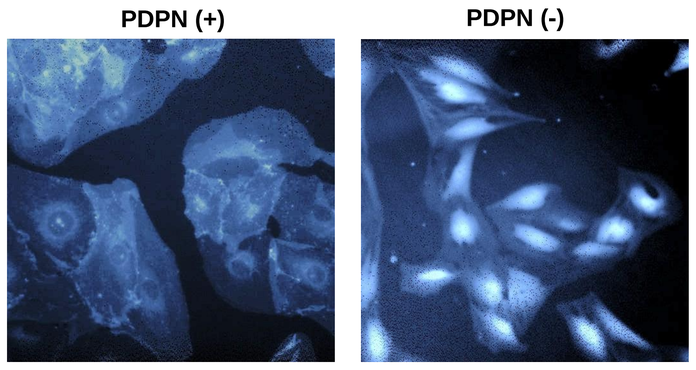
Expression of podoplanin

Podocytes transfected with CFP-PDPN vector expressed high levels of podoplanin compared to control podocytes.



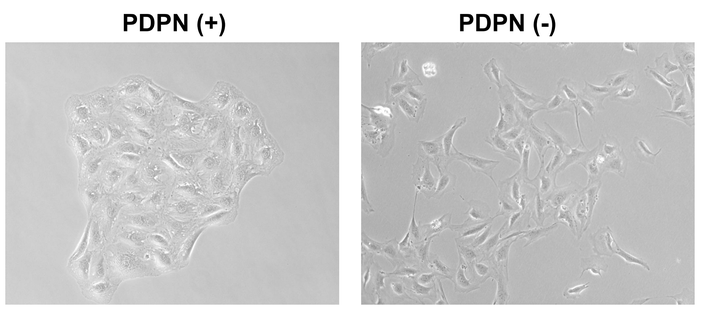
Localization of CFP-podoplanin

While CFP is localized at mainly nucleus or cytoplasm in control podocytes, it is localized at the plasma membrane in PDPN overexpressing podocytes.



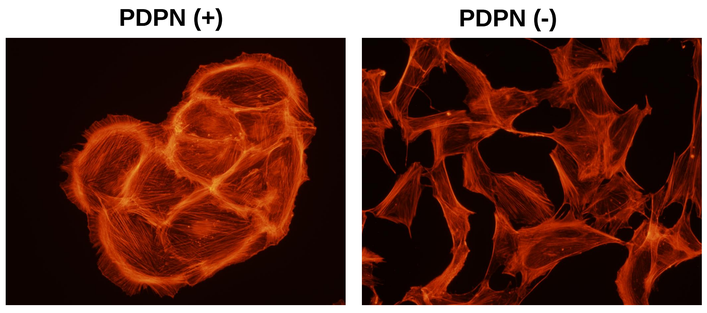
Podoplanin overexpression induced morphological changes in podocytes

Morphologically, the control podocytes were similar to wild-type podocytes possessing some processes and staying apart from adjacent cells. The podoplanin overexpressing podocytes, however, possessed less process and grew in aggregates with a simplified cell shape. This morphological change seems to be similar to flattening of foot processes in vivo.



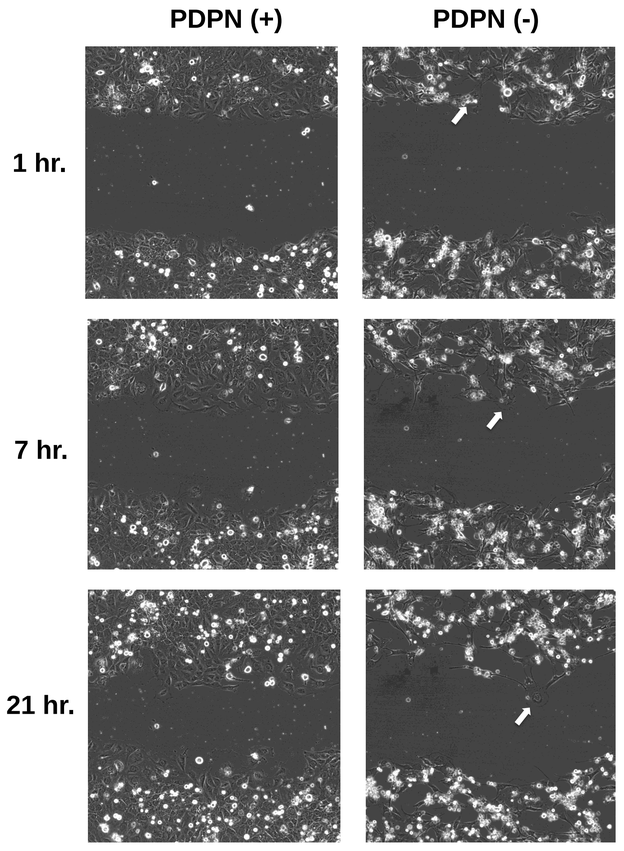
Podoplanin overeppressing podocytes have reorganized actin cytoskeleton

Podoplanin overexpressing podocytes possessed increased peripheral actin bundle compared with control podocytes.

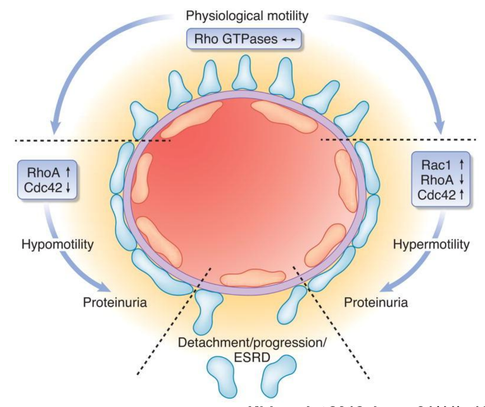


Podoplanin overexpression in podocytes caused hypomotility

After scratching into the cell layer, the intercellular connection of control podocytes have got loose and cells have moved dynamically (arrow). On the other hand, podoplanin overexpressing podocytes were still clustered together and static. Podoplanin overexpression in rat podocytes might cause hypomotility.

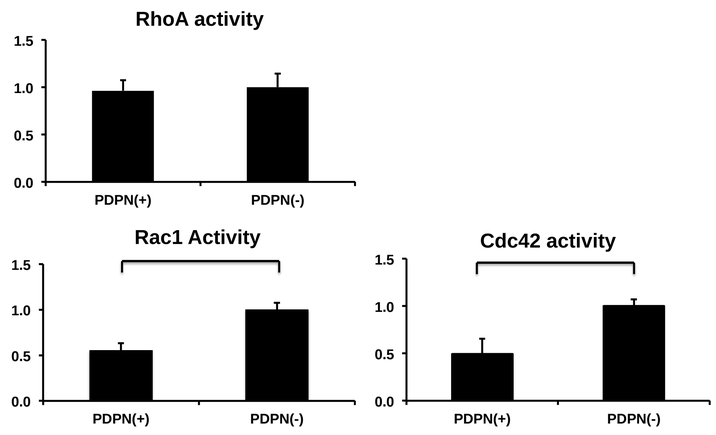


Podocyte GTPases regulate kidney filter dinamics



Kidney Int 2012 June; 81(11): 1053–1055.

Activity of Rac1 and Cdc42 was decreased in podoplanin overexpressing podocytes



Some studies suggest the relationship between podoplanin, ERM protein and Rho-family of small GTPases

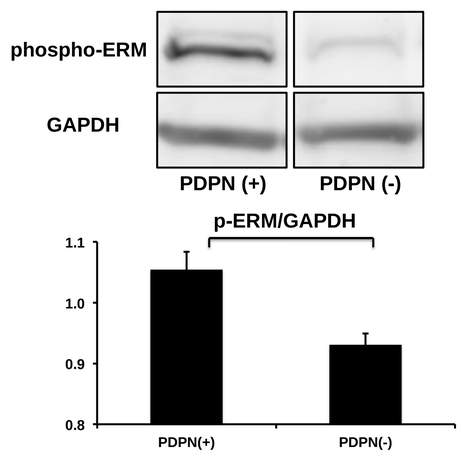
Tumor invasion in the absence of epithelial-mesenchymal transition: Podoplanin-mediated remodeling of the actin cytoskeleton.
Andreas Wicki et al.
Cancer Cell 2006 Apr; 9(4): 261–72.

Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition.
Ester Martín-Villar et al.
J Cell Sci 2006 Nov 1; 119(Pt 21): 4541-53.

T1alpha/podoplanin is essential for capillary morphogenesis in lymphatic endothelial cells.
Angels Navarro et al.
Am J Physiol Lung Cell Mol Physiol 2008 Oct; 295(4): L543-51.

Polarized migration of lymphatic endothelial cells is critically dependent on podoplanin regulation of Cdc42.
Angels Navarro et al.
Am J Physiol Lung Cell Mol Physiol 2011 Jan; 300(1): L32-L42.

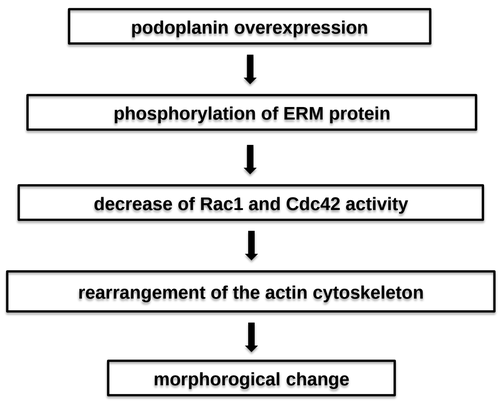
Ezrin/Radixin/Moesin(ERM) protein was strongly phosphorylated in podoplanin overexpressing podocytes



CONCLUSIONS

Podoplanin overexpression in rat podocytes may decrease activity of Rac1 and Cdc42 through the phosphorylation of ERM proteins, cause rearrangement of the actin cytoskeleton, lead to hypomotility of podocyte and result in flattening of foot processes.

Proposed model for the morphological changes in rat podocytes induced by podoplanin overexpression is showed in the figure.



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