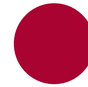
View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Copenhagen University Research Information System

UNIVERSITY OF COPENHAGEN



Characterisation of stromal-cellular mechanotransduction through syndecan-4

Hollosi, Peter; Grossi, Alberto; Couchman, John Robert

Publication date:
2010

Document version
Early version, also known as pre-print

Citation for published version (APA):
Hollosi, P., Grossi, A., & Couchman, J. R. (2010). *Characterisation of stromal-cellular mechanotransduction through syndecan-4*. Poster session presented at FECTS meeting, .

Download date: 07. apr.. 2020

Characterisation of stromal-cellular mechanotransduction through syndecan-4

Peter Hollosi, Alberto Grossi, John R. Couchman

Biotech Research and Innovation Centre (BRIC), Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark

Abstract

Interaction of adherent cells with the surrounding matrix is a prerequisite for tissue homeostasis. Detailed understanding of the molecular composition, dynamics of assembly and disassembly of such sites is therefore important. Focal adhesions are macromolecular assemblies present in most mammalian cell types, where mechanical forces and regulatory signals are transmitted in both directions through the plasma membrane. A cell surface heparan sulphate proteoglycan, syndecan-4, is a transmembrane component of focal adhesions along with integrins, where it serves as a direct physical link between a number of extracellular matrix proteins and actin microfilaments. Syndecan-4 knock out mice have delayed healing of skin wounds and impaired granulation tissue angiogenesis. Cells null for syndecan-4 are characterised by decreased cell motility and distinct changes in their cytoskeleton, including a lack of α-smooth muscle actin in stress fibres and disorganised α-actinin. Unlike in wild type cells, detergent extraction of living null cells removes most α-actinin, an indicator of distinctly different organisation. We have shown that syndecan-4 binds α-actinin directly and now provide evidence that syndecan-4 in focal adhesions triggers α-actinin to relocate to those cell-matrix adhesion sites. This allows tension to be transmitted across the plasma membrane, shown by F-actin bundling with α-smooth muscle actin positivity. Re-introduction of syndecan-4 to null cells restores normal cytoskeleton phenotype and rate of cell migration. We map the binding site for syndecan-4 cytoplasmic domain in α-actinin to spectrin repeat 4, utilising solid phase binding assays and recombinant peptides. Moreover, phosphorylation of syndecan-4 on its sole serine residue, known to influence cytoplasmic domain conformation, has no effect on α-actinin binding. In summary, syndecan-4 could be a transducer of mechanical forces in tissues through direct linkage to microfilaments.

Introduction

Most mammalian cell types adhere to the surrounding extracellular matrix through large macromolecular complexes termed focal adhesions. Syndecan-4, a ubiquitously expressed cell surface heparan sulphate proteoglycan is selectively enriched at those sites[1] necessary for their formation[2, 3, 4, 5, 6]. Through its extracellular glycosaminoglycan chains, syndecan-4 binds various elements of the stroma, while the cytoplasmic domain of its core protein serves as scaffold for other focal adhesion components, including the actin crosslinking protein α-actinin[7] (**Fig. 1**).

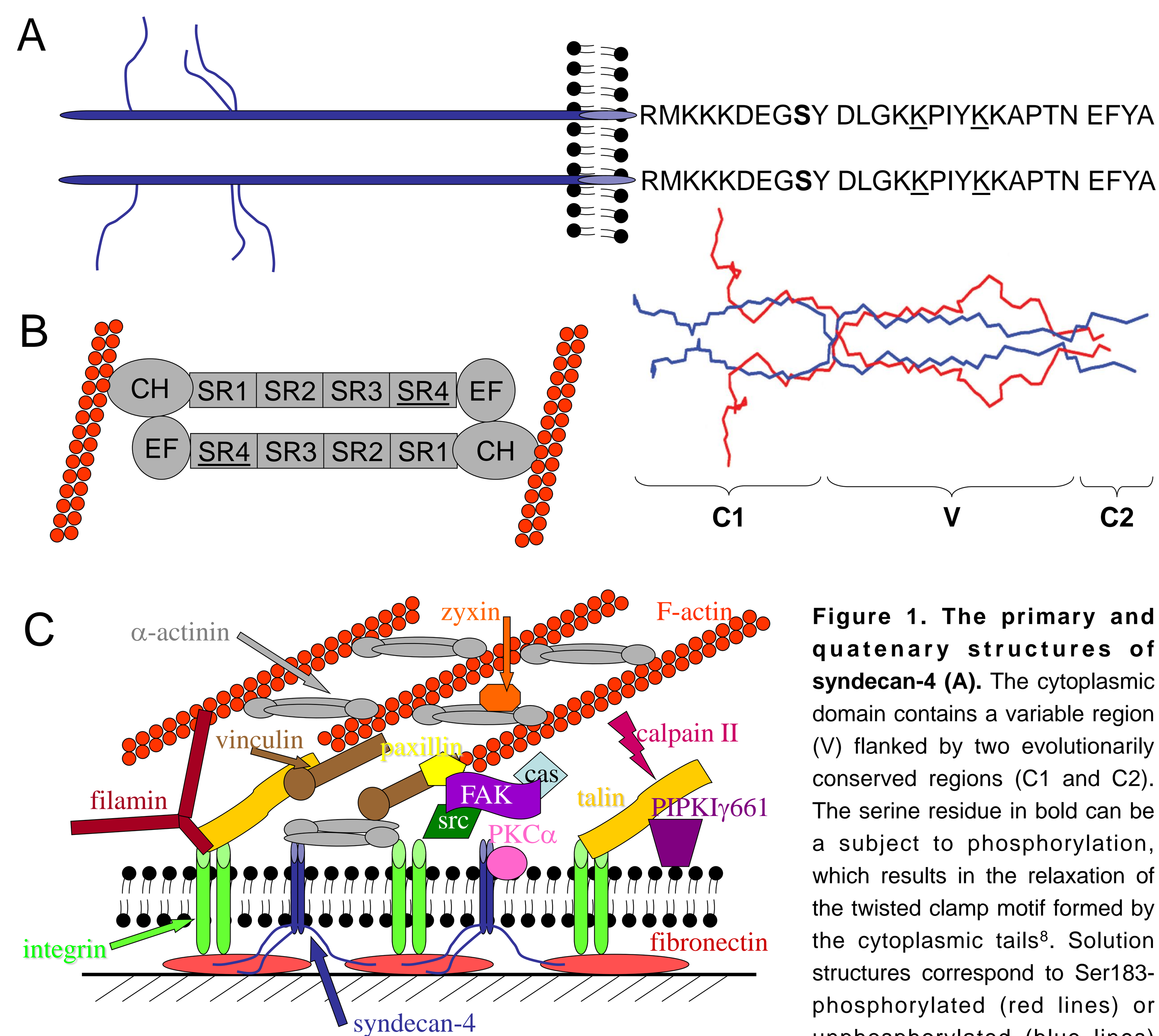


Figure 1. The primary and quaternary structures of syndecan-4 (A). The cytoplasmic domain contains a variable region (V) flanked by two evolutionarily conserved regions (C1 and C2). The serine residue in bold can be a subject to phosphorylation, which results in the relaxation of the twisted clamp motif formed by the cytoplasmic tails[8]. Solution structures correspond to Ser183-phosphorylated (red lines) or unphosphorylated (blue lines) states. Lysine residues underlined are essential for interaction with α-actinin[7]. **The functional unit of α-actinin (B).** Two α-actinin proteins form an antiparallel dimer with actin-binding domains at either end, comprised of a tandem calponin homology domain (CH) from one molecule and a calmodulin-like domain (EF) from the other. The N- and C-termini are separated by four spectrin repeats (SR). **Schematic organisation of the focal adhesion (C).**

Objective

The aims of this study were to establish the role of syndecan-4 in targeting α-actinin to focal adhesions, and refine our knowledge on their interaction and its impact on the cytoskeleton.

Materials and methods

Rat embryo fibroblasts (REFs), syndecan-4 null (sdc4 KO) and matching wild type mouse embryonic fibroblasts (MEFs)[9] were transfected with plasmid constructs coding for human α-actinin 1, isoform a (GenBank: BC003576.1) and rat syndecan-4 (GenBank: M81786). Living cells were detergent extracted and prepared for immunostaining as published previously[10]. The antibodies used were for α-actinin (cl. BM75.2, Sigma), α-smooth muscle actin (α-SMA, cl. 1A4, Sigma), vinculin (cl. hVIN-1, Sigma), and zyxin (Sigma). F-actin was visualised using Alexa-conjugated phalloidin (Molecular Probes). For the solid phase binding and competition assays, the amounts of bound peptides were quantified using the TMB ONE colorimetric peroxidase assay kit (Kem-En-Tec Diagnostics).

Results

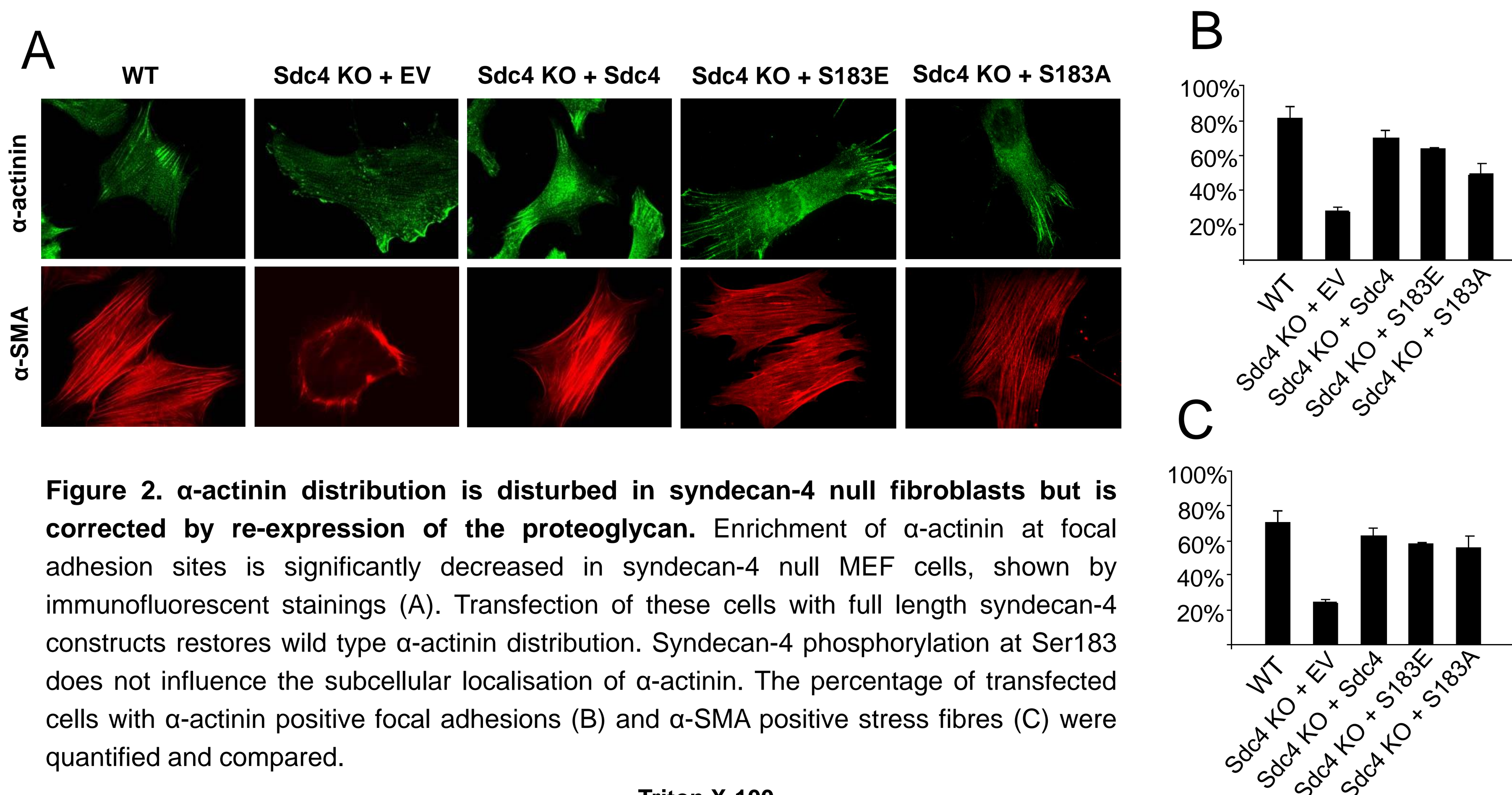


Figure 2. α-actinin distribution is disturbed in syndecan-4 null fibroblasts but is corrected by re-expression of the proteoglycan. Enrichment of α-actinin at focal adhesion sites is significantly decreased in syndecan-4 null MEF cells, shown by immunofluorescent stainings (A). Transfection of these cells with full length syndecan-4 constructs restores wild type α-actinin distribution. Syndecan-4 phosphorylation at Ser183 does not influence the subcellular localisation of α-actinin. The percentage of transfected cells with α-actinin positive focal adhesions (B) and α-SMA positive stress fibres (C) were quantified and compared.

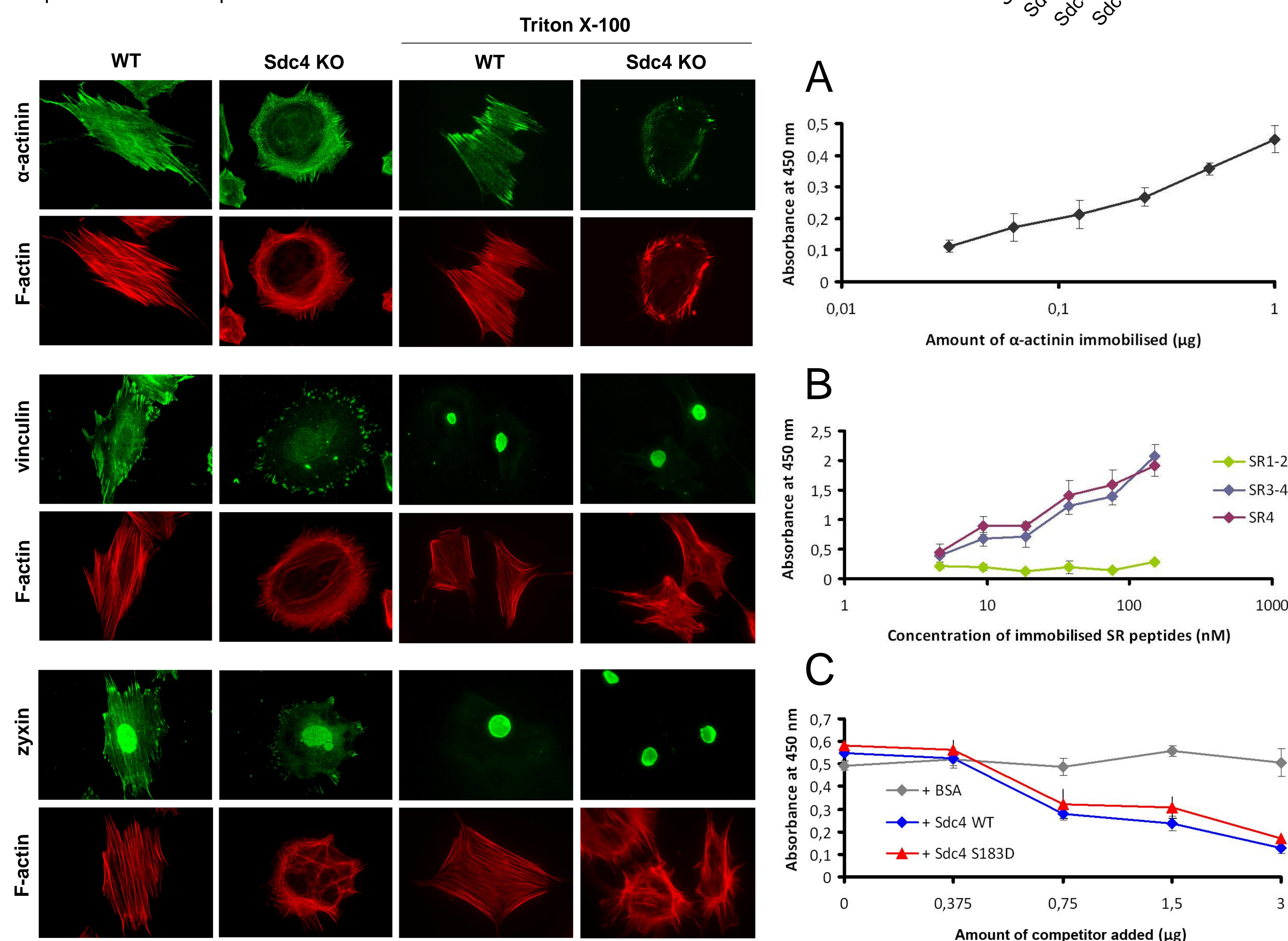


Figure 3. The presence of syndecan-4 prevents extraction of α-actinin by low concentration Triton-X100 treatment. Living MEF cells plated on fibronectin were subjected to mild (0.2%) Triton-X100 extraction. Immuno-fluorescent staining of the detergent resistant remnants revealed that in WT cells α-actinin is resistant to the extraction, as opposed to syndecan-4 null cells, where α-actinin is washed away. Vinculin and zyxin, two other focal adhesion components were extracted regardless of the presence of syndecan-4.

A

Absorbance at 450 nm

Amount of α-actinin immobilised (μg)

B

Absorbance at 450 nm

SR1-2

SR3-4

SR4

Concentration of immobilised SR peptides (nM)

C

Absorbance at 450 nm

+ BSA

+ Sdc4 WT

+ Sdc4 S183D

Amount of competitor added (μg)

Figure 4. α-actinin binds syndecan-4 through its SR4 domain, with the same affinity towards Ser183-phosphorylated or unphosphorylated forms. Solid phase binding assays were used to characterise the binding of α-actinin to syndecan-4. Full length α-actinin binds syndecan-4 (A) and the binding site lies within its SR4 domain (B). Wild type and Ser183-pseudophosphorylated syndecan-4 peptides compete equally for the α-actinin binding site (C).

Conclusion

Syndecan-4 binds the spectrin repeat 4 domain of α-actinin, and directs it to sites of focal adhesion. The interaction does not depend on the phosphorylation status of syndecan-4 at Ser183, and is important in the transmission of traction forces exerted on the stroma generated by stress fibres.

Discussion

We demonstrate that in syndecan-4 null fibroblasts α-actinin shows decreased localisation to focal adhesions, accompanied by decreased number of α-SMA positive stress fibres, recently reported by our group[11]. By mild extraction with Triton-X100 and solid phase binding assays we now show that syndecan-4 is responsible for directing α-actinin to focal adhesions, regardless of its phosphorylation status at its sole serine residue[8, 13]. Incorporation of α-SMA into stress fibres, a reaction to tension exerted on the matrix[12], is strongly promoted by linking syndecan-4 to stress fibres through α-actinin, indicating an important role for syndecan-4 as a cellular mechanotransducer.

Acknowledgements

Danmarks Grundforskningsfond
Danish National Research Foundation

Danish Medical Research Council
Haensch Fond
Vilhelm Pedersen Fond (Novo Nordisk Fonden)
Lundbeck Fonden

References

1 Woods A and Couchman JR. 1994 *Mol Biol Cell*
2 Woods A *et al.* 1986 *EMBO*
3 Bloom L *et al.* 1999 *Mol Biol Cell*
4 Saoncella S *et al.* 1999 PNAS
5 Woods A *et al.* 2000 Arch Biochem Biophys
6 Morgan MR *et al.* 2007 Nat Rev Mol Cell Biol
7 Greene DK *et al.* 1993 J Biol Chem
8 Koo BK *et al.* 2006 J Mol Biol
9 Ishiguro K *et al.* 2000 J Biol Chem
10 Woods A *et al.* 1985 J Biol Chem
11 Gopal S *et al.* 2010 J Biol Chem
12 Hinz B 2006 Eur J Cell Biol
13 Chaudhuri P *et al.* 2005 Circ Res

Correspondance should be addressed to: peter.hollosi@bric.ku.dk