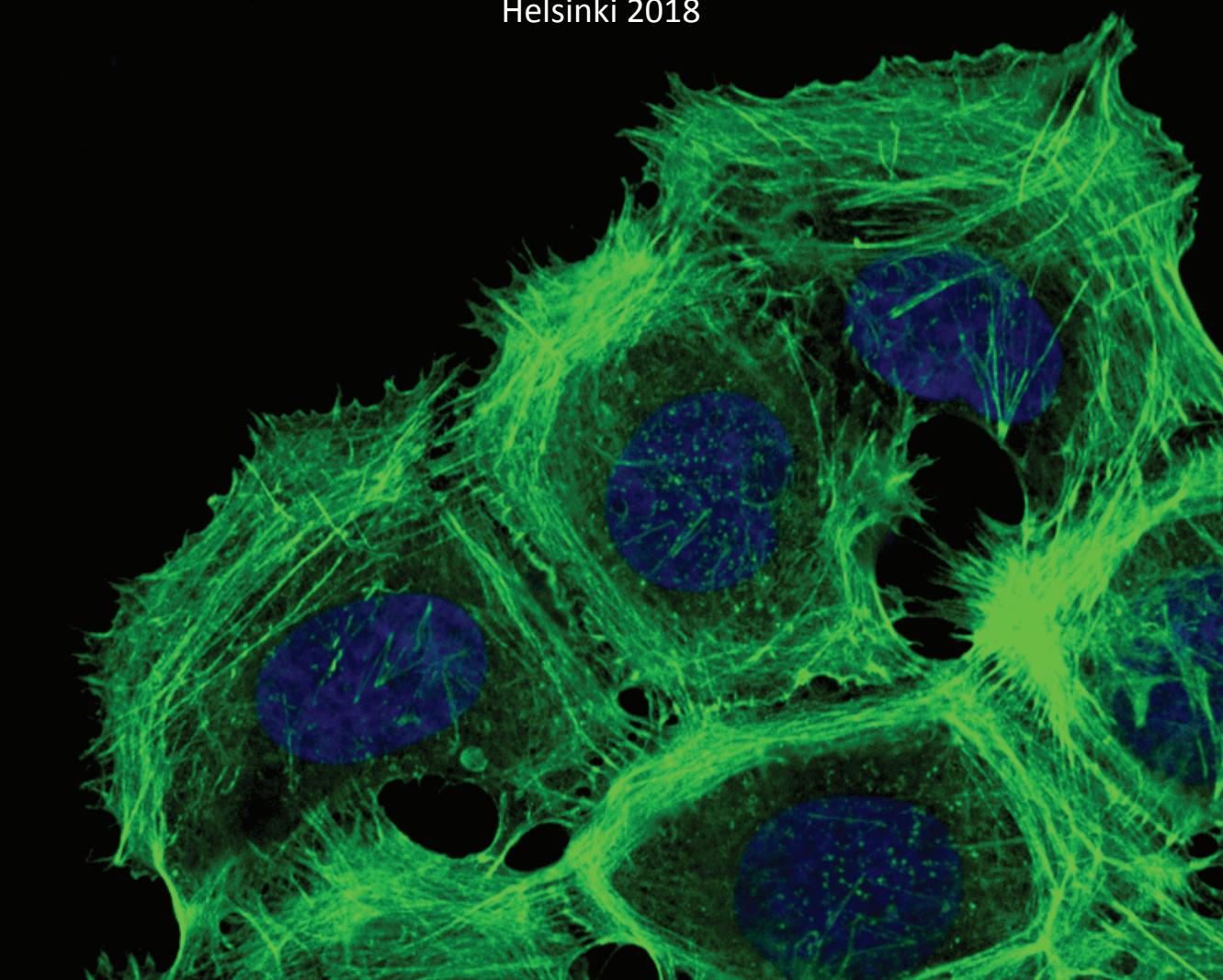
Involvement of non-muscle α-actinins and NUAK2 kinase in regulating actin stress fibers

Bianca Kovac

Faculty of Biological and Environmental Sciences
University of Helsinki
Helsinki 2018



Involvement of non-muscle α-actinins and NUAK2 kinase in regulating actin stress fibers

Bianca Kovac

Division of Genetics
Department of Biosciences
Faculty of Biological and Environmental Sciences
and
Research Programs Unit
Faculty of Medicine
and
Doctoral Programme in Integrative Life Science

University of Helsinki
Finland

ACADEMIC DISSERTATION

To be presented for public examination with the permission of the
Faculty of Biological and Environmental Sciences of the
University of Helsinki, in Haartman Institute,
Lecture Hall 2, Haartmaninkatu 3, Helsinki
on February 16th 2018, at 12 noon.

HELSINKI 2018

Thesis supervisors

Tea Vallenius, M.D., Ph.D.
Research Programs Unit
Faculty of Medicine
University of Helsinki, Finland

Professor Tomi P. Mäkelä
Research Programs Unit and
Helsinki Institute of Life Science
University of Helsinki, Finland

Thesis advisory committee

Professor Olli Carpén
Department of Pathology
University of Helsinki, Finland

Docent Maria Vartiainen
Institute of Biotechnology
University of Helsinki, Finland

Thesis reviewers

Professor Jari Ylänne
Department of Biological and
Environmental Science
University of Jyväskylä, Finland

Docent Pirta Hotulainen
Minerva Institute for Medical Research
University of Helsinki, Finland

Thesis opponent

Associate Professor Sanna Lehtonen
Department of Pathology
University of Helsinki, Finland

Custodian

Professor Juha Partanen
Division of Genetics
Department of Biosciences
Faculty of Biological and Environmental Sciences
University of Helsinki, Finland

Cover image: Confocal image of the wound edge of migrating human osteosarcoma cells stained with F-actin (green) and Hoechst (blue) to visualize the actin cytoskeleton and nucleus, respectively.

ISBN 978-951-51-4053-1 (paperback)
ISBN 978-951-51-4054-8 (PDF)
http://ethesis.helsinki.fi
Press: Hansaprint Oy
Helsinki 2018

“The important thing is not to stop questioning.
Curiosity has its own reason for existing.”

- Albert Einstein

To my loving family

TABLE OF CONTENTS

ABBREVIATIONS **6**

LIST OF ORIGINAL PUBLICATIONS **8**

ABSTRACT **9**

REVIEW OF THE LITERATURE **10**

1. Actin stress fibers and cell migration **10**

1.1 Components of the actin cytoskeleton 10

1.2 Actin stress fibers in non-muscle cells 11

1.3 Architecture of actin stress fibers 12

1.4 Modes of migration 15

1.5 Physiological relevance of actin stress fibers 16

2. Cell adhesion molecules as mechanosensors **17**

2.1 Cell adhesion molecules 17

2.2 Integrin based cell-matrix adhesions 18

2.3 Mechanosensing through cell-matrix adhesions during cell migration 19

2.4 Cadherin based adherens junctions 20

2.5 Plasticity of E-cadherin cell-cell adhesions 22

3. Rho GTPases as actin stress fiber regulators **24**

3.1 Cdc42, Rac1 and RhoA as master regulators 24

3.2 Dynamics of actin stress fiber 26

3.3 Assembly and regulation of actin stress fiber subtypes 28

3.4 Contractility of actin stress fibers 30

4. α-actinins in non-muscle cells **31**

4.1 Family of α-actinins 31

4.2 The structure of α-actinins 31

4.3 Functions of non-muscle α-actinin-1 and α-actinin-4 33

4.4 α-actinin-1 and α-actinin-4 in disease and cancer 36

AIMS OF THE STUDY **39**

MATERIALS AND METHODS **40**

RESULTS AND DISCUSSION **45**

1. NUAK2 kinase regulates actin stress fibers and cell contractility (I) **45**

1.1 Association of NUAK2 and MRIP on actin stress fibers 45

1.2 NUAK2 promotes central actin stress fibers and cell contractility 45

1.3 Actin stress fibers are regulated by NUAK2 expression 48

2. α-actinin-1 regulates actin stress fibers and cell plasticity in mesenchymal cells (II) **49**

2.1 α-actinin-1 and α-actinin-4 assemble distinct actin stress fibers 49

2.2 α-actinin-1 modulates cell-matrix adhesions in mesenchymally migrating cells 50

2.3 α-actinin-1 promotes cell migration and early cell spreading independently of myosin phosphorylation 51

2.4 Rac1 signaling regulates actin stress fibers assembled by α-actinin-1 52

3. α-actinin-1 contributes to cancer progression (III) **54**

3.1 α-actinin-1 is upregulated in various cancers 54

3.2 α-actinin-1 regulates epithelial cell plasticity and promotes cell migration 54

3.3 α-actinin-1 and α-actinin-4 have different roles in cancer 55

CONCLUDING REMARKS **59**

ACKNOWLEDGEMENTS **60**

REFERENCES **63**

ABBREVIATIONS

ab	antibody
ABD	actin binding domain
ACTN1	*alpha-actinin-1* gene
ACTN4	*alpha-actinin-4* gene
AMPK	AMP-activated protein kinase
Arp2/3	actin related protein 2/3
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
Cdc42	cell division control protein 42
cDNA	complementary deoxyribonucleic acid
CDH1	*E-cadherin* gene
CLP36	C-terminal LIM protein
CMTP	congenital macrothrombocytopenia
C-terminus	carboxyl terminus
DAAM	disheveled-associated activators of morphogenesis
E-cadherin	epithelial cadherin
ECM	extracellular matrix
EMT	epithelial-to-mesenchymal transition
Ena/VASP	enabled/vasodilator-stimulated phosphoprotein
ER	estrogen receptor
ERK1/2	extracellular signal-regulated kinases 1/2
F-actin	filamentous actin
FAK	focal adhesion kinase
FHOD	formin homology domain containing protein
FMN	original “namesake” formins
FMNL	formin-like nucleator
FSGS	focal and segmental glomerulosclerosis
G-actin	globular actin
GAP	GTPase activating proteins
GDI	guanine nucleotide dissociation inhibitors
GEF	guanine nucleotide exchange factor
GFP	green fluorescent protein
HER2	human epidermal growth factor receptor 2
HDAC7	histone deacetylase 7
INF	inverted formins
kDa	kilodalton
LIMK	LIM-motif containing kinase
LINC	linker of nucleoskeleton and cytoskeleton complex
LKB1	liver kinase B1 (also known as STK11)
mDia	mammalian homologue of *Drosophila diaphanous*

MEF	mouse embryonic fibroblasts
MEF2	myocyte enhancer factor 2
MET	mesenchymal-to-epithelial transition
MLC	myosin light chain
MLCK	myosin light chain kinase
MLCP	myosin light chain phosphatase
mRNA	messenger ribonucleic acid
MRIP	myosin phosphatase-Rho interacting protein
MYH9	*myosin II A* gene
MYPT1	myosin phosphatase targeting subunit 1
N-cadherin	neuronal cadherin
NF-kB	nuclear factor kappa-light chain enhancer or activated B cells
N-terminus	amino terminus
NPF	nuclear promoting factor
NUAK2	sucrose-non-fermenting AMPK related kinase (also known as SNARK)
PAK	p21-activated kinase
P-cadherin	placental cadherin
PJS	Peutz-Jeghers Syndrome
PP1	protein phosphatase 1
PR	progesterone receptor
Rac1	Ras-related C3 botulinum toxin substrate 1
RhoA	Ras homology gene family member A
Rho GTPases	Ras-related small GTPases
ROCK	Rho-associated coiled-coil kinase
siRNA	small interfering RNA
TGF-β	transforming growth factor β
TMA	tissue microarray
VE-cadherin	vascular epithelium cadherin
WASP	Wiskott-Aldrich Syndrome protein
WAVE	WASP-family verprolin-homologous protein

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their Roman numerals. In addition, some unpublished data are presented.

I. Tea Vallenius*, Kari Vaahtomeri*, **Bianca Kovac**, Ana-Maria Osiceanu, Martta Viljanen and Tomi P Mäkelä (2011). An association between NUAK2 and MRIP reveals a novel mechanism for regulation of actin stress fibers. *J Cell Sci, 2011. 124:384-93.* * Equal contribution

II. **Bianca Kovac**, Jessica L Teo, Tomi P Mäkelä and Tea Vallenius (2013). Assembly of non-contractile dorsal stress fibers requires alpha-actinin-1 and Rac1 in migrating and spreading cells. *J Cell Sci, 2013, 126:263-73.*

III. **Bianca Kovac**, Tomi P Mäkelä and Tea Vallenius (2017). Increased alpha-actinin-1 destabilizes E-cadherin based adhesions and associates with poor prognosis in ER negative breast cancer. *Submitted manuscript.*

The author´s contribution to each publication:

I. BK performed and analyzed immunofluorescence experiments, assisted in writing materials and methods. This publication is also included in the thesis of Dr. Kari Vaahtomeri.

II. BK planned, performed and analyzed majority of the experiments, and participated in writing the publication together with the other authors.

III. BK planned, performed and analyzed majority of the experiments, and participated in writing the manuscript together with the other authors.

ABSTRACT

Actin cytoskeleton is essential in generating mechanical forces together with the associated adhesions and transmitting signals that impact processes such as cell migration. Cell migration is necessary for numerous biological processes including wound healing and embryonic development. Moreover, aberrant cell migration promotes cancer invasion and metastasis. Cell migration events require dramatic spatial and temporal reorganization of the actin cytoskeleton that involves coordinated formation and regulation of multiple structures such as actin stress fibers. Actin stress fibers are dynamic structures, which differ in their subcellular localization, connection to substratum and their dynamics. However, these actin stress fibers are less characterized in terms of the molecules required for assembly-disassembly and the signaling pathways involved in regulating their functions in mesenchymal and epithelial cells. This thesis focuses on characterizing key players and signaling pathways involved in regulating actin stress fiber assembly-disassembly, cell adhesion and contractility. Understanding these cell plasticity changes is essential as in cancer context they are likely to be deregulated thus leading to increased migratory and invasive potential of the cells.

During this thesis study, NUAK2 a novel serine-threonine kinase was identified to associate with myosin phosphatase Rho-interacting protein (MRIP) on actin stress fibers. Association between NUAK2 and MRIP increases cells contractility and promotes formation of actin stress fibers through phosphorylation of myosin light chain (MLC). The identified NUAK2-MRIP association reveals a novel mechanism for the maintenance of actin stress fibers. Our findings implicate NUAK2 as an important regulator of cell contractility and actin stress fiber assembly. Thus providing further knowledge of how actin stress fibers and cell contractility can be regulated in mesenchymal cells.

To further characterize the specificity of molecules required for the assembly of actin stress fibers, we studied the function of most abundant actin crosslinking proteins in non-muscle cells, α-actinin-1 and α-actinin-4. Our findings reveal that specifically α-actinin-1 and not α-actinin-4, is required to assemble dorsal stress fibers found at the leading edge of mesenchymal cells. In addition, loss of α-actinin-1 modulates cell-matrix adhesions leading to decreased cell migration without altering cells contractility. Contrary to traditional views, dorsal stress fibers assembled by α-actinin-1 are non-contractile and are induced by Rac1 signaling. Rac1 is essential in regulating polymerization of actin filaments. Thus suggesting that force required for cell migration is at least partially generated through actin polymerization. Interestingly, we found α-actinin-1 to be upregulated in various cancers and especially associates with decreased survival in estrogen receptor (ER) negative breast cancer patients. In mammary epithelial cells, α-actinin-1 levels regulate epithelial cell plasticity, reorganize actin stress fibers and destabilize cell-cell adhesions accompanied with increased cell migration. This thesis extends the knowledge of especially α-actinin-1 in regulating actin stress fiber assembly and cell plasticity in both epithelial and mesenchymal cells. Furthermore, identifying α-actinin-1 as a candidate prognostic biomarker in ER negative breast cancer patients.

REVIEW OF THE LITERATURE

The actin cytoskeleton is an essential component in creating motility-driving forces in co-operation with associated adhesions during cell migration (Aguilar-Cuenca et al., 2017; Heath and Dunn, 1978; Olson and Sahai, 2009). Cell migration is necessary for numerous biological processes including wound healing, embryonic development and immune response (Horwitz and Webb, 2003). Deregulation of cell migration promotes progression of many diseases including cancer invasion and metastasis (Fife et al., 2014; Hall, 2009; Yamaguchi and Condeelis, 2007). Process of cell migration involves a cascade of events including changes in cell morphology and polarization, formation of protrusions at the leading edge, attachment and interaction with the extracellular matrix, production of forces required for cell body movement and tail retraction (Ananthakrishnan and Ehrlicher, 2007; Kirfel et al., 2004; Lauffenburger and Horwitz, 1996; Ridley et al., 2003; Sheetz, 1994). These alterations during cell migration require dramatic spatial and temporal reorganization of the actin cytoskeleton that involves coordinated formation and regulation of multiple structures such as actin stress fibers and cell adhesions (Heath and Holifield, 1991; Olson and Sahai, 2009). The following review of the literature contains discussion of actin stress fibers, cell adhesions, Rho GTPase signaling cascade, actin crosslinking protein, α-actinin, and their involvement in regulating cell plasticity and migration.

1. Actin stress fibers and cell migration

1.1 Components of the actin cytoskeleton

The cytoskeleton is a network of protein filaments consisting of three major components named actin filaments, microtubules and intermediate filaments. In cells, these filaments are highly integrated and function in a well-orchestrated manner and have a large dedicated subset of accessory proteins that modify their dynamics and structure (Fletcher and Mullins, 2010). Actin, a globular 42 kDa protein, is the most abundant protein in most eukaryotic cells and form dynamic actin filaments structures (Dominguez and Holmes, 2011; Holmes et al., 1990; Kabsch et al., 1990). There are six actin genes identified in *Homo sapiens,* four muscle actins (α-skeletal, α-cardiac, α-smooth muscle and γ-smooth muscle actin) and two non-muscle actins (β-actin and γ-actin) that are ubiquitously expressed (Mounier et al., 1997; Rubenstein, 1990; Vandekerckhove and Weber, 1978). These actin genes share high degree (93%) of protein sequence identity but nevertheless functionally are very diverse due to interactions with specific subsets of actin binding proteins (Mounier et al., 1997; Perrin and Ervasti, 2010). Actin filaments are formed through head-to-tail polymerization of actin monomers (G-actin) and exist as two helical interlaced strands of filamentous actin subunits (F-actin) (Dominguez and Holmes, 2011; Schleicher and Jockusch, 2008).

Actin filaments are arranged into different actin-based entities and contribute to remarkable variety of activities depending on its selective interactions with various actin binding proteins and spatial localization within the cell (Chhabra and Higgs, 2007; Pollard and Cooper, 2009). Actin filaments can organize into different networks in a cell such as branched actin networks (lamellipodial actin), parallel actin networks (filopodial actin) and anti-parallel actin filament networks (contractile actin stress fibers). The described actin networks are presented in Figure 1. These networks act as mechanical elements to drive cell shape changes and migration (Blanchoin et al., 2014; Ridley, 2011). At the cell front, actin assembly drives the extensions of flat, dynamic membrane protrusions called lamellipodia and finger-like protrusions called filopodia (Abercrombie et al., 1971). Lamellipodia consists of dense and branched actin filament network and is involved in pushing forward the plasma membrane and interaction with various signaling molecules (Koestler et al., 2008; Pollard and Borisy, 2003). Filopodia is composed of parallel actin filaments and is important for probing the microenvironment, nutrient transport and sensory processes (Mattila and Lappalainen, 2008). In the more stable lamella region, localized behind the lamellipodium, cell forms adhesion structures such as focal adhesions that couples the extracellular matrix to the actin and myosin mediated contractility network to provide the mechanical force (Ponti et al., 2004). In the lamella region and rear of the cell, these dynamic structures composed of anti-parallel contractile structures are named actin stress fibers. Actin stress fibers have an important role in mechanical response and force generation during cell migration and tail retraction (Le Clainche and Carlier, 2008; Letort et al., 2015; Parsons et al., 2010).

1.2 Actin stress fibers in non-muscle cells

Actin stress fibers were first detected in cultured cells by light microscope as dark lines and described as thin, cytoplasmic “tension striae” or “stress fibers” (Lewis, 1924). Furthermore, under the electron microscope detected as more prominent, straight bundles varying in size with broadened termini pointed to cell membrane (Abercrombie et al., 1971; Buckley and Porter, 1967; McNutt et al., 1971; Perdue, 1973). Actin stress fibers are thought to resemble sarcomeric-like structures found in muscle cells based on their protein composition, interacting proteins and ability to contract both *in vitro* and *in vivo* (Clark et al., 2002; Kreis and Birchmeier, 1980; Peterson et al., 2004; Sanger et al., 1983). Supporting evidence originated from early immunofluorescence studies indicating that actin localizes as continuous line along the fibers (Lazarides and Weber, 1974), whereas myosin and tropomyosin demonstrate an overlapping periodic staining (Lazarides, 1975a; Weber and Groeschel-Stewart, 1974). α-actinin is also periodically distributed along the actin stress fibers (Lazarides, 1975b) but not overlapping with myosin or tropomyosin demonstrating that they are alternately spaced (Gordon, 1978).

In general, actin stress fibers are bundled through myosin and actin filament crosslinking proteins such as α-actinin to build long, straight, contractile fibers with alternating polarities (Cramer et al., 1997; Lazarides, 1975b; Weber and Groeschel-Stewart, 1974). The contractility of actin stress fibers is mainly mediated by the myosin II activity. Three myosin isoforms have been identified in mammalian cells, myosin IIA, IIB and IIC. Myosin IIA and IIB are predominant isoforms whereas myosin IIC is expressed in more restricted subset of cells including neural cells, breast and lung cells (Jana et al., 2006; Vicente-Manzanares et al., 2009). These contractile actin stress fibers are formed when they are stably anchored to the substrate such as focal adhesions, which connect the extracellular matrix to the actin cytoskeleton (Chrzanowska-Wodnicka and Burridge, 1996; Giuliano and Taylor, 1990; Pellegrin, 2007). Actin filaments are dynamic structures having a fast-growing barbed end (+ end) and a slow-growing pointed end (- end). Actin stress fibers are regulated in several ways including actin filament nucleation, elongation, severing, capping, crosslinking and actin monomer sequestration (Pollard and Cooper, 2009; Welch and Mullins, 2002). These events are controlled through various actin-binding proteins and regulated most notably by Rho family GTPases that act as GTP-dependent molecular switches (discussed in section 3).

1.3 Architecture of actin stress fibers

Actin stress fibers have been extensively studied in migrating and spreading cells where they have been found to differ in their subcellular localization, connection to substratum and assembly mechanism. Actin stress fibers were initially subcategorized based on their subcellular localization and association with focal adhesions in mouse fibroblasts studies (Small et al., 1998). More recently, this view has been expanded and actin stress fibers are further characterized according to their dynamics, assembly mechanisms, myosin II abundance and function (Ang et al., 2010; Burnette et al., 2011; Feng et al., 2013; Gateva et al., 2014; Hotulainen and Lappalainen, 2006; Khatau et al., 2009; Maninova and Vomastek, 2016; Naumanen et al., 2008; Oakes et al., 2012; Schulze et al., 2014; Skau et al., 2016; Skau et al., 2015; Tee et al., 2015; Tojkander et al., 2015; Tojkander et al., 2012; Tojkander et al., 2011; Vallenius, 2013). These actin stress fiber subtypes are named: dorsal stress fibers, transverse arcs, ventral stress fibers and more recently identified subtype, perinuclear actin cap. The actin stress fibers subtypes are presented in Figure 1.

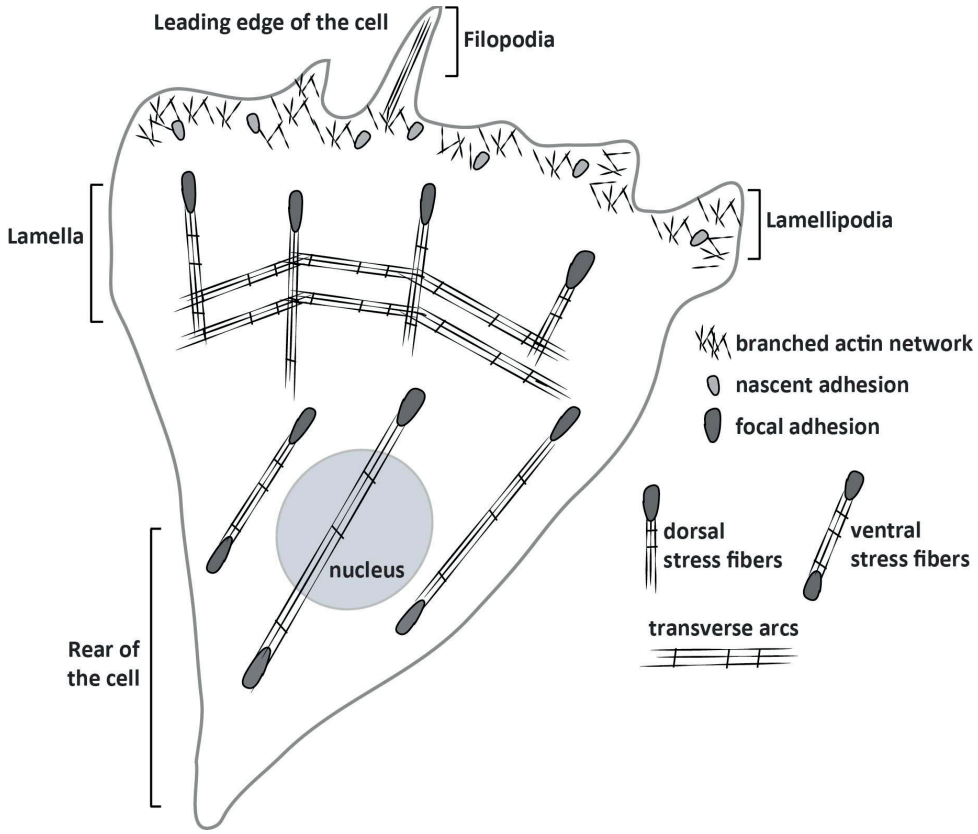


Figure 1. Schematic representation of a mesenchymally migrating cell. A cell can sense the environment initially at the leading edge of the cell with finger-like protrusions named filopodia consisting of parallel actin filaments. Lamellipodia consists of branched actin filament network and nascent adhesions. In the lamella region focal adhesions mature and actin stress fibers are formed. There are four actin stress fibers (anti-parallel actin filaments) subtypes characterized in mesenchymally migrating cells. Dorsal stress fibers elongate from a focal adhesion, transverse arcs are connected to the dorsal stress fibers, and ventral stress fibers are attached to a focal adhesion from both ends and usually persist at the rear of the cell. Perinuclear actin cap fibers assemble from dorsal fibers, transverse arcs, and peripheral ventral stress fibers and are localized above the nucleus.

Dorsal stress fibers and transverse arcs are found at the leading edge of a cell, where they are orientated perpendicular to each other. Dorsal stress fibers are anchored from one end to a focal adhesions and the other end often interacts with transverse arcs (Figure 1). This network is suggested to form a mechanical link between the transverse arc and maturing focal adhesion, where dorsal stress fibers act as a structural template for the maturation of integrin based focal adhesions (Hotulainen and Lappalainen, 2006; Naumanen et al., 2008; Oakes et al., 2012; Parsons et al., 2010; Small et al., 1998; Tojkander et al., 2012; Tojkander et al., 2011; Vallenius, 2013). More recently it has been reported that tensin-containing fibrillar adhesions together with dorsal stress fibers form a specific adhesion-associated actin structures that mediates generation and remodeling of the extracellular matrix (Skau et al., 2015). Fibrillar adhesions are mature focal adhesions that are responsible for fibronectin fibrillogenesis (Katz et al., 2000).

Transverse arcs are curve-shaped and do not interact with focal adhesions. They are contractile actomyosin bundles displaying a periodic α-actinin and myosin II pattern and are assembled from dorsal stress fibers that originate in the lamellipodium. Therefore they are thought to serve as a structural element underlying the connection between the lamellipodium and the lamella during directed cell migration. Transverse arcs orientate parallel with the leading edge and are continuously moving towards the nucleus in a retrograde flow manner. Furthermore transverse arcs require dorsal stress fibers to create a contractile network that generates a flat lamella in cells (Burnette et al., 2011; Burnette et al., 2014; Feng et al., 2013; Heath, 1983; Hotulainen and Lappalainen, 2006; Small et al., 1998; Tee et al., 2015).

Ventral stress fibers are contractile and terminate to focal adhesions at their both ends (Figure 1). These fibers are localized at the ventral surface of the cell where they promote formation of front-to-rear polarity and trail retraction during cell migration (Ang et al., 2010; Chen, 1981; Hotulainen and Lappalainen, 2006; Naumanen et al., 2008; Small et al., 1998; Tojkander et al., 2012; Tojkander et al., 2011; Vallenius, 2013; Vicente-Manzanares et al., 2008). Contractile ventral stress fibers formation is thought to assemble through the endwise joining of preexisting network of dorsal stress fibers and transverse arcs. This process is mechanosensitive and spatio-temporally coordinated (Hotulainen and Lappalainen, 2006; Schulze et al., 2014; Tojkander et al., 2015; Tojkander et al., 2012). In addition, ventral stress fibers may also be generated from the fusion of short focal adhesion attached actin stress fibers (Zimerman et al., 2004). More recently it has been suggested that ventral stress fibers can be formed from the perinuclear actin cap following nucleus displacement (Kim et al., 2012; Maninova et al., 2017).

Perinuclear actin cap consists of contractile stress fibers positioned above the nucleus where they associate with nuclear envelope protein named the linker of nucleoskeleton and cytoskeleton (LINC) complex, which physically links actin fibers with nuclear lamina (Khatau et al., 2009; Maninova et al.,

2017; Maninova and Vomastek, 2016). Perinuclear actin cap is reported to assemble from dorsal fibers, transverse arcs, and peripheral bundles (Maninova and Vomastek, 2016). Proposed function for perinuclear actin cap is that it mediates the interplay between cell shape, nuclear shape and cell adhesion (Khatau et al., 2009; Maninova et al., 2017). Furthermore, perinuclear actin cap fibers are extremely dynamic and can protect the nucleus from physical damage in confined three-dimensional matrices during migration. Additionally, actin cap is important in transducing mechanical cues to the nucleus (Burridge and Guilluy, 2016; Kim et al., 2012; Skau et al., 2016).

1.4 Modes of migration

Migrating and stationary cells have differences in actin stress fibers. Initially it was considered that actin stress fibers are not required for cell migration per se (Burridge, 1981; Herman et al., 1981), but instead contribute to migration by generating force to release the tail thus promoting migration (Crowley and Horwitz, 1995). This observation was based on findings that actin stress fibers are absent from highly migratory cells such as leukocytes and single cell *Dictyostelium discoideum* amoeba (Valerius et al., 1981; Yumura et al., 1984). Currently this observation is explained through a broader understanding of cells owing different migration modes that require changes in the cytoskeletal organization, morphology patterns and cell-matrix interactions (Friedl and Wolf, 2003). Additionally, it is considered that cells with actin stress fibers must have dynamic coupling between adhesion strength and traction force (Parsons et al., 2010). For a cell to be able to migrate forward it should obtain stronger adhesion and traction force at the leading edge than at the rear of the cell, thus the force is derived from both retrograde actin flow and myosin-generated tension (Burridge and Guilluy, 2016; Case and Waterman, 2015).

The broad classification of the different migration modes involves either single (amoeboid or mesenchymal) or collective (a group of cells) cell migration (Friedl and Alexander, 2011; Friedl and Wolf, 2003; Friedl and Wolf, 2010; Sahai, 2005). Characteristics for amoeboid migration mode are low adhesion forces, sparse actin stress fibers and ability to adapt cell shape and squeeze through tissue gaps. Migrating leukocytes, neutrophils and lymphoma cancer cells are examples of cell types utilizing this migration mode (Friedl and Alexander, 2011; Lammermann and Sixt, 2009). In contrast, cells adopting mesenchymal migration mode are elongated, fan-shaped cells with prominent actin stress fibers and the ability to degrade and remodel the extracellular matrix while migrating. Cell utilizing mesenchymal migration mode are for example fibroblasts and fibrosarcoma cancer cells. During migration these cells undergo the migration cycle: formation of protrusions, cell-matrix adhesions, contractile actin stress fibers and rear retraction (Ananthakrishnan and Ehrlicher, 2007; Friedl and Wolf, 2003; Ridley et al., 2003).

Collective cell migration is essential in events such embryological development, wound healing and branching morphogenesis (Vaughan and Trinkaus, 1966). Cells migrate as a sheet of several cells,

maintaining adhesive cell-cell contacts. Cells at the front generate the front-to-rear asymmetry, whereas cells located at the rear of the cell remain largely none motile (Friedl and Wolf, 2003; Haeger et al., 2014). Furthermore, it is important to emphasize that cells can modify their migration modes in response to different conditions. During developmental events such as gastrulation, transition of collectively migrating epithelial cells to mesenchymal migratory phenotype, known as epithelial-to-mesenchymal transition (EMT), is essential (Thiery et al., 2009). This migratory event is critical also during cancer metastasis, where it is thought to provide cancer cells migratory potential from primary tumor to secondary sites (metastasis) (Friedl et al., 2012; Friedl and Wolf, 2010; Thiery et al., 2009).

1.5 Physiological relevance of actin stress fibers

In cultured cells, actin stress fibers are prominent, dynamic and well-studied structures. Whereas their role and existence *in vivo* and in three-dimensional environment has been somewhat controversial due to changes in the microenvironment and substrate stiffness, which was thought to cause loss of actin stress fibers (Burridge and Chrzanowska-Wodnicka, 1996; Mochitate et al., 1991). Nevertheless, the increased understanding that biochemical and mechanical interactions between cell and the surrounding environment have an impact on actin stress fiber abundance, structure and organization has allowed the researchers to use versatile approaches in their investigations and to better understand the role of actin stress fibers under a variety of conditions. Advances in three-dimensional culture models and in various techniques such as super-resolution imaging, traction force microscopy, atomic force microscopy, molecular biosensors, femtosecond laser ablation, structures illumination microscopy (SIM) and single-cell micropatterning, has made it possible to study the mechanical and structural properties of actin stress fibers as well as cell-matrix adhesions (Beach et al., 2014; Burnette et al., 2014; Doyle et al., 2015; Fischer et al., 2009; Kassianidou and Kumar, 2015; Kubow and Horwitz, 2011; Kumar and Weaver, 2009; Lee and Kumar, 2016; Lu et al., 2008b; Worth and Parsons, 2008). These advantages are providing excellent tools to understand physical models of how actin stress fibers contract and contribute to overall mechanics of the cell.

Current findings in various tissues and *in vivo* suggest that actin stress fibers (actin cables, actin filaments, actin bundles) are essential in endothelial cells regulating blood flow tension and mechanical stress (e.g. aortic valve and aorta), in platelet activation in case of endothelial injury, in tendons monitoring tensile load, in directing the morphogenesis of the pharyngeal pouches as well as in processes such as wound healing, embryonic epithelial sheet closure and mammary duct contraction (Gudjonsson et al., 2005; Jacinto et al., 2002; Lu et al., 2008a; Quinlan et al., 2004; Ralphs et al., 2002; Tanaka and Itoh, 1998; Tomasek et al., 2002; van Nieuw Amerongen and van Hinsbergh, 2001; Wong et al., 1983). Furthermore, actin fibers are present in dendritic spines where they underlie the stabilization of memories after learning, in neural growth cones and in immunological synapses of T lymphocytes. In addition, actin stress fibers have been shown to develop under pathological conditions such as atherosclerosis, hypertension and ischemia (Hotulainen and

Hoogenraad, 2010; Kwon et al., 2002; Schaefer et al., 2008; van Nieuw Amerongen and van Hinsbergh, 2001; Yi et al., 2012). More recent findings demonstrate that actin stress fibers are crucial in maintaining the filtration barrier in the kidney and their disruption leads to podocyte injuries (Suleiman et al., 2017). Additionally, actin stress fibers can promote cell stiffening and proliferation of pre-invasive breast cancer cells thus could drive tumor growth during premalignant stages (Tavares et al., 2017). In general, actin stress fibers are crucial for various functions by enabling cells to sense and respond to mechanical stimuli, remodeling cell adhesions as well as epithelial and endothelial barriers and promoting migration and invasion of cancer cells (Martin and Parkhurst, 2004; Millan et al., 2010; Olson and Sahai, 2009; Pellegrin, 2007; Tojkander et al., 2012).

2. Cell adhesion molecules as mechanosensors

2.1 Cell adhesion molecules

During cell migration and in events such as tissue morphogenesis and embryogenesis, cell adhesion is a critical event allowing cells to sense and respond to the mechanical cues and transduce appropriate signals (De Pascalis and Etienne-Manneville, 2017; Edelman, 1986; Gumbiner, 1996). Cell adhesion is facilitated through various cell adhesion molecules, which are cell surface proteins involved in mediating the interactions between neighboring cells or between a cell and the extracellular matrix (ECM). Typically a cell adhesion unit consists of a transmembrane cell adhesion molecule, intracellular adaptor proteins, signaling proteins and the cytoskeleton (Katz et al., 1991; Makrilia et al., 2009). Transmembrane adhesion receptor selectively interacts with a ligand (cell/ECM) and binds several cytoplasmic adaptor proteins and signaling proteins located at the intracellular surface of the plasma membrane. These intracellular clusters of proteins connect the adhesion receptor to the cytoskeleton, which in turn coordinates cellular functions and transduces signals (Aplin et al., 1998; Han and de Rooij, 2016; Miyoshi and Takai, 2008). The transmembrane adhesion receptors can be broadly grouped into four distinct families based on their structural and functional similarities: integrins, cadherins, selectins and immunoglobulin (Ig) superfamily (Katz et al., 1991; Makrilia et al., 2009). Furthermore, these transmembrane adhesion receptors can form unique intercellular junctions required for distinct functions through various adaptor proteins and cytoskeletal components. In general, cells can form fluid-tight seals between cells (tight junctions), anchor cells to the extracellular matrix (focal adhesions), form cell-cell adhesions (adhesive junctions) or form junctions that allow diffusion of ions and small molecules between adjacent cells (gap junctions) (Farquhar and Palade, 1963; Kawauchi, 2012).

Integrins and cadherins are major surface transmembrane receptors modulating adhesive cell-matrix and cell-cell junctions, respectively. They can act as mechanotransduction receptors, respond to environmental constrains and affect cell migration and tissue remodeling (Bachir et al., 2017; Geiger et al., 2001; Martinez-Rico et al., 2010). Both integrin and cadherin interact with several cytoplasmic

proteins, connecting them to the cytoskeleton and activating signaling pathways. Actin filaments and intermediate filaments together with integrin and cadherin can give rise to versatile adhesive junctions. Intermediate filaments give rise to hemidesmosomes and desmosomes at cell-matrix and cell-cell contacts, respectively. Hemidesmosomes are integrin-linked adhesions and play an important role during tissue morphogenesis and wound healing. Whereas desmosomes are cadherin associated adhesions and are essential providing mechanical strength and tissue integrity in tissues such as skin and heart (Green and Jones, 1996; Schmidt and Koch, 2007). Focal adhesions are formed at cell-matrix interphase through integrin (Hynes, 2002), and adherens junctions are formed at cell-cell contacts through cadherin (Niessen and Gottardi, 2008). Actin cytoskeleton plays an essential role in regulating both focal adhesions and adherens junctions by providing contractile forces required for cell migration and maintaining physical association between cells (Brieher and Yap, 2013; Ciobanasu et al., 2012).

2.2 Integrin based cell-matrix adhesions

Structurally defined adhesion sites at the cell-matrix interphase were initially identified in cultured fibroblasts and found to be important in cell spreading and migration (Abercrombie and Dunn, 1975; Izzard and Lochner, 1980). Integrin is found to be a major transmembrane receptor family by which cells attach to the extracellular matrix and also in some cases to adjacent cells (Hynes, 1987). Integrin receptor is a heterodimer consisting of α and β subunits with a large extracellular domain responsible for ligand binding, a transmembrane domain and a cytoplasmic domain (Hynes, 1992; Schwartz et al., 1995). There are several α and β subunit isoforms that can give rise to various combinations depending on the binding specificity that integrin receptor has to different extracellular matrix components such as fibronectin, collagen, laminin and vitronectin (Burridge and Chrzanowska-Wodnicka, 1996; Fath et al., 1989; Geiger et al., 2001). Furthermore, cytoplasmic domain of the integrin receptor contains a large number of proteins that can strengthen the mechanical link between the extracellular matrix and the actin cytoskeleton as well as participate in the adhesion-mediated signaling to regulate the coordination of cell protrusion, adhesion and contraction (Zaidel-Bar et al., 2003; Zamir and Geiger, 2001).

Integrin based adhesions (adhesomes) are multiprotein complexes consisting of over 160 distinct components (Geiger et al., 2009; Geiger and Yamada, 2011; Zaidel-Bar et al., 2007). Integrin mediated interactions with the extracellular matrix can trigger formation of different matrix adhesions. These adhesions can undergo dynamic structural changes that are regulated by the matrix rigidity and are driven by mechanical forces generated through the actin stress fibers and by external forces applied to the cells (Geiger and Yamada, 2011; Riveline et al., 2001). Distinct types of adhesions include focal adhesions, fibrillar adhesions, podosomes and invadopodia. Actin stress fibers form a continuous structural network that is mechanically coupled to the extracellular matrix through focal adhesions. This dynamic network allows cells to sense the matrix and generate tension required during cell

migration (Lee and Kumar, 2016; Parsons et al., 2010; Ridley et al., 2003). Fibrillar adhesions are an additional form of adhesion sites that are tensin-enriched and are involved in the fibronectin fibrillogenesis (Katz et al., 2000; Pankov et al., 2000). Podosomes and invadopodia both form ringlike, actin-rich membrane adhesion structures. Podosomes are prominent structures found in different monocyte derivatives where they are involved in matrix modulation and matrix invasion by cancer cells. Invadopodia structures are associated with degradation of the extracellular matrix in cancer cell invasion and metastasis (Gimona et al., 2008).

2.3 Mechanosensing through cell-matrix adhesions during cell migration

Cellular mechanosensing is produced and transmitted through focal adhesions consisting of discrete protein clusters that are located at the basal surface of cells (Bershadsky et al., 2006; Burridge and Guilluy, 2016; Geiger and Yamada, 2011). Moreover, cell migration requires continuous formation and disassembly of focal adhesions (adhesion turnover) to occur in coordinated manner during which adhesions are going through several morphological and compositional changes (Gardel et al., 2010; Worth and Parsons, 2008). Initial adhesion formation takes place at the leading edge of protrusions, in the lamellipodial region, whereas disassembly occurs both at the cell rear and at the base of protrusions (Webb et al., 2002). The initial form of adhesions, nascent adhesions, resembles a dot-like structure that is formed in the lamellipodial region of the cell (Figure 1). Nascent adhesions are short-lived, transient structures that either disappear or develop into mature focal adhesions (Nobes and Hall, 1995; Zaidel-Bar et al., 2003). Formation of nascent adhesions requires actin polymerization coupled with the retrograde flow of F-actin (Alexandrova et al., 2008; Giannone et al., 2007). Furthermore, formation and turnover of nascent adhesions persists in the absence of non-muscle myosin II mediated tension (Choi et al., 2008; Vicente-Manzanares et al., 2007). Focal complex represents an initial form of a mature adhesion that differs from nascent adhesions in size and myosin II dependency (Choi et al., 2008). Focal complexes are transient structures found at the boundary between lamellipodium and lamellum. As lamellipodium moves forward during cell migration, focal complexes mature into larger, elongated focal adhesions. In general, focal complexes serve as a physical platform to slow down the retrograde movement of the lamellipodial actin filament bundles (Riveline et al., 2001; Vicente-Manzanares and Horwitz, 2011).

Focal adhesions represent a fully mature form of adhesions that have a slow turnover and are found in protrusions and cell body. Maturation of focal adhesions is a mechanosensitive process induced by the actomyosin force generated in the lamella region (Figure 1). Structurally, focal adhesions are elongated, streak-like structures associated with actin stress fibers (Bershadsky et al., 2006; Ciobanasu et al., 2012; Heath and Dunn, 1978). Formation of focal adhesions requires myosin II mediated tension but more importantly it requires an actin stress fiber template crosslinked by α-actinin. α-actinin mediates the formation of the actin stress fiber template, which in turn facilitates the recruitment and stable association of focal adhesion proteins required for the compositional

maturation (Choi et al., 2008; Oakes et al., 2012; Parsons et al., 2010). Actin stress fibers associated with focal adhesions grow and incorporate new components, mainly at the focal adhesion–stress fiber interface (Endlich et al., 2007). Focal adhesions act as a "molecular clutch" mechanism that provides dynamic links and bidirectional signaling conduits between the actin cytoskeleton and extracellular environment (Bachir et al., 2017; Case and Waterman, 2015; Gardel et al., 2010; Geiger et al., 2009; Schwartz, 2010). More than 100 focal adhesion specific proteins have been identified, including mechanosignalling proteins (e.g. paxillin, FAK, Src, p130Cas), mechanosensing proteins (e.g. vinculin, talin, zyxin), and actin regulators (e.g. α-actinin, VASP), which mediate inside-out and outside-in signaling, microenvironmental sensing, and coordinated cell migration (Geiger and Yamada, 2011; Hu et al., 2007; Jansen et al., 2017; Kanchanawong et al., 2010; Kuo et al., 2011; Zamir and Geiger, 2001).

2.4 Cadherin based adherens junctions

Adherens junctions are cell-cell adhesion complexes that mediates cell recognition, adhesive interactions between cells, morphogenesis and tissue integrity. These cell-cell interactions are dynamically remodeled and essential during embryogenesis, tissue regeneration and wound repair (Harris and Tepass, 2010; Meng and Takeichi, 2009). In polarized epithelial cells, adherens junctions (zonula adherens) were initially characterized as part of the tripartite junctional complex localized at the apical/basolateral border region, between the tight junctions (zonula occludens) and desmosomes (macula adherens), where they maintain structural and functional integrity of epithelia. Based on ultrastructural observations, the adherens junctions were described as a region at the interface of two adjacent cells with opposing membranes (Farquhar and Palade, 1963). Furthermore the use of deep-etch electron microscopy revealed that the intercellular space of adherens junctions is filled with rod-shaped molecules connecting the membranes and the cytoplasmic side of the adherens junction is associated with a dense actin filament network (Hirokawa and Heuser, 1981; Miyaguchi, 2000). In general, adherens junctions perform multiple functions in cells including initiation and stabilization of cell-cell adhesions, regulation of the actin cytoskeleton, intracellular signaling and transcriptional regulation (Hartsock and Nelson, 2008). Adherens junctions are usually composed of transmembrane adhesion receptors (e.g. cadherin), several membrane proteins that link the adhesive components with the cytoskeleton (e.g. catenins) and the cytoskeletal network anchoring the adhesive components (e.g. actin filaments) (Niessen and Gottardi, 2008).

Cadherin is a large superfamily of calcium-dependent adhesion molecules that play a key role in dynamic cell–cell contact formation, remodeling of junctions and tissues thus are crucial for maintaining overall tissue integrity (Gumbiner, 2005; Halbleib and Nelson, 2006; Niessen et al., 2011). In addition to the mechanical function, cadherin molecules can activate signaling cascades that regulate cytoskeletal organization, cell cycle progression and differentiation as well as act as force sensors to mediate intercellular tension (Leckband and de Rooij, 2014; Lecuit et al., 2011; Yonemura, 2011). Cadherin molecules were originally identified as cell surface glycoproteins responsible for cell-

cell adhesions during development of mouse and chick embryo (Gallin et al., 1983; Peyrieras et al., 1983; Yoshida-Noro et al., 1984). Cadherin molecules are expressed in almost all vertebrate tissues and form primarily homophilic cell-cell interactions that are concentrated at adherens junctions. These cadherin based adherens junctions have been identified and found essential in wide variety of animal species (Hulpiau and van Roy, 2009; Oda and Takeichi, 2011). Cadherin superfamily can be broadly categorized into groups including: major cadherins (32 members), protocadherins (65 members) and cadherin-related cadherins (17 members) (Gul et al., 2017). Classical cadherin group is a large cadherin family found in the major cadherin group consisting of type I and type II classical cadherins. This classical cadherin superfamily is the most extensively studied. Most common type I classical cadherins include epithelial (E-cadherin), neuronal (N-cadherin) and placental (P-cadherin) cadherins, whereas vascular epithelium (VE-cadherin) cadherin represents a common type II classical cadherin (Angst et al., 2001; Halbleib and Nelson, 2006; Saito et al., 2012).

Domain structures of type I and II classical cadherins are similar, each with an ectodomain composed of five tandem extracellular cadherin (EC) repeats, a single-pass transmembrane region and a cytoplasmic domain region. Through the EC repeat domains cells can mediate homophilic ligation and initiate intercellular contacts that are formed by clusters of *trans*- or *cis*-dimers of cadherins on opposing cells. This event is calcium dependent, which is required for the correct conformational organization of the cadherin extracellular domain (Gumbiner, 2005; Halbleib and Nelson, 2006; Harrison et al., 2011; Pokutta et al., 1994). The cytoplasmic domain binds to different cytoplasmic signaling molecules and cytoskeletal proteins, which in turn can locally regulate actin cytoskeleton organization, cadherin stability and intracellular signaling pathways that control gene transcription (Hartsock and Nelson, 2008; Leckband and de Rooij, 2014; Mege and Ishiyama, 2017; Perez-Moreno and Fuchs, 2006).

Cadherin adhesions recruit numerous proteins, collectively known as “cadhesomes”, to form large molecular complexes closely associate with the actin cytoskeleton. This allows cells to modulate their adhesive properties in response to intrinsic or external cues in a temporally and spatially controlled manner (Guo et al., 2014; Zaidel-Bar, 2013). More than 170 proteins have been reported to associate with cadherin based adherens junctions, including mechanosignaling proteins (e.g. β-catenin, p120-catenin), mechano-sensing proteins (e.g. α-catenin, vinculin) and actin regulators (e.g. α-actinin, EPLIN). In addition, these cadhesome-associated proteins can interact with each other, which in turn increases the complexity of the network further. This mechanosensing and adhesion remodeling is essential and allows cell shape changes into tissue dynamics to occur in events such as wound healing and morphogenesis (Bertocchi et al., 2017; Lecuit, 2005; Twiss and de Rooij, 2013; Xia and Kanchanawong, 2017).

2.5 Plasticity of E-cadherin cell-cell adhesions

E-cadherin (epithelial cadherin) was initially identified as a key transmembrane adhesion receptor involved in calcium dependent cell-cell adhesion in V79 Chinese hamster lung cells (Takeichi, 1977). Since its identification E-cadherin has been extensively studied and shown to play pivotal roles in epithelial cell behavior and tissue formation (van Roy and Berx, 2008). An essential part of E-cadherin function in mediating adhesion formation and maturation occurs through the cytoplasmic binding partners and the actin cytoskeleton (Mege and Ishiyama, 2017; Nelson, 2008). Binding of actin filaments to the cytoplasmic region of E-cadherin is crucial for guiding cadherin clustering and assembly, and providing tension mediated stabilization and maturation of cell adhesions (Hong et al., 2013; Maruthamuthu et al., 2010; Ratheesh and Yap, 2012). This in turn allows the force-sensing and force-generating capabilities of the adhesions to participate in complex tissue rearrangements and functions (Cavey and Lecuit, 2009). Cytoplasmic domain of E-cadherin binds to members of catenin protein family, such as β-catenin and p120-catenin. p120-catenin primarily regulates the adhesion stability by controlling E-cadherin retention at the cell surface (Davis et al., 2003). β-catenin binds initially to the cytoplasmic region of E-cadherin to form cadherin/catenin complex, which in turn binds the F-actin binding protein α-catenin (Buckley et al., 2014; Huber et al., 2001). Binding and interaction of tension transducer α-catenin to the cadherin/catenin, links the complex to the actin cytoskeleton and recruits additional cytoplasmic proteins such as vinculin, α-actinin and EPLIN (Abe and Takeichi, 2008; Hazan et al., 1997; Knudsen et al., 1995). Especially recruitment of vinculin occurs and is controlled through force dependent conformational switch of α-catenin and this association stabilizes E-cadherin adhesive clusters (Thomas et al., 2013; Yao et al., 2014; Yonemura et al., 2010). E-cadherin cell adhesion complexes are functional mechanosensors that probe the mechanical environment and transmit force to allow changes in the mechanics of the cell-cell adhesions (le Duc et al., 2010).

In epithelial tissues, E-cadherin is a key mediator of stable cell-cell adhesions and is an essential regulator of tissue integrity. These specialized adhesions between cells allow morphogenetic changes such as the movement of epithelial sheets and the formation of tubes during development to occur (Lecuit and Yap, 2015; Niessen et al., 2011). Epithelial-to-mesenchymal transition (EMT) is a fundamental process during embryonic development and physiological response to injury, where epithelial sheets undergo dynamic cell rearrangements. During this process epithelial cells loose their apico-basal polarity, reorganize their actin cytoskeleton, gain mesenchymal-like properties including increased migratory potential and the stable cell-cell adhesions are disrupted and rearranged. EMT is notably correlated with downregulation of E-cadherin. EMT is reversible and most adult tissues and organs arise from a series of EMT conversions as well as from the reversed process, mesenchymal-to-epithelial transition (MET). In pathological conditions such as fibrosis and cancer progression, EMT and its intermediate states are considered as central driving forces of cancer cell dissemination and tumor progression. Plasticity of EMT and MET is controlled by many signaling pathways that are

involved in normal and pathological conditions (Nieto et al., 2016; Thiery et al., 2009; Ye and Weinberg, 2015).

Recent findings demonstrate a partial EMT resulting in hybrid epithelial and mesenchymal phenotypes in cells. During this phenotypic plasticity, retention of E-cadherin and E-cadherin based adherens junctions are required for collective invasion and migration of cancer cells. This hybrid phenotype has been detected also in circulating tumor cells in the peripheral blood of patients with metastatic carcinoma (Chao et al., 2012; Jolly et al., 2016; Lambert et al., 2017; Lecharpentier et al., 2011; Rodriguez et al., 2012). In the light of partial EMT, rearrangements of the actin cytoskeleton and E-cadherin based cell-cell adhesions are essential. During the course of malignant cancer progression, neoplastic transformation leads to prominent changes in the organization of actin cytoskeleton and the stability of E-cadherin mediated adherens junctions. These morphological and structural transformations are shown to induce collective cell migration and invasion of tumor cells, as well as metastatic outgrowth (Ayollo et al., 2009; Gloushankova et al., 2017; Rubtsova et al., 2015).

Epithelial and junctional remodeling of E-cadherin based adherens junctions is a dynamic event. This process requires rearrangement of dynamic contacts through actomyosin-induced tension as well as structural and compositional changes of the actin cytoskeleton (Biswas and Zaidel-Bar, 2017; Takeichi, 2014). Actomyosin based contractility maintains the shape and function of adherens junctions, is involved in the formation and disassembly of adhesions as well as supports the structural integrity of epithelial tissues (Lecuit and Yap, 2015; Leerberg et al., 2014; Smutny et al., 2010). Ultrastructural studies have allowed characterizing three types of adherens junctions that depend on the adhesion dynamics and actin filaments associated with them. These include linear, punctate and tricellular adherens junction (Takeichi, 2014; Twiss and de Rooij, 2013; Yonemura, 2017). At linear adherens junctions in mature epithelial sheets, actin filaments form circumferential actin bundles that form a continuous line and run parallel to the plasma membrane. E-cadherin colocalizes with these actin filaments to organize the adherens junction (zonula adherens). Linear adherens junction is considered as a mature and stable adhesion that can contract by the interaction with myosin and are found in highly polarized epithelial cells (Leerberg et al., 2014; Mege et al., 2006). Tricellular adherens junctions are formed at the corner where three or more cells meet and are essential in formation of epithelial barrier. Actin filaments bundles in this type of adhesion are associated with plasma membrane at high angles (Ikenouchi et al., 2005; Oda et al., 2014).

Punctate adherens junctions are dynamic, mechanosensing sites that remodel the junction through the actin cytoskeleton thus allowing regulation of E-cadherin stability and mobility. This junctional remodeling is essential in functions such as morphogenesis, epithelial barrier formation, wound healing and tumorigenesis (Twiss et al., 2012; Vaezi et al., 2002). Punctate adhesions are especially interesting considering partial EMT where plasticity of cell-cell contacts retaining E-cadherin and

changes in the actin cytoskeleton provide cells with increased migration and invasion potential (Gloushankova et al., 2017). Punctate junctions are also known as adhesion zipper, nascent, spot or focal adherens junctions depending on the cell type they are found (e.g. keratinocytes, fibroblasts, epithelial or endothelial cells). Punctate adherens junctions are found near the edge of cell colony, where cells come into contact with each other and small adhesive E-cadherin puncta are formed. Stability and mobility of these punctate junctions depend on two actin populations with different dynamic properties. Radial actin fibers associate with the plasma membrane in a perpendicular manner and connect to the E-cadherin puncta. Additionally, a predominant network of thin, contractile arc-like bundles around the junctional cortex is constantly recycled and connects to the radial actin fibers (Cavey et al., 2008; Huveneers et al., 2012; Kobielak et al., 2004; Miyake et al., 2006; Vasioukhin et al., 2000; Yonemura et al., 1995; Zhang et al., 2005).

3. Rho GTPases as actin stress fiber regulators

3.1 Cdc42, Rac1 and RhoA as master regulators

Rho family of GTPases constitutes a distinct family within the superfamily of Ras-related small GTPases and is found in all eukaryotic cells. Rho GTPases act as GTP-dependent molecular switches that contribute to several cellular processes including organization of actin cytoskeleton, cell adhesion, cell polarity and cell migration (Figure 2). The mammalian Rho family of small GTPases consists of 20 proteins that are divided into eight subfamilies, which are classified as typical or atypical depending on their mode of regulation. Cdc42, Rac1 and RhoA belong to the typical Rho GTPases and are recognized as the most important and well-studied regulators of the actin cytoskeleton (Hall and Nobes, 2000; Heasman and Ridley, 2008). Rho GTPases are activated through ligand stimulated transmembrane receptors, whose hydrolysis cycle is modulated by guanine nucleotide exchange factors (GEFs), GTPase activating proteins (GAPs) and guanine nucleotide-dissociation inhibitors (GDIs), thus are considered to act as molecular switches. Moreover, activated GTPases can interact with variety of different downstream targets including actin binding proteins, kinases and other scaffolding molecules that can regulate actin filament rearrangements (Haga and Ridley, 2016; Hall and Nobes, 2000). The actin binding proteins can be categorized into groups that interact with monomeric actin (sequestering and depolymerizing proteins), with F-actin (nucleation, capping and severing proteins) or with actin filament assemblies (elongation, crosslinking, myosin contraction and membrane attachment proteins). This cascade of events can lead to the formation of different cytoskeletal networks such as focal adhesions, filopodia, lamellipodia, and actin stress fibers (Figure 1 and 2) (Etienne-Manneville and Hall, 2002; Spiering and Hodgson, 2011).

Cdc42 has been shown to have a role in yeast budding, epithelial and migratory polarity as well as fate specification during cell migration. Cdc42 has been implicated in chemotaxis and directed migration of several cell types such as macrophages, T cells and fibroblasts. Cdc42 is present on the plasma

membrane and localizes predominantly to the Golgi complex where it is thought to regulate vesicle trafficking. At the leading edge, Cdc42 controls the direction in response to extracellular cues and formation of membrane protrusions such as filopodia. Filopodia are highly dynamic finger-like actin rich protrusions that are important for sensing the environment and during formation of epithelial cell-cell contacts (Etienne-Manneville, 2004; Heasman and Ridley, 2008; Nobes and Hall, 1995). Rac1 has been shown to mediate lamellipodia formation, which are broad sheets rich in polymerizing actin, as well as formation of membrane ruffling in various cells such as macrophages, T cells, epithelial cells and fibroblasts. In addition, Rac1 activity appears to be essential in regulating actin stress fibers, cell adhesion, cell migration and also play a role in cell spreading (Guo et al., 2006; Heasman and Ridley, 2008; Ridley, 2001; Ridley et al., 1992). Both Cdc42 and Rac1 functions are regulated through actin-nucleating factors (e.g. Arp2/3) or through binding of p21-activated kinase (PAK) family members. PAKs are family of serine/threonine kinases that function as effector proteins for Cdc42 and Rac1. PAKs regulate the formation and stability of filopodia and lamellipodia structures either through affecting directly actin polymerization or by phosphorylating LIM-motif containing kinase (LIMK) required for actin filament stability thus regulating actin turnover. PAKs can also affect the phosphorylation of myosin light chain kinase (MLCK), which leads to decreased myosin light chain (MLC) phosphorylation thus subsequent actin stress fiber and focal adhesion dissolution (Burridge and Wennerberg, 2004; Rane and Minden, 2014; Sanders et al., 1999).

RhoA stimulates the actomyosin contraction in the cell body and the resulting tension drives the formation of stress fibers and focal adhesions (Chrzanowska-Wodnicka and Burridge, 1996; Ridley and Hall, 1992). RhoA mediated formation of focal adhesions and contractile actin stress fibers is partly mediated through direct increase of myosin light chain (MLC) phosphorylation or by activating Rho-associated coiled-coil kinase (ROCK). ROCK is important in regulating actin stress fiber contractility and activation of ROCK kinase increases MLC levels by inhibiting MLC phosphatase and simultaneously inactivating actin depolymerization at the lamella through LIMK activation thus leading to actin filament stabilization (Figure 4). In addition, RhoA can directly activate actin-nucleating factors (e.g. formins), which have been proposed to induce actin assembly during stress fiber formation thus play a role in lamellipodia formation (Jaffe and Hall, 2005; Kimura et al., 1996; Riento and Ridley, 2003; Watanabe et al., 1999). RhoA has been found to be active at the rear of the cell and in focal adhesions at the anchoring sites of actin stress fibers. Moreover, in migrating cells the activity of RhoA and ROCK is needed for proper regulation of rear retraction. Whereas Rac1 activity is concentrated at the cell edge and mediates formation of focal complexes in the lamellipodia region (Nobes and Hall, 1995; Pertz et al., 2006; Spiering and Hodgson, 2011). Rac1 can drive cell migration by promoting lamellipodia formation, whereas RhoA signals to the ROCK kinase promoting the formation of actin stress fibers and generation of the actomyosin contractile forces required for cell migration (Kimura et al., 1996; Ridley, 2001; Ridley et al., 1992). Figure 2 illustrates the summary of Cdc42, Rac1 and RhoA functions in cells.

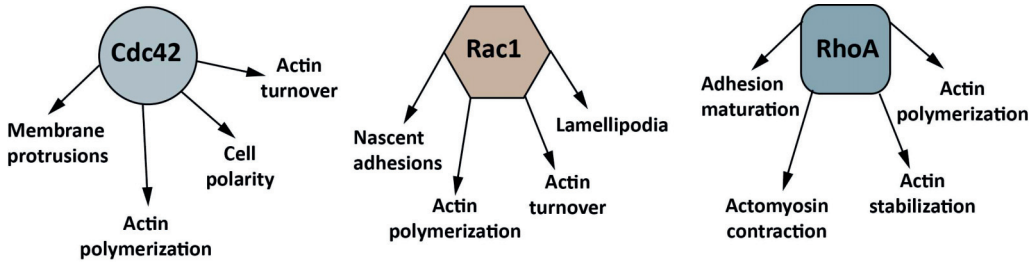


Figure 2. Cdc42, Rac1 and RhoA as master regulators. An overview of Cdc42, Rac1 and RhoA main functions in cells in regulating e.g. actin stress fibers, adhesions, cell polarity and cell contractility. The specific molecules involved in these processes are described in the text.

Tumor cells can exhibit mesenchymal and amoeboidal migration modes that are interconvertible. Interestingly, Rac1 activation controls plasticity of tumor cell movement and is required for mesenchymal type of migration. Whereas amoeboid migration requires high levels of actomyosin contractility driven by ROCK (Sanz-Moreno et al., 2008). Similar examples of Rac1 and RhoA reciprocal regulation occur also during cell protrusions and during neurite growth cone extensions (Kozma et al., 1997; Machacek et al., 2009). More recent studies reveal that RhoA activity is also essential at the leading edge of the cell where ROCK can stabilize the leading lamella during cell protrusions and that myosin-based contractility is also required at the front of invading carcinoma cells *in vivo* (Wyckoff et al., 2006). Furthermore inhibition of ROCK has shown to lead to a switch in the Rho GTPase and activation of RhoA/ROCK at the leading edge coordinates the coupling of Cdc42 and Rac1 to the actin cytoskeleton (El-Sibai et al., 2008). These events highlights the importance of spatial and temporal regulation between RhoA, Rac1 and Cdc42 that allow multiple mechanisms regulating membrane protrusions, actin stress fibers and different modes of cell migration in response to extracellular cues.

3.2 Dynamics of actin stress fiber

Actin stress fibers are dynamic structures that can polymerize and elongate at the barbed end (+ end), whereas depolymerization and shortening of filaments occurs at the pointed end (- end). Polymerization – depolymerization is tightly controlled by variety of regulatory proteins including monomer binding proteins (e.g. profilin, thymosin), capping proteins (e.g. tropomodulin, CapZ), severing proteins (e.g. gelsolin), depolymerizing proteins (e.g. ADF/cofilin), and actin filament stabilizing proteins that prevent depolymerization (e.g. tropomyosin) (dos Remedios et al., 2003; Pollard and Cooper, 2009; Welch and Mullins, 2002). In addition to the polymerization – depolymerization processes of actin filaments, there are several of other actin filament binding proteins that can regulate actin stress fibers. Actin filament crosslinking proteins such as α-actinin are

an example of such proteins that can contribute to the regulation of actin stress fibers (discussed in section 4).

Initiation of actin polymerization also known as nucleation occurs at the barbed end of actin filaments. It involves the formation of actin monomers that function as a template to the elongation of the new filament. *De novo* nucleation of actin filaments is controlled by actin-nucleating proteins, including the actin-related protein 2/3 (Arp2/3) complex and formins (Lee and Dominguez, 2010). Arp2/3 complex is the first major nucleator to be discovered and is composed of seven subunits. Arp2/3 complex can assemble and disassemble actin filaments especially in the leading edge of migrating cell. Arp2/3 complex creates new barbed ends that allow rapid growth of actin filaments. Furthermore, Arp2/3 complex catalyzes polymerization of a new filament from the side of an existing filament at a 70-degree angle to form a Y-branched network. This type of branched and dense actin network is used to assemble actin structures such as lamellipodia and focal adhesions (Figure 1).

Elongation of the branched actin filaments at the lamellipodium can push the cell membrane forward and thus drive cell migration (Goley and Welch, 2006; Pollard and Borisy, 2003; Welch and Mullins, 2002). Arp2/3 complex itself is not an efficient nucleator and it requires additional activity through the family of nucleation promoting factors (NPF), including Wiskott-Aldrich Syndrome protein (WASP), neuronal WASP (N-WASP) and WASP-family verprolin-homologous protein (WAVE) also known as SCAR (suppressor of cyclic AMP repressor). WAVE and WASP drive Arp2/3-mediated actin filament branching, and thus rapid actin polymerization, by increasing the number of free barbed ends. Arp2/3 complex activity is inhibited by coronin that promotes debranching of actin filaments and recycling of the Arp2/3 complex. Similarly, ADF/cofilin can dissociate the Arp2/3 complex binding from the actin filaments and cause depolymerization (Firat-Karalar and Welch, 2011; Goley and Welch, 2006). Cdc42 activates N-WASP and Rac1 activates WASP/WAVE family proteins that in turn activate Arp2/3 complex to induce lamellipodia and ruffle formation in cells through nucleation of actin filaments (Ladwein and Rottner, 2008).

The second major class of actin nucleators identified is formins, which can also act as elongation factors. Formins are multidomain proteins that function as dimers to promote nucleation of unbranched filaments and can assemble diverse actin structures including stress fibers, cytokinetic actin rings and actin cables *in vivo*. Formins associate with growing barbed ends while preventing capping proteins such as gelsolin and CapZ from terminating polymerization. 15 mammalian formin genes can be broadly classified into seven different groups, including the Diaphanous formins (mDia), the formin-like (FMNL), the disheveled-associated activators of morphogenesis (DAAM), delphilin, the inverted formins (INF), the formin homology domain containing proteins (FHOD) and the original “namesake” formins (FMN). All members contain a FH2 domain that mediates actin assembly and are reported to have distinct functions (Aspenström, 2010; Goode and Eck, 2007; Pollard and Cooper,

2009). More recently identified third group of nucleators include Spire, Cordon bleu (Cobl) and Leiomodin (Lmod) that nucleate actin by a mechanism that involves actin monomer recruitment to form polymerization seeds (Firat-Karalar and Welch, 2011).

Once actin filaments are nucleated, filaments grow freely at their barbed ends until monomer pools are depleted and/or capping proteins terminate elongation. Actin elongation factors associate and move with the growing barbed ends of filaments, shielding them from capping proteins and controlling the rate of elongation (Chesarone and Goode, 2009). Such elongation factors include the family of formins and Ena/VASP (enabled/vasodilator-stimulated phosphoprotein). Ena/VASP proteins are ubiquitously expressed in mammals and localize to areas of dynamic actin reorganization such as focal adhesions, cell-cell contacts, filopodia tips and lamellipodia. Ena/VASP proteins contribute to cell migration, cell adhesion, endocytosis and intracellular pathogen motility (Krause et al., 2003).

3.3 Assembly and regulation of actin stress fiber subtypes

Transverse arcs can be generated by endwise annealing of Arp2/3 nucleated actin filaments that are crosslinked by α-actinin together with myosin IIA containing actin bundles at the lamella (Burnette et al., 2011; Cai et al., 2010; Hotulainen and Lappalainen, 2006; Tee et al., 2015). Additional assembly mechanism for transverse arcs is through mDia2 nucleated actin filaments that are decorated with tropomyosin 4, thus promoting the assembly of myosin II bundles during transverse arcs formation (Tojkander et al., 2011). Tropomyosins are actin-binding proteins that prevent actin filament depolymerization at pointed ends and can inhibit ADF/cofilin promoted actin filament disassembly (Broschat, 1990; Ono and Ono, 2002).

The assembly of dorsal stress fibers has been shown to partially depend on mDia1 and is crosslinked by α-actinin, an actin filament crosslinking protein (Hotulainen and Lappalainen, 2006; Oakes et al., 2012). FMN2 formin nucleator was found to be critical in generating perinuclear actin and focal adhesion network. This FMN2 mediated network allows protecting the nucleus and DNA from damage and cell death during two-dimensional and invasive three-dimensional migration. In addition FMN2 can promote metastasis of melanoma cells to lung (Skau et al., 2016). Moreover, INF2, another formin-family nucleator, was found to be critical at the focal adhesion and dorsal stress fiber junction where it promotes actin polymerization and centripetal elongation of adhesion associated actin filaments to form dorsal stress fibers. INF can also promote elongation and maturation of focal adhesions into tensin-containing fibrillar adhesions that mediates generation and remodeling of ECM together with dorsal stress fibers (Skau et al., 2015). It has also been demonstrated that palladin, a multidomain protein that associates with actin stress fibers in a variety of cell types, can function as a dynamic scaffolding protein to promote the assembly of dorsal stress fibers by VASP recruitment (Azatov et al., 2016; Gateva et al., 2014). In addition, elongation of dorsal stress fibers is mediated through phosphorylation of VASP and ADF/cofilin mediated disassembly of dorsal stress fibers is

important for the maturation of ventral stress fibers (Tojkander et al., 2015). Recently, it was discovered that FHOD1 plays a central role in the spatial and temporal coordination of actin stress fiber dynamics. FHOD1 can promote formation of transverse arcs by restricting the length of dorsal stress fibers as well as coordinating turnover of mature contractile ventral stress fibers (Schulze et al., 2014).

DAAM1, an actin nucleator, can promote the assembly of myosin IIB rich ventral stress fibers without requiring RhoA binding to associate with the actomyosin network. DAAM1 is required for centrosome repositioning and polarity during cell migration (Ang et al., 2010; Vicente-Manzanares et al., 2008). More recently, it was discovered that formation of contractile ventral stress fibers is a mechanosensitive process that requires phosphorylation of VASP. Interestingly, it was demonstrated that AMP-activated protein kinase (AMPK) can mediated phosphorylation of VASP that is essential for the formation and stabilization of ventral stress fibers and consequently can inhibit dorsal stress fiber assembly at the focal adhesions (Blume et al., 2007; Tojkander et al., 2015). Moreover, AMPK has been suggested to directly modulate actomyosin fiber dynamics by phosphorylating myosin light chain (Lee et al., 2007). These are interesting findings as AMPK is also a major regulator of metabolism and can be activated by the serine-threonine kinase, liver kinase B1 (LKB1; also known as STK11) (Lizcano et al., 2004). LKB1 is a tumor suppressor that was initially identified as a gene mutated in the inherited disorder Peutz-Jeghers syndrome (PJS), which predisposes to a wide spectrum of benign and malignant tumors (Hemminki et al., 1998). LKB1 is a master kinase with a large repertoire of 14 substrates that are phosphorylated and activated by LKB1 thus enabling LKB1 to regulate various functions such as cell growth and cell polarity (Shackelford and Shaw, 2009). Interestingly, actin stress fibers in *Lkb1*$^{-/-}$ mouse embryonic fibroblasts were dramatically decreased. This loss was accompanied with deficient focal adhesion maturation as well as decreased contractility (Vaahtomeri et al., 2008).

Generally, the LKB1 substrate kinases are interesting targets in understanding their function and possible contribution in regulating the actin cytoskeleton, cell adhesion and thus tumorigenesis. Such possible candidates include the NUAK2 kinase, also known as sucrose-non-fermenting AMPK-related kinase (SNARK), which has been implicated in regulating actin dynamics. NUAK2 was initially identified as the fourth AMPK-related kinase and discovered as an ultraviolet induced gene in rat keratinocytes that is essential in glucose deprived conditions and other forms of metabolic stress (Lefebvre et al., 2001). In addition, overexpression of NUAK2 was found to induce cell-cell detachment and disruption of actin stress fibers during glucose starvation (Suzuki et al., 2003). Based on NUAK2 mice studies (heterozygous *Snark*$^{+/-}$), NUAK2 can regulate glucose transport in skeletal muscle as mice develop premature onset of obesity as well as hyperglycemia and hyperinsulinemia, which in turn predisposes for colorectal tumorigenesis (Tsuchihara et al., 2008). Interestingly, NUAK2 was also found to have anti-apoptotic activities that is induced in apoptosis resistant tumor cells triggered through the death receptor, CD95, thus causing increased invasion of the tumor cells (Legembre et al., 2004). More

recently, NUAK2 was reported to affect tumor growth, migration and clinical outcome of patients of human melanoma (Namiki et al., 2011). In general, various kinases regulating the actin dynamics are interesting as they potentially can be used as drug therapy targets and have valuable clinical applications in various pathological conditions.

3.4 Contractility of actin stress fibers

RhoA functions as an important regulator of actin stress fiber contractility via a complex network of protein interactions (Katoh et al., 2001). Myosin regulatory light chain (MLC or MRLC) of myosin II is a key molecule that monitors the assembly-disassembly balance and contractility of actin stress fibers (Bresnick, 1999). RhoA can activate ROCK, which directly phosphorylates MLC to induce the ATPase activity of myosin II thus promoting assembly of contractile actin stress fibers (Figure 4) (Amano et al., 1996; Katoh et al., 2001). In addition MLC phosphorylation is regulated by myosin light chain kinase (MLCK) and myosin phosphatases. MLCK is $Ca^{2+/}$calmodulin dependent kinase that can phosphorylate MLC, which then leads to increased contractility of actin stress fibers. MLCK is regulated by the elevated intracellular calcium that results in calmodulin binding to MLCK thus activating MLCK through conformational changes (Riento and Ridley, 2003; Sellers et al., 1981; Vicente-Manzanares et al., 2009). MLCK phosphorylates myosin IIA dependent actomyosin assemblies in the lamella region, whereas ROCK affects contractility of actin stress fibers located in the center of cells. This differential localization means that actomyosin structures in the cell center are more stable than those in the lamella region, which are more responsive to upstream stimuli (Tan et al., 2008; Totsukawa et al., 2000).

Myosin light chain phosphatase (MLCP) is a trimeric complex consisting of the 37 kDa protein phosphatase 1 (PP1) catalytic subunit, a 130 kDa myosin phosphatase targeting subunit 1 (MYPT1 or MBS) and a small 20 kDa subunit (Alessi et al., 1992). MYPT1 is a major regulator of myosin phosphatase activity because it binds both the catalytic subunit and the phosphorylated myosin thus leading to actin stress fiber disassembly. MYPT1 can be phosphorylated by ROCK, which results in the inhibition of myosin phosphatase activity resulting in increased actomyosin assembly and contractility (Kimura et al., 1996). In addition to ROCK, MYPT1 can be phosphorylated by various other kinases (e.g. 14-3-3 proteins) (Koga and Ikebe, 2008). Furthermore MYPT1 can interact with additional proteins to promote its activity. An actin filament binding protein MRIP (myosin phosphatase-Rho interacting protein, MRIP or p116Rip) can directly bind to RhoA and also MYTP1 on actin stress fibers. The association between MRIP and MYPT1 participates in the recruitment of MLCP and leading to more efficient dephosphorylation of MLC and disassembly of actin stress fibers (Koga and Ikebe, 2005; Mulder et al., 2003; Surks et al., 2003). These mechanisms are schematically illustrated in Figure 4 of the results section.

4. α-actinins in non-muscle cells

4.1 Family of α-actinins

α-actinin is conserved and ubiquitously expressed cytoskeletal protein that was initially discovered as a component of skeletal muscles and described as an actin crosslinking protein (Ebashi and Ebashi, 1964). Later on, α-actinin was also identified and characterized in non-muscle cells (Mimura and Asano, 1978). α-actinin belongs to a family of structurally related proteins, including spectrin, dystrophin and utrophin, which regulate the organization of actin cytoskeleton in a cell type specific manner (Broderick and Winder, 2005). α-actinin is present in multiple subcellular regions of both muscle and non-muscle cells, including cell-matrix, cell-cell adhesion sites, cellular protrusions and stress fiber dense regions (Otey and Carpen, 2004). At present, four isoforms of human *α-actinin* genes have been identified. α-actinin-2 and α-actinin-3 are muscle specific isoforms found in striated, cardiac and smooth muscle cells. They stabilize and form part of the contractile machinery by anchoring actin filaments at the Z-disks of sarcomeres in striated muscles and dense bodies in smooth muscle cells (Beggs et al., 1992; Endo and Masaki, 1984).

α-actinin-1 and α-actinin-4 (Honda et al., 1998; Lazarides, 1975b) represent the non-muscle α-actinins and are expressed in a variety of non-muscle cells. α-actinin-1 localizes along actin stress fibers with a periodic distribution reminiscent of muscle sarcomeres as well as at focal adhesions sites and at cell-cell contacts (Edlund et al., 2001; Knudsen et al., 1995; Lazarides, 1975b; Wehland et al., 1979). In comparison, α-actinin-4 is more concentrated on edge of cell clusters, in circular dorsal ruffles, and its cytoplasmic localization has been associated with enhanced migration (Araki et al., 2000; Honda et al., 1998). Apart from the mechanical role, α-actinins have important roles in the cell by linking the cytoskeleton to different transmembrane proteins, regulating activity of numerous receptors, serving as a scaffold to connect the cytoskeleton to diverse signaling pathways (Foley and Young, 2014; Otey and Carpen, 2004).

4.2 The structure of α-actinins

α-actinins native structure is a homodimer with a subunit molecular weight of 93-103 kDa and is visualized in the electron microscopy studies as a long, narrow, rod-like molecule that is composed of two anti-parallel α-actinin monomers (Blanchard et al., 1989; Podlubnaya et al., 1975; Sjöblom et al., 2008). The domain structure of α-actinin has been solved separately by X-ray crystallography in several studies (Djinovic-Carugo et al., 1999; Drmota Prebil et al., 2016; Franzot et al., 2005; Ylänne et al., 2001). In addition, high-resolution structure of the α-actinin-2 dimer from striated muscle has been determined thus allowing exploring functional implications of α-actinins at the biochemical and cellular level (Ribeiro Ede et al., 2014). All members of the α-actinin family have a common domain structure: at N-terminal an actin binding domain (ABD) followed by a central rod domain containing four spectrin repeats and at C-terminal domain containing at least two calcium binding motifs

(Blanchard et al., 1989; Virel and Backman, 2007). The domain structure of α-actinin is presented in Figure 3.

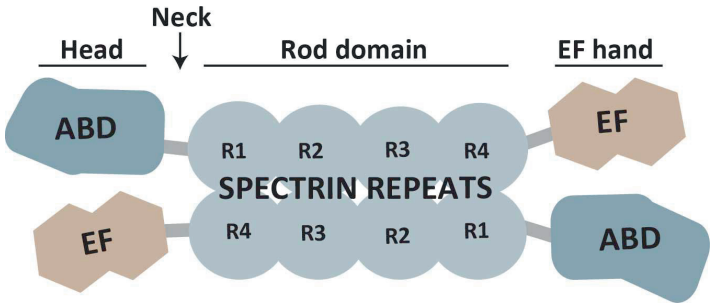


Figure 3. Schematic illustration of α-actinin dimer structure. Actin-binding domain (ABD) is at the N-terminal (head region), followed by a central rod domain consisting of four spectrin repeats (R1-R4) and the EF-hand motifs are at the C-terminal. The flexible neck region between ABD and rod domain is indicated with an arrow.

The ABD is the most conserved domain within the protein family. ABD is composed of two calponin homology domains, which gives the α-actinin the ability to crosslink with actin filaments (McGough et al., 1994). Moreover, electron microcopy studies suggest that the N-terminal ABD can have several different conformations through the movements of flexible neck region between the ABD and spectrin repeats. This neck region is also sensitive to proteolytic cleavage (Winkler et al., 1997). The central rod-like domain consists of four spectrin repeats that are important for α-actinin dimerization. The spectrin repeats provide significant elasticity allowing α-actinin to resist mechanical strain.

In addition, the rod domain is twisted and curved axially thus providing stability and allowing formation of high-affinity binding sites. Moreover, the rod domain determines the distance and the orientation of the crosslinking with actin filaments and serves as a docking site for many receptors, signaling and adaptor proteins. This is essential and allows isoform-specific protein-protein interactions (Djinovic-Carugo et al., 1999; Franzot et al., 2005; Ylänne et al., 2001). At the C-terminal domain there are two calcium binding EF-hand motifs that in non-muscle α-actinins regulates actin binding activity, whereas in muscle isoforms the EF-hand motifs are non-functional and thereby bind actin filaments in a calcium insensitive manner (Noegel et al., 1987; Sjöblom et al., 2008; Tang et al., 2001). The loss of calcium sensitivity has thought to rise from alternative splicing of exon 19, where exon 19b and 19a splice variants are calcium insensitive and sensitive, respectively. Additionally, alternative splicing on exon 8 allows α-actinin splice variants to have tissue expression differences (Foley and Young, 2013).

Analysis of the rod domain and spectrin repeats shows that the dimer interphase is highly conserved across all α-actinins (Djinovic-Carugo et al., 2002). Muscle α-actinins are known to be able to form homodimers and heterodimers based on *in vitro* and *in vivo* studies (Chan et al., 1998). On the basis of amino acid sequence similarities between α-actinin-1 and -4 and their actin-binding properties it seems likely that α-actinin-1 and -4 form homodimers. More recently, extensive cell line studies provided evidence for a significant degree of heterodimer formation between α-actinin-1 and -4 (Foley and Young, 2013; Foley and Young, 2014). Formation of homodimers and heterodimers between α-actinin-1 and -4 may mediate and explain isoform-specific functions and specific protein interactions.

4.3 Functions of non-muscle α-actinin-1 and α-actinin-4

Non-muscle α-actinin-1 and -4 are ubiquitously expressed and share 80% nucleotide similarity and 87% amino acid identity. Despite their similarities these two non-muscle α-actinins display diverse roles in cytoskeletal organization, cell motility, cell adhesion, subcellular localization and binding partners (Foley and Young, 2014; Honda et al., 1998). α-actinin-1 was first identified through immunofluorescence analysis from primary rat embryo cells and primary human skin fibroblasts (Lazarides, 1975b). α-actinin-4 was identified much later through cDNA studies (Honda et al., 1998). Both α-actinin-1 and -4 are most commonly known as structural components of actin stress fibers and their ability to crosslink actin filaments. Nevertheless, increasing amount of evidence based on numerous studies highlight the similarities but more importantly the diversity and differences these two proteins have in regulating biological functions as well as in pathological conditions. It is well known that the actin cytoskeleton plays a major role in cell migration and that α-actinin is a central component involved in this function, as it is one of the major links between actin filaments and focal adhesions (Gluck and Ben-Ze'ev, 1994; Le Clainche and Carlier, 2008).

Both α-actinins are localized in lamellipodial extensions in migrating cells and along actin stress fibers (Sen et al., 2009; Vallenius et al., 2000). Radial actin fibers enriched in α-actinin-1 are required to mediate cellular chirality required for establishing left-to-right asymmetry in cells (Tee et al., 2015). In melanoma cell line studies, α-actinin-4 has been shown to crosslink radial actin fibers, which α-actinin-1 can partially compensate. Furthermore, α-actinin-4 dependent amoeboid morphology is essential for maintaining invasion of melanoma cells whereas α-actinin-1 is exhibiting more mesenchymal morphology (Shao et al., 2014). More recent study demonstrates that both α-actinin-1 and -4 are essential in participating in the assembly of invadopodia structures in carcinoma cells (Yamaguchi et al., 2017).

Cell adhesion plays a critical role in malignant transformation through dynamic interactions between the ECM and the actin cytoskeleton. α-actinin-1 is localized at focal adhesions, where it associates with cytoplasmic domain of beta-1 integrin (Otey et al., 1990), and α-actinin-4 is particularly enriched

at the leading edge of invading cells (Honda et al., 1998). Hence, it can be assumed that α-actinins are essential in sensing ECM stiffness during adhesion maturation. α-actinin-1 associates also with vinculin, which is an important mechanosensing protein (Wachsstock et al., 1987). Recently, it was reported that mechanical stress causes a conformational change in the α-actinin rod domain resulting in stabilizing interactions with the vinculin thus proposing α-actinin to act as a mechanosensor at adhesion sites (Shams et al., 2012). Several α-actinin-1 specific studies demonstrate that α-actinin-1 is considered to be a critical player during focal adhesion maturation. Firstly, α-actinin-1 is specifically required to orchestrate the formation and maturation of nascent adhesions in a tension independent and dependent manner, respectively. Adhesion maturation process requires also radial actin stress fibers assembled by α-actinin-1 to act as a template (Choi et al., 2008; Oakes et al., 2012). A resent study utilizing overexpression approach of both α-actinin-1 and -4 in colorectal cancer cells demonstrate that both α-actinins are localized at focal adhesions and in some conditions it is possible that α-actinins could compete with each other to occupy the focal complexes and thus determining the differential protein-protein interactions, adhesion maturation and invasion abilities (Fukumoto et al., 2015).

In colon cancer cells, specific phosphorylation of α-actinin-1 at tyrosine-12 (Y12) plays a crucial role in pressure-induced cell adhesion and mechanotransduction by facilitating the enhanced phosphorylation of focal adhesion kinase, FAK (Craig et al., 2007a). These results are interesting as cancer cells may be exposed to increased extracellular pressure in the tumor microenvironment and metastatic progression is also dependent on the adhesion of tumor cells to distant tissues. In pressure stimulated conditions, α-actinin-1, but not α-actinin-4, is shown to mediate mechanical signals at focal adhesions by activating extracellular signal-regulated kinase, ERK1/2 (Craig et al., 2007b). Interestingly, *in vivo* studies of murine surgical wounds where α-actinin-1 is downregulated show that murine tumor cell wound implantation is inhibited and tumor-free survival is increased (Craig et al., 2008).

α-actinin-4 has been reported to mediate pressure stimulation of proliferation within growing tumors by partly binding to transcription factors such as NF-kB (Downey et al., 2011). Separate studies have demonstrated that p65 and p50 subunits of NF-kB can bind to α-actinin-4 in the nucleus and act as selective transcriptional co-activator (Aksenova et al., 2013; Babakov et al., 2008). These are interesting observations as only α-actinin-4 and not α-actinin-1 has been reported to localize in the nucleus. α-actinin-4 has nuclear specific functions; it can undergo nucleocytoplasmic shuttling, associate with INO80 chromatin remodeling complex and regulate cell cycle related genes (Kumeta et al., 2010). More recently, α-actinin-4 has been found to participate in mRNA processing functions in the nucleus such as pre-mRNA packing, maturation and trafficking (Khotin et al., 2010). α-actinin-4 has been identified as a novel and atypical coactivator in the nucleus that regulates transcription networks including histone deacetylase 7 (HDAC7), myocyte enchancer factor 2 (MEF2) and estrogen

receptor α (ERα), to control cell growth and cell proliferation (Chakraborty et al., 2006; Khurana et al., 2011; Khurana et al., 2012b). In light of this evidence it is proposed that α-actinin-4 has a dual role both in the cytoplasm and the nucleus.

In regard to cell migration, α-actinin-4 has a more established role that has been extensively reported in comparison to α-actinin-1. Nevertheless, depending on cell or tissue type it has been reported that α-actinin-1 and -4 can either promote or inhibit cell migration. In glioma cells, both α-actinins can regulate cell migration speed and contribute to ECM rigidity sensing (Sen et al., 2009). Studies in keratinocytes report that both α-actinins are controlling and modulating cell migration through focal adhesions and lamellipodial dynamics (Hamill et al., 2015; Hamill et al., 2013). Whereas a study in astrocytoma cell lines found that only α-actinin-4 is required for cell migration (Quick and Skalli, 2010). Some *in vivo* studies of 3T3 and SVT2 cells expressing either low or high levels of α-actinin-1 injected into nude or syneneic BALB/c mice, respectively report that α-actinin-1 can modulate the tumorigenicity of these cells (Gluck and Ben-Ze'ev, 1994; Gluck et al., 1993).

In colorectal cancer cell lines as well as in oral squamous cell carcinoma, induction or loss of α-actinin-4 expression has been found to be essential in regulating spreading of filopodia and cell migration, respectively (Honda et al., 2005; Yamada et al., 2010). In addition, silencing of α-actinin-4 in fibroblasts has been reported to increase cell contractility and cell spreading while decreasing cell migration, focal adhesions and cell proliferation (Shao et al., 2010a). More recently, it was suggested that both α-actinin-1 and -4 are essential for breast cancer cell migration, invasion and metastasis through interaction with CLP36 (Liu et al., 2015). This is an interesting observation as CLP36 is a PDZ and LIM protein family member that has been shown to associate with both α-actinins on actin stress fibers. In addition CLP36 is required for actin stress fiber formation and focal adhesion assembly (Bauer et al., 2000; Tamura et al., 2007; Vallenius et al., 2000). The molecular mechanism that links the changes in α-actinin expression and cell migration to the tumorigenic ability is extremely interesting. Studies on isoform-specific binding partners could provide further understanding on how α-actinins contribute in regulating cell migration and thus participate in promoting metastasis in tumor cells.

α-actinin-1 is also localized at adherens junctions, where α-actinin-1 binds to α-catenin, and this interaction anchors the cadherin-catenin complex to the cytoplasmic actin cytoskeleton (Knudsen et al., 1995). Vinculin, the mechanosensing protein, also binds to α-catenin under force conditions. Applied tension allows α-catenin to undergo conformational changes for vinculin to bind and stabilize α-catenin. This in turn allows transforming force into a sustainable biochemical signal (Yao et al., 2014; Yonemura et al., 2010). Binding of actin filaments to the cadherin-catenin complex is essential as actin filaments can guide the cadherin-catenin cluster assembly, stability and movement (Hong et al., 2013). α-actinin-4 is also found present at the adherens junctions but instead to α-catenin, α-

actinin-4 binds to β-catenin. Their interaction occurs in the absence of E-cadherin. In addition, it is reported that colocalization of α-actinin-4 and β-catenin in cytoplasm is elevated in the infiltrative colorectal cancer (Hayashida et al., 2005). Remodeling of adherens junctions is essential in many biological and pathological conditions thus it is essential to understand whether α-actinins have specific or common functions and how they are regulated. Taken together, all the reviewed functional α-actinin-1 and -4 studies indicate that both α-actinins can associate with variety of distinct proteins in different subcellular structures and are regulated through several mechanisms that are only partially understood.

4.4 α-actinin-1 and α-actinin-4 in disease and cancer

Missense mutations in the *α-actinin-4* gene cause an inherited autosomal-dominant form of focal segmental glomerulosclerosis (FSGS) (Kaplan et al., 2000). FSGS is a kidney pathology with multiple causes that results in proteinuria and weakening kidney function and can lead to renal failure. Three disease-causing mutations (K228E, T232I, S235P) have been identified and are reported to result in α-actinin-4 protein with an increased affinity for actin (Weins et al., 2005; Weins et al., 2007). α-actinin-4 interaction with actin can also be regulated through phosphorylation of α-actinin-4 upon epidermal growth factor (EGF) exposure (Shao et al., 2010b). Moreover, these FSGS-linked α-actinin-4 mutations failed to potentiate transcriptional activation by nuclear hormone receptors in podocytes (Khurana et al., 2012a). Two separate α-actinin-4 mice model studies show that α-actinin-4 deficient mice are viable but have abnormal morphology of the podocytes and severe glomerular disease as well as increased lymphocyte chemokinesis and chemotaxis (Kos et al., 2003; Michaud et al., 2003). Interestingly, histological analysis from these mice indicates abnormalities only in the kidney that lead to death after several months of age without α-actinin-1 being able to compensate. Podocytes are highly differentiated epithelial cells within the glomerulus (filtrating unit of the kidney) that can extend lamellapodia (known as primary processes) to wrap the capillary walls of the glomerulus. These primary processes can further branch into smaller, actin-rich foot processes that can form unique cell-cell junctions (known as slit diaphragm) with neighboring podocytes. This forms a physical filtration barrier and prevents large proteins into the urine. α-actinin-4 is present at these foot processes and is responsible for bundling actin filaments (Faul et al., 2007; Khurana et al., 2012a). In addition, expression of α-actinin-4 was altered also in children with nephrotic syndrome (Guan et al., 2003) as well as in human diabetic nephropathy (Kimura et al., 2008). More recently, α-actinin-1 distribution was detected in mesangial cells in patients with IgA nephropathy, focal segmental glomerusclerosis, minimal change disease and thin glomerular basement membranes. Mesangial cells are specialized smooth muscle like-cells found in the glomerular basement membrane that provides capillary support and contractility (Faul et al., 2007; Yang and Glass, 2008). Taken together, these results indicate a major function for α-actinin-4 in the kidney but still specific functions between the two α-actinins.

Interestingly, α-actinin-1 disease-causing missense mutations have been also identified. These mutations can cause dominantly inherited forms of congenital macrothrombo-cytopenia (CMTP) disease of moderate severity in French and Japanese families. The missense mutations identified are at the ABD and C-terminal calcium binding motifs suggesting that the noted mutations affect the actin binding properties by enhancing F-actin association. CMTP is characterized by reduced numbers of blood platelets and the presence of abnormal large platelets (Gueguen et al., 2013; Kunishima et al., 2013; Murphy et al., 2016; Yasutomi et al., 2016). These findings are supporting previous observations of α-actinin-1 in platelet activation (Izaguirre et al., 1999), and even suggesting a possible role for α-actinin-1 in platelet formation. Interestingly, mutations in α-actinin-1 and -4 affecting actin binding properties can modulate cellular dynamics and force generation thus suggesting a mechanism by which physical defects can lead to human diseases (Ehrlicher et al., 2015).

Since the initial discovery of α-actinin-4 and its correlation with poor prognosis in breast cancer (Honda et al., 1998), aberrant α-actinin-4 expression has been reported in many tumor types and has been linked to poor outcomes of several cancers (Honda, 2015). In colon cancer, overexpression of α-actinin-4 promotes lymph node metastasis in immunodeficient mice. α-actinin-4 is found to mostly accumulate at the leading edge of the invasive front (Honda et al., 2005). An interesting finding regarding α-actinin-1 in colon cancer was made where exon 19a splicing variant was found to be predominantly high in colon cancer tumor samples in comparison to 19b, which was predominant in normal samples (Gardina et al., 2006). Additionally, α-actinin-1 exon 19a splice variant is increased in advanced stages of colon cancer as well as bladder and prostate cancers reflecting cancer cell specific splicing events (Thorsen et al., 2008). In comparison, α-actinin-4 exon 8b alternative splice variant has been described as an independent prognostic factor for small cell lung cancer and high-grade neuroendocrine lung tumors (Honda et al., 2004; Miyanaga et al., 2013). Alternative splicing can enhance proteome diversity and modulate cancer-associated proteins thus identifying and validating these findings could promote diagnostic implications.

Amplification of *α-actinin-4* gene was first found in pancreatic ductal adenocarcinoma and amplification correlates with poor survival (Kikuchi et al., 2008; Welsch et al., 2009). In ovarian cancer, α-actinin-4 is overexpressed as well as *α-actinin-4* gene is amplified, both are associated with metastatic form of cancer and in some cases with tumor chemoresistance (Barbolina et al., 2008; Yamamoto et al., 2007; Yamamoto et al., 2009; Yamamoto et al., 2012). More recently, *α-actinin-4* gene was found to be amplified also in stage I and II oral tongue cancer (Kakuya et al., 2017). Consequently, α-actinin-4 is proposed as a predictive biomarker in ovarian, pancreatic and oral tongue cancers. So far, there has not been any reported studies that *α-actinin-1* gene would be amplified in cancers. In addition, copy number increased of *α-actinin-4* gene has been associated with poor prognosis and metastatic phenotypes in various cancers e.g. salivary gland carcinoma (Watabe et al., 2014).

Immunohistochemical analysis of human tumors demonstrates that the cytoplasmic localization of α-actinin-4 can be utilized to predict infiltrative phenotypes and poor clinical prognosis in colon, ovarian and pancreatic cancers (Barbolina et al., 2008; Honda et al., 2005; Kikuchi et al., 2008). On the contrary, in neuroblastomas and in prostate cancer as well as prostate cancer cell line studies it has been reported that α-actinin-4 might act as a tumor suppressor (Hara et al., 2007; Nikolopoulos et al., 2000). Interesting α-actinin-1 observations has been made through proteomic biomarker approaches that can be utilized to predict cancer aggressiveness and lethality despite biopsy-sampling error. In these settings decreased expression of α-actinin-1 was associated with the prostate cancer aggressiveness and lethality (Shipitsin et al., 2014). In comparison, high expression of α-actinin-1 in high-grade osteosarcoma associates with poor prognosis (Fellenberg et al., 2007). In addition, high α-actinin-1 expression is reported in a gene expression profiling study to be increased in aggressive ductal breast cancer compared to better prognosis and rare medullary breast cancer (Bertucci et al., 2006).

So far, α-actinin-1 role in cancer has not yet been so extensively reported in comparison to α-actinin-4. Nevertheless, the functional *in vitro* and *in vivo* studies are encouraging to the researchers to investigate the role of α-actinin-1 in cancer context as well as identifying signaling pathways regulating its functions. Noteworthy is, that α-actinin-1 disease-causing mutations were just recently discovered. Whereas α-actinin-4 has already a large repertoire of *in vitro* and *in vivo* reported studies even though α-actinin-4 was discovered much later. Hopefully the growing evidence supporting α-actinin-1 and -4 isoform-specific functions and the advances in the methods to dissect these differences more accurately will allow exciting new findings to be made.

AIMS OF THE STUDY

Actin cytoskeleton has an essential role in regulating cell migration in co-operation with cell adhesion during cancer invasion and metastasis. Actin stress fibers are dynamic structures within the actin cytoskeleton that has multiple functions. However, they are less characterized in terms of the molecules required for their assembly-disassembly as well as regulatory pathways involved. This study was undertaken to characterize key players involved in regulating actin stress fiber assembly, cell adhesion and contractility in mesenchymal and epithelial cells.

First aim of the study was to identify and characterize key players involved in actin stress fiber assembly-disassembly process that is regulated by the myosin regulatory light chain (MLC) pathway (publication I). MLC pathway is one of the main signaling pathways involved in assembly-disassembly balance of actin stress fibers as well as cell contractility.

Second aim of the study was to investigate the specificity of molecules required for the assembly of actin stress fibers and to identify signaling pathways involved. This was conducted by studying the two abundant actin crosslinking proteins, α-actinin-1 and α-actinin-4 in mesenchymal cells. Furthermore, investigate whether α-actinin-1 or α-actinin-4 have distinct functions (publication II).

Third aim of the study was to investigate the function of α-actinin-1 and α-actinin-4 in modulating actin stress fibers and cell-cell adhesions in epithelial cells as well as their contribution in cancer (publication III). Understanding these cell plasticity changes is essential as in cancer context the regulation is altered leading to increased migratory and invasive potential of the cells.

MATERIALS AND METHODS

The materials and methods used in this study are listed below, and described more in detail in the original publications, which are here referred to using Roman numerals.

Table 1. Methods used in this study

Method	Publication
Antibody blocking in cells and tissues	III
Cell culture	I, II, III
Data mining (e.g. patient survival analysis)	III
Expression plasmid transfection	I, II, III
Generation of stable cell lines	III
Inhibitor treatments (e.g. Y27632, blebbistatin)	I, II
Image analysis	I, II, III
Image quantification	I, II, III
Immunoblotting	III
Immunofluorescence analysis	I, II, III
Immunohistochemistry	III
Immunoprecipitation analysis	I
Live cell imaging	II, III
Microscopy	I, II, III
Migration assay	II, III
Plasmid purification	II, III
Quantification of protein microarray patient lysates	III
Quantification of TMA samples	III
RNA extraction	I
siRNA mediated silencing	I, II, III
Spreading assay	II
Statistical analysis	I, II, III
Three-dimensional matrigel assay	III
Transwell migration assay	II
Western blotting analysis	I, II, III

Table 2. Primary antibodies used in this study

Name, clone	Description, use	Source/reference	Used in
α-actinin, A5044	Mouse monoclonal ab	Sigma	II
α-actinin-1, A1-A341	Rabbit polyclonal ab	(Kovac et al., 2013)	II, III
α-actinin-4, ALX -210-356	Rabbit polyclonal ab	Alexis Biochemicals	II, III
β-actin, A1978	Mouse monoclonal ab	Sigma	II
γ-actin	Mouse monoclonal ab	Provided by Dr. Chaponnier, (Dugina et al., 2009)	II
CDK2, sc-163	Rabbit polyclonal ab	Santa Cruz	II
E-cadherin, U3254	Rat monoclonal ab for immunofluorescence analysis	Sigma	III
E-cadherin, 3195S	Rabbit monoclonal ab for Western blotting analysis	Cell Signaling	III
ERα, sc-8002	Mouse monoclonal ab	Santa Cruz	II
GAPDH, 14C10 and 2118S	Rabbit monoclonal ab	Cell Signaling	I, II, III
GFP, TP401	Rabbit polyclonal ab	Torrey Pines	I
GFP, ab290	Rabbit polyclonal ab	Abcam	III
GST, ab9085	Rabbit polyclonal ab	Abcam	I
GST, AP308P	Mouse monoclonal ab	Chemicon Int.	I
HA, 12CA5	Mouse monoclonal ab	Babco	I
Laminin, L9393	Rabbit polyclonal ab	Sigma	III
MLC, M4401	Mouse monoclonal ab	Sigma	I, II
Mypt1, 2634	Rabbit polyclonal ab	Cell signaling	I
Phospho-MLC, ser19, 3671	Rabbit polyclonal ab	Cell signaling	I, II
Vinculin, V9131	Mouse monoclonal ab	Sigma	I, II, III

Table 3. Cell lines used in this study

Cells	Description	Source/reference	Used in
HeLa	Human cervical cancer cell line	ATCC	I
U2OS	Human osteosarcoma cells	ATCC	I, II
MEF	Mouse embryonic fibroblasts, immortalized with a C-terminal fragment of p53	(Vaahtomeri et al., 2008)	II
EpH4	Mouse mammary epithelial cells	ATCC	III
NMuMG	Mouse mammary epithelial cells	ATCC	III
MDA-MB-231	Human breast adenocarcinoma	ATCC	III
MDA-MB-361	Human breast adenocarcinoma	ATCC	III
HCC1937	Human breast ductal carcinoma	ATCC	III
HCC1954	Human breast ductal carcinoma	ATCC	III
Hs578T	Human breast carcinoma	ATCC	III
BT-20	Human breast carcinoma	ATCC	III
T-47D	Human breast ductal carcinoma	ATCC	III
MCF-7	Human breast adenocarcinoma	ATCC	III
HCC1428	Human breast adenocarcinoma	ATCC	III

Table 4. Expression plasmids and peptides used in this study

Plasmid/peptide	Description	Source	Used in
ACTN1 ab peptide	DHYDSQQTND	Storkbio Ltd	III
ACTN4 ab peptide	MGDYMAQEDDW	Storkbio Ltd	III
pcDNA6.2/N-EmGFP-DEST-ACTN1	ACTN1 N-terminal Em-GFP-tag	ORFeome library	II, III
pcDNA6.2/N-EmGFP-DEST-ACTN4	ACTN4 N-terminal Em-GFP-tag	ORFeome library	II, III
pcDNA3/N-EmGFP-DEST-hCDH1	hCDH1, N-terminal Em-GFP-tag	Addgene (Gottardi et al., 2001)	III
pcDNA-DEST53-MRIP	MRIP, N-terminal GFP tag	(Vallenius et al., 2011)	I
pcDNA6.2/N-EmGFP-DEST-MRIP	C-terminal MRIP clones identified in Yeast two hybrid, N-terminal Em-GFP-tag	(Vallenius et al., 2011)	I
pDEST27-MRIP	MRIP, N-terminal GST-tag	(Vallenius et al., 2011)	I
pcDNA6.2/N-EMGFP-DEST-MYH9	MYH9 N-terminal Em-GFP-tag	ORFeome library	II
pDEST32-NUAK2	NUAK2 fused with GAL DNA binding domain	Invitrogen	I
pEBG-NUAK2	NUAK2, N-terminal HA-and GST-tag	Provided by Dr. Alessi, (Lizcano et al., 2004)	I
pEBG-NUAK2-T208A	NUAK2-T208A, N-terminal HA- and GST-tag	Provided by Dr. Alessi, (Lizcano et al., 2004)	I
pEBG-NUAK2-T208E	NUAK2-T208A, N-terminal HA- and GST-tag	Provided by Dr. Alessi, (Lizcano et al., 2004)	I
GFP-Rac1-V12	Constitutively active Rac1 with GFP-tag	Provided by Dr. Debant, (Bellanger et al., 2000)	II
GFP-RhoA-V14	Constitutively active RhoA with GFP-tag	Provided by Dr. Debant, (Bellanger et al., 2000)	II

Table 5. siRNA oligos used in this study

si-Oligos	Description	Source	Used in
Human ACTN1	L-011195, J-011195-05, J-011195-06	Dharmacon	II, III
Human ACTN4	L-011988, J-011988-08, J-011988-09	Dharmacon	II, III
Mouse actn1	L-066191	Dharmacon	II
Human MRIP	L-014102, J-014102-09, J-012102-10	Dharmacon	I
Human MYPT1	L-011340, J-011340-07, J-011340-08	Dharmacon	I
Human NUAK2	L-005374, J-005374-8, J-005374-9	Dharmacon	I
Non-targeting oligo	D-001206-13	Dharmacon	I, II, III
Human RAC1	L-003560	Dharmacon	II

RESULTS AND DISCUSSION

1. NUAK2 kinase regulates actin stress fibers and cell contractility (I)

1.1 Association of NUAK2 and MRIP on actin stress fibers

Generation of force required for cell migration can be produced through the assembly of contractile actin stress fibers. Moreover, actomyosin contractility can dictate cell differentiation and defects in tensional homeostasis during tissue differentiation can promote tumorigenesis (Clark et al., 2007). Motivated by this and several studies suggesting that LKB1 tumor suppressor and its substrate kinases (e.g. AMPK, NUAK2) are involved in regulating actin stress fibers, it was of great interest that NUAK2 interacted with the actin binding protein MRIP in two independent yeast two hybrid screens carried out in the laboratory (I, Figure 1). MRIP is a large protein considered to function as a molecular scaffold between the actin cytoskeleton and MLCP that promotes actin stress fiber disassembly. This is based on findings that MRIP can bind to F-actin on its N-terminus and to RhoA and MYPT1 with its C-terminus. The association between MRIP and MYPT1 participates in dephosphorylation of MLC and inactivation of RhoA, which leads to disassembly of actin stress fibers (Koga and Ikebe, 2005; Mulder et al., 2004; Surks et al., 2003; Surks et al., 2005).

Subsequent localization analyses of NUAK2 were performed to study the role of NUAK2 on actin stress fibers. These experiments were carried out in HeLa cells as MRIP was earlier characterized in these cells (Koga and Ikebe, 2005; Xia et al., 2005). NUAK2 was observed on actin stress fibers where MRIP is also localized further supporting the interaction observed. Furthermore, NUAK2 expressing cells showed dotted cytoplasmic and nuclear staining (I, Figure 1, S2 and S3). Interestingly, localization of NUAK2 on actin stress fibers is MRIP dependent as NUAK2 fails to localize on actin stress fibers following loss of MRIP. In addition, NUAK2-MRIP association on actin stress fibers is independent of MYPT1 (I, Figure 3). Here, we have identified a novel association between NUAK2 and MRIP on actin stress fibers that could contribute to actin stress fiber regulation. MRIP has been reported to regulate actin stress fiber assembly and disassembly by either being downregulated or overexpressed, respectively (Koga and Ikebe, 2005; Surks et al., 2005).

1.2 NUAK2 promotes central actin stress fibers and cell contractility

To investigate whether NUAK2 contributes to the regulation of actin stress fibers, we utilized quantification of F-actin intensities of NUAK2 overexpressing cells. Interestingly, these cells exhibited significantly increased F-actin staining, frequently located in central actin stress fibers (I, Figure 1 and S1). Based on more detail actin stress fiber characterization in polarized mesenchymal cells and division into various actin stress fiber subtypes, these fibers could represent perinuclear actin cap fibers as they are prominently localized above the nucleus (Khatau et al., 2009; Small et al., 1998). Perinuclear actin cap fibers are reported to be important in protecting the nucleus from physical damage in confined three-dimensional matrices during migration as well as in transducing mechanical

cues to the nucleus (Kim et al., 2012; Skau et al., 2016). These are interesting observations regarding the noted nuclear staining of NUAK2. Interestingly, it was recently reported that NUAK2 could act as a transcriptional modulator of gene expression in response to stress in the nucleus (Kuga et al., 2008). In comparison to NUAK2, AMPK kinase was reported to be essential for the formation and stabilization of ventral stress fibers (Blume et al., 2007; Tojkander et al., 2015). Suggesting that these two kinases could regulate actin stress fiber subtypes differently. NUAK2 can regulate actin stress fiber functions partially in a kinase independent manner as both kinase deficient T-loop mutant NUAK2-T208A and constitutively active NUAK2-T208E mutant (Lizcano et al., 2004) prompted actin stress fibers in a comparable manner to the control cells (I, Figure 1 and S1). Consistent with the increased actin stress fibers upon overexpression of NUAK2, downregulation of NUAK2 led to significant reduction of both actin stress fibers and phosphorylated MLC, measuring the cells contractility. Furthermore, it has been reported that NUAK2 can induce cell-cell detachment and disruption of actin stress fibers also during glucose starvation (Suzuki et al., 2003). Our results are suggesting that NUAK2 together with MRIP association promote actin stress fibers in a kinase-independent manner.

Considering a possible pathway that NUAK2 is utilizing for regulating the actin stress fiber dynamics, MLCP is an obvious candidate as inhibition of MLCP activity maintains the assembly of actin stress fibers. To this end, we treated cells with ROCK specific inhibitor, Y27632 that leads to rapid disassembly of actin stress fibers in normal cells. Following the treatment, NUAK2-expressing cells were partially resistant to the inhibitor, exhibiting long centrally localized actin fibers (I, Figure 4). On the other hand, treatment with cytochalasin D, which blocks monomer addition to the barbed ends, did not affect the loss of actin stress fibers. These results suggest that NUAK2 inhibits MRIP-MLCP-mediated central actin stress fiber disassembly. In comparison, AMPK kinase was reported to mediate the ventral stress fibers through VASP phosphorylation (Blume et al., 2007; Tojkander et al., 2015). These results suggest distinct pathways for NUAK2 and AMPK in regulating actin stress fibers. Furthermore, NUAK2 mediated actin stress fibers disruption together with decreased MLC phosphorylation is dependent on both MRIP and MYPT1 (I, Figure 7). Our results suggest that NUAK2 requires MRIP and MYPT1 to regulate actin stress fibers, but the localization of NUAK2 on actin stress fibers only requires MRIP. MRIP acts as a scaffold protein that can mediate and regulate MLC both in a negative and positive manner. Thus revealing a novel mechanism for NUAK2 and MRIP in actin stress fiber and cell contractility regulation. These findings are schematically illustrated in Figure 4. Regulation of actin stress fiber assembly-disassembly is important as actomyosin contractility driven by traditional ROCK in cancer context promotes tumor cell migration and invasion (Riento and Ridley, 2003). In general, various kinases regulating the actin dynamics are interesting as they potentially can be used as drug therapy targets and have valuable clinical applications in various pathological conditions.

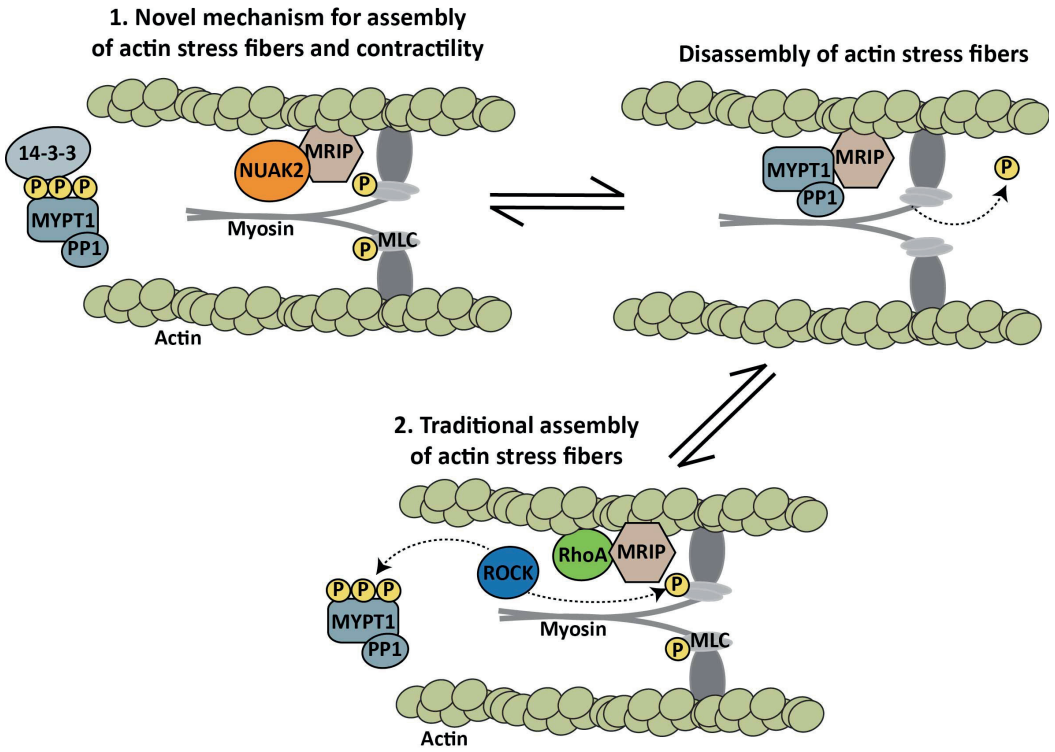


Figure 4. Schematic illustration of the proposed novel actin stress fiber assembly and cell contractility mechanism. NUAK2 associates with MRIP on actin stress fibers, phosphorylating MLC of the myosin and leading to actin fiber assembly and increased contractility. NUAK2 can inhibit the MLCP complex including MYPT1 and PP1 subunits, which leads to sequestration of MLCP by 14-3-3. Disassembly of actin fibers occurs when MYPT1 and PP1 associates with MRIP and dephosphorylates MLC. Traditional view of actin fiber assembly occurs through ROCK that phosphorylates MLC and MYPT1 thus keeping the MCLP away from actin stress fibers and MRIP associated with RhoA can promote actin fiber assembly.

1.3 Actin stress fibers are regulated by NUAK2 expression

NUAK2 was observed to promote actin stress fibers partially in a kinase independent manner. This observation together with previous studies, where it was reported that NUAK2 levels are inducible by various cellular stresses e.g. hyperosmotic pressure and elevation of cellular AMP as well as tumor necrosis factor (TNF)-alpha (Lefebvre and Rosen, 2005; Yamamoto et al., 2008), led us to investigate whether NUAK2 expression levels can act as a regulator in NUAK2 specific functions. Interestingly, NUAK2 mRNA and protein levels were high in exponentially growing HeLa cells with prominent actin stress fibers, whereas in serum starved conditions NUAK2 levels and actin stress fibers were attenuated. Furthermore, direct evidence that actin stress fibers can directly regulate NUAK2 levels was obtained from cells treated with ROCK kinase inhibitor Y27632, myosin II ATPase inhibitor blebbistatin and actin binding drug cytochalasin D, which all caused rapid loss of actin stress fibers and significantly decreased NUAK2 mRNA levels (I, Figure 5). This type of rapid regulation could be due to specific transcription and promoter factors that could promote or repress NUAK2 functions. These observations are interesting in regard to the noted nuclear localization of NUAK2 and its reported nuclear function (Kuga et al., 2008). The strong regulation of NUAK2 levels by actin stress fibers and vice versa identifies a positive feedback loop for actin stress fiber maintenance. This could be considered as an alternative model to the traditional RhoA-ROCK pathway that regulates actin stress fiber assembly and contractility through phosphorylation of MYPT1 (Kimura et al., 1996).

Kinase dependent function of NUAK2 was recently reported in a study were NUAK2 was shown to directly bind and phosphorylate MYPT1 indicating that NUAK2 can regulate and inhibit the phosphatase also in a kinase-dependent manner (Yamamoto et al., 2008). Furthermore, a closely related kinase, NUAK1 was shown to inhibit MLCP and promote cell detachment by regulating the myosin phosphatase complex and phosphorylating MYPT1 on 14-3-3 binding site (Zagorska et al., 2010). Thus the NUAK family can regulate MLCP and MYPT1 in a kinase-dependent and kinase-independent manner. Taken together, our results identify NUAK2 as an actin stress fiber regulator, specifically central actin stress fibers. NUAK2 can modulate assembly-disassembly of actin stress fibers and cell contractility through its novel association with MRIP. In addition, NUAK2 has a positive feedback loop that regulates actin dynamics and contributes to actin stress fiber maintenance. These are interesting findings as generation of force can be produced through cells contractility that comes from the assembly-disassembly of actin stress fibers (Clark et al., 2007). It is important to maintain a balance between external forces and contractility forces inside the cell because if deregulated it can lead to subsequent increased stiffness, cell detachment and increased cell migration.

2. α-actinin-1 regulates actin stress fibers and cell plasticity in mesenchymal cells (II)

2.1 α-actinin-1 and α-actinin-4 assemble distinct actin stress fibers

In mesenchymally migrating cells prominent actin stress fibers containing α-actinin crosslinking protein and myosin II motors together with a leading edge and contractile rear are specific features that can promote cell migration and cell spreading (Friedl and Wolf, 2010; Pollard and Borisy, 2003). This prompted us to study the specificity of molecules required for the assembly of distinct actin stress fiber subtypes characterized in mesenchymally migrating cells by studying the function of the two abundant actin crosslinking proteins in non-muscle cells, α-actinin-1 and -4. Subsequent localization analysis of α-actinin-1 and -4 were conducted in U2OS cells due to their well-defined actin stress fiber subtypes (Hotulainen and Lappalainen, 2006; Oakes et al., 2012; Vallenius et al., 2000). α-actinin-1 and -4 staining was noted on all actin stress fiber subtypes (II, Figure 1). Interestingly, differential staining pattern was observed on dorsal stress fibers found at the leading edge. α-actinin-1 is prominently localized along the whole dorsal stress fiber (II, Figure 1), in comparison to α-actinin-4 that is abundant only at the base of the dorsal stress fiber that emerges from the focal adhesion (II, Figure 1). Our findings demonstrate significant selectivity of α-actinins in decorating the dorsal stress fibers with α-actinin-1 being most abundant, whereas both α-actinins are localized on transverse arcs and ventral stress fibers.

In order to investigate whether the observed difference in α-actinin-1 and -4 localization on dorsal stress fibers would correlate with their functions, we performed small interfering RNA (siRNA) mediated downregulation of α-actinin-1, α-actinin-4 or both. α-actinin-1 depletion leads to almost complete loss of dorsal stress fibers without affecting transverse arc or ventral stress fiber formation, whereas α-actinin-4 did not cause any detectable changes in the actin stress fibers (II, Figure 2). Loss of dorsal stress fibers was noted also in mouse embryonic fibroblasts (MEFs) and this loss could not be rescued by ectopic expression of α-actinin-4. Our results suggest that dorsal stress fibers are assembled through homodimer formation of α-actinin-1, whereas transverse arcs and ventral stress fibers could possibly be assembled through heterodimer formation between the two α-actinins. Thus supporting the findings of the study that reported a significant degree of heterodimer formation between the two α-actinins (Foley and Young, 2013). Furthermore, silencing of both α-actinins simultaneously led to nearly undetectable actin stress fibers indicating a major role for both α-actinins in assembling actin stress fibers in U2OS cells.

Consistent with our findings, additional studies have reported α-actinin-1 to be a required for the assembly of dorsal stress fiber (also known as radial fibers) (Oakes et al., 2012). In addition, α-actinin-1 is reported to be critical for dorsal stress fiber establishment and in coupling transverse arcs and perinuclear actin cap fibers (Maninova and Vomastek, 2016). Especially the tyrosine phosphorylation (Y12) of α-actinin-1 is identified to be critical for dorsal stress fiber formation (Feng et al., 2013).

Interestingly, this phosphorylation site does not appear to be a major phosphorylation site in α-actinin-4 (Shao et al., 2010b). More recently, palladin has been identified as an important scaffolding protein for the formation of α-actinin-1 mediated dorsal stress fibers that can modulate force generation and mechanosensitivity of tumor associated fibroblasts (Azatov et al., 2016; Gateva et al., 2014). Taken together, our results suggest that α-actinin-1 and -4 can assembly distinct actin stress fibers in mesenchymal cells and α-actinin-1 is specifically required for the assembly of dorsal stress fibers.

2.2 α-actinin-1 modulates cell-matrix adhesions in mesenchymally migrating cells

Interestingly, loss of dorsal stress fibers in α-actinin-1 silenced cells was accompanied with impaired adhesion maturation. α-actinin-1 silenced cells resulted in significantly smaller adhesion size accompanied with increased amount of adhesions specifically at the leading edge in comparison to the rear of the cell (II, Figure 2). Our observations are consistent with findings made in CHO.K1 cells where α-actinin-1 is required for the formation of nascent adhesions (Choi et al., 2008). Formation and turnover of nascent adhesions persists in the absence of non-muscle myosin II mediated tension, commonly stained with paxillin to detect this type of adhesions. In comparison, vinculin staining is used to detect mature focal adhesions that are myosin II dependent (Choi et al., 2008; Pasapera et al., 2010; Vicente-Manzanares et al., 2007). In addition, α-actinin-1 assembled dorsal stress fibers are reported to act as a structural template facilitating focal adhesion maturation over a wide range of tensions and through lamellar retrograde flow driving the elongation of dorsal stress fibers accompanied with focal adhesion maturation (Hotulainen and Lappalainen, 2006; Oakes et al., 2012). Moreover, α-actinin-1 has been shown to affect the composition of mature focal adhesions by impairing the activation of FAK and paxillin phosphorylation at the leading edge (Oakes et al., 2012).

In colon cancer cells α-actinin-1 can enhance the phosphorylation of FAK in pressure-induced conditions resulting in increased cell adhesion and mechanotranduction (Craig et al., 2007a). FAK and paxillin are both mechanosignalling proteins that are important at adhesion sites where they require myosin II mediated activity and recruitment of vinculin to reinforce the cytoskeletal ECM linkage and thus drive maturation of focal adhesion (Pasapera et al., 2010). Vinculin is an important mechanosensing protein that associates with α-actinin-1 (Wachsstock et al., 1987). Interestingly, it was reported that mechanical stress causes a conformational change in the α-actinin rod domain resulting in stabilizing interactions with vinculin thus proposing α-actinin to act as a mechanosensor at adhesion sites (Shams et al., 2012). Dorsal stress fibers together with focal adhesions can form a specific integrated adhesion-actin structure complex that mediates generation and remodeling of ECM (Skau et al., 2015). Furthermore, dorsal stress fibers have been suggested to be essential in controlling three-dimensional shape of crawling cells, where dorsal stress fibers generate forces on the substrate through their attachment to focal adhesions on one end and transverse arcs on the other end (Burnette et al., 2014). Our results suggest that α-actinin-1 and dorsal stress fibers are

necessary for the maturation of cell-matrix adhesions, which are essential mechanosensing sites during cell migration events.

2.3 α-actinin-1 promotes cell migration and early cell spreading independently of myosin phosphorylation

Actin stress fibers have an established function in regulating cell migration for example by formation of front-to rear polarity or promoting rear traction (Ang et al., 2010; Ridley, 2001; Vicente-Manzanares et al., 2008). Motivated by this, we investigated the role of α-actinin-1 and dorsal stress fibers in cell migration. Wound-healing assay revealed that loss of α-actinin-1 leads to a significant decrease in cell wound closure rate in comparison to control cells. These results are accompanied with decreased transmigration of cells through matrigel-coated transwells (II, Figure 3). α-actinin-4 silencing also results in decreased cell migration as expected (Honda, 2015). Interestingly, even though α-actinin-1 silenced cells migrated significantly slower, their phosphorylated MLC levels did not change. From previous studies it has been proposed that myosin IIA and IIB have opposite roles in regulating cell migration (Sandquist et al., 2006). It is known that myosin IIA is essential for adhesion maturation and cell edge retraction at the leading edge (Even-Ram et al., 2007; Vicente-Manzanares et al., 2007), whereas myosin IIB is localized at the central and rear of the cell (Lo et al., 2004; Vicente-Manzanares et al., 2008). Our results suggest that dorsal stress fibers assembled by α-actinin-1 promote cell migration at least partially though actin polymerization as cell contractility is not altered during cell migration.

Early cell spreading is an event where myosin II activity is low and actin polymerization at the cell periphery has a major role. During early spreading, dorsal stress fibers (radial fibers) assemble from peripheral adhesions (Cai et al., 2006; Cai et al., 2010). Thus it was of great interest to analyze α-actinin-1 depleted cells in early cell spreading. Interestingly, dorsal stress fibers fail to form in α-actinin-1 downregulated cells during early cell spreading (II, Figure 5). Time-lapse analysis of spreading cells showed that α-actinin-1 depleted cells resulted in delayed spreading, with a smaller cell circumference during the early stages of cell spreading. Consistent with our observations, it was reported that dorsal stress fibers (radial fibers) enriched in α-actinin-1 are required to mediate cellular chirality when plated on circular adhesive islands. This process is required for establishing left-to-right asymmetry in cells and is essential during embryonic development (Tee et al., 2015). These functional observations suggest that dorsal stress fibers assembled by α-actinin-1 are required for cell migration and early cell spreading.

Requirement of dorsal stress fibers for cell migration in a myosin II independent manner prompted us to investigate the role of MLC and myosin II in decorating dorsal stress fibers. Interestingly, phosphorylated MLC staining is not evident on dorsal stress fibers except in regions close to transverse arcs, neither myosin IIA nor IIB was detected on dorsal stress fibers (II, Figure 4). To further

evaluate the lack of myosin on dorsal stress fibers we treated cells with ROCK kinase inhibitor, Y27632, or myosin II ATPase inhibitor, blebbistatin. Strikingly, dorsal stress fibers are significantly more resistant to the treatments in comparison to dramatic loss of transverse arcs, ventral stress fibers as well as focal adhesions. Focal adhesions were stained with vinculin, which has been reported to be myosin II dependent (Pasapera et al., 2010). Interestingly, α-actinin-1 staining remains on the dorsal stress fibers following the treatments, indicating that myosin II driven tension is not required for the α-actinin-1 localization on dorsal stress fibers. Traditionally it is considered that actin stress fibers contain myosin II motors together with actin crosslinking protein. Our findings strongly supports that dorsal stress fibers are unipolar entities assembled by α-actinin-1, lacking myosin II motors thus are non-contractile. Interesting *in vivo* findings regarding injury induced actin cytoskeleton rearrangements in the podocytes, identify and report the existence of non-contractile dorsal stress fibers *in vivo*, especially in healthy foot processes. These non-contractile dorsal stress fibers are required to stabilize capillary structures in the podocytes against the hydrostatic pressures of the blood (Suleiman et al., 2017). These findings are exciting as both α-actinins are found to contribute and cause disease pathology of the glomerulus and podocytes (Kaplan et al., 2000; Yang and Glass, 2008). Moreover, it is interesting and encouraging that distinct actin stress fiber subtypes can be distinguished *in vivo* and found to contribute to different cellular functions.

2.4 Rac1 signaling regulates actin stress fibers assembled by α-actinin-1

Considering possible upstream regulators of dorsal stress fibers Rac1 and RhoA are obvious candidates. More specifically Rac1, as it is a critical regulator of actin polymerization (Ridley, 2011), whereas RhoA is involved in contractility mediated cell migration (Parsons et al., 2010). Interestingly, cells expressing GFP-tagged constitutively active Rac1 (Rac1 V12) leads to prominent dorsal stress fibers, which are increased in amount and are also significantly longer. Furthermore, downregulation of Rac1 leads to loss of dorsal stress fibers. The stimulation of dorsal stress fibers by constitutively active Rac1 is α-actinin-1 dependent. In comparison, cells expressing GFP-tagged constitutively active RhoA (RhoA V14) leads to increased ventral stress fibers as expected (II, Figure 6). Taken together our results suggest that α-actinin-1 and Rac1 are critical molecules required for the assembly of dorsal stress fibers in migrating and spreading cells. Further highlights the noted spatial and temporal regulation between Rac1 and RhoA to allow assembly of distinct actin stress fibers that contribute to different modes of cell migration interconverting from amoeboid (RhoA) to mesenchymal (Rac1) (El-Sibai et al., 2008; Sanz-Moreno et al., 2008).

Several studies have further characterized dorsal stress fibers and identified several actin nucleators that are involved in dorsal stress fiber assembly from which most of them belong to the formin-family of actin nucleators that promote polymerization of unbranched actin filaments. mDia1 and mDia2 have both been suggested to nucleate dorsal stress fibers and interestingly both associate with Rac1 (Hotulainen and Lappalainen, 2006; Lammers et al., 2008; Oakes et al., 2012). Furthermore, INF2

formin was reported to be essential at the focal adhesion and dorsal stress fiber junction where it promotes actin polymerization and centripetal elongation of adhesion associated actin filaments to form dorsal stress fibers (Skau et al., 2015). VASP and ADF/cofilin have been reported to mediate dorsal stress fiber elongation and disassembly, respectively. In addition, FHOD can restrict the length of dorsal stress fibers by promoting formation of transverse arcs (Gateva et al., 2014; Schulze et al., 2014; Tojkander et al., 2015). Taken together, all the findings suggest that dorsal stress fibers are essential leading edge actin stress fibers associated with the mechanosensing cell-matrix (focal adhesions). This in turn allows sensing of the microenvironment and through distinct regulatory molecules promoting nucleation, elongation or crosslinking can lead to specific functions such as cell migration, cell spreading, ECM remodeling. Our findings provide further evidence for isoform-specific functions between α-actinin-1 and -4 thus suggesting that α-actinin-1 and -4 are required for distinct but partially overlapping functions in mesenchymally migrating cells. Figure 5 contains a summary of our findings in mesenchymally migrating cells regarding actin stress fiber subtypes and how α-actinin-1 or/and -4 assemble these fibers and contribute to different functions.

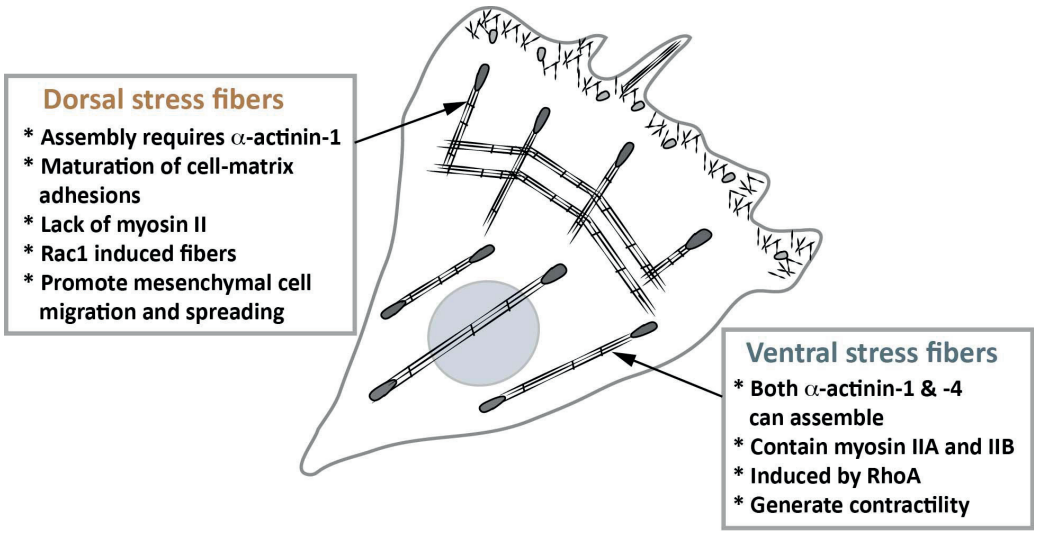


Figure 5. Summary of actin stress fiber subtypes assembled by α-actinin-1 or/and α-actinin-4 and their functions in mesenchymally migrating cells.

3. α-actinin-1 contributes to cancer progression (III)

3.1 α-actinin-1 is upregulated in various cancers

Uncontrolled cell migration promotes progression of many diseases such as cancer and metastasis (Fife et al., 2014). In our previous study, we reported that α-actinin-1 promotes cells migration in mesenchymal cells (publication II). Moreover, its close relative α-actinin-4 has a well-established role in cell migration and cancer (Honda, 2015). This motivated us to study the role of α-actinin-1 in cancer progression. As previously reported, α-actinin-4 has been extensively studied in various cancers and associated with poor prognosis. The findings that α-actinin-4 is upregulated in various cancers have been revealed gradually through numerous single studies using both mRNA expression changes and staining approaches (Barbolina et al., 2008; Honda et al., 1998; Honda et al., 2005; Kakuya et al., 2017; Kikuchi et al., 2008; Welsch et al., 2009). In our study, we utilized publicly available, constantly expanding mRNA expression data both from healthy and cancer tissues (Kilpinen et al., 2008). Consistent with previous studies α-actinin-4 is upregulated in various cancers. Interestingly, also α-actinin-1 expression shows increased levels in several cancers compared to healthy controls (III, Figure 1). Many of these tissues overlap with α-actinin-4 including breast and colon. In this thesis work we selected to investigate the role of α-actinin-1 in breast cancer further.

To strengthen the noted mRNA findings of α-actinin-1, we performed immunohisto-chemistry and Western blotting analysis of commercially available human breast cancer material (tissue microarrays (TMA) and lysate array). Both of these analysis used were consistent with the data mining results, i.e. the expression of α-actinin-1 is increased often in breast cancer. Furthermore, α-actinin-4 immunohistochemistry and Western blotting analysis were also increased consistent with previous reports. Our results prompted us to continue investigating the role of α-actinin-1 in breast cancer context.

3.2 α-actinin-1 regulates epithelial cell plasticity and promotes cell migration

Upregulation of α-actinin-1 in breast cancer patient samples prompted us to investigate consequences of its ectopic expression in mammary epithelial cells both in two- and three-dimensional culture conditions. The use of three-dimensional cultures is essential as it is considered an important modeling tool for epithelial cancers and allowing investigation of mechanisms associated with tumor initiation and progression (Debnath and Brugge, 2005). In two-dimensional culture conditions, ectopic expression of α-actinin-1 promoted migration of sheet of epithelial cells that can be considered as simple model for collectively migrating epithelial cells. In three-dimensional cultures, α-actinin-1 expressing cells resulted in irregular, larger and unpolarized epithelial structures compared to control cells (III, Figure 2 and S2). These results imply that the increased expression of α-actinin-1 promotes morphological cell plasticity, which is characteristic for tumor cells acquiring metastatic potential.

In support of the increased morphological plasticity, immunofluorescence staining of mammary epithelial cells expressing ectopic α-actinin-1 revealed major reorganization of the actin cytoskeleton and cell-cell contacts (III, Figure 2). Notable finding is that the key cell-cell adhesion molecule, E-cadherin, is still present and maintain adjacent epithelial cells together. However, the E-cadherin distribution in α-actinin-1 expressing cells is distinct compared to control cells. In normal mammary epithelial cells E-cadherin distributes linearly, whereas in α-actinin-1 expressing cells this linearity is lost, and E-cadherin is punctate. Previous studies indicate that a close crosstalk between E-cadherin and the actin cytoskeleton regulates the stability of cell-cell contacts (Lecuit and Yap, 2015; Mege and Ishiyama, 2017). This suggests that actin stress fibers assembled by α-actinin-1 can regulate the stability of E-cadherin based adhesions. The loss of E-cadherin linear distribution at cell-cell contacts together with the noted increase in morphological cell plasticity propose a lack of epithelial to mesenchymal changes. Instead our results suggest that the increased expression of α-actinin-1 is involved in partial EMT, where rearrangements of the actin cytoskeleton, retention of E-cadherin at cell-cell adhesions contribute to collective cell migration and metastatic outgrowth (Ayollo et al., 2009; Gloushankova et al., 2017; Lambert et al., 2017).

In epithelial cells, this type of observed punctate junctions are considered as dynamic and mechanosensing sites that allow remodeling of adherens junctions found essential in functions such as morphogenesis and epithelial barrier formation (Twiss et al., 2012; Vaezi et al., 2002). Furthermore, It has been previously reported that the stability and mobility of these punctate junctions depend on two actin populations with different dynamics (Cavey et al., 2008; Zhang et al., 2005). In agreement with this, we also detect two distinct actin populations or actin stress fiber subtypes. Actually, these subtypes resemble markedly actin stress fiber subtypes identified at lamella region in mesenchymally migrating cells (Figure 1 and publication II) (Hotulainen and Lappalainen, 2006; Small et al., 1998). Further studies are required to compare actin stress fiber subtypes in mesenchymal and epithelial cells, but these findings suggest more general principles how actin stress fibers regulate integrin and E-cadherin based adhesions. Taken together, both of the findings highlight the importance of α-actinin-1 in regulating cell-matrix and cell-cell adhesions in mesenchymal and epithelial cells, respectively. Overall, our results in two- and three-dimensional environment suggest α-actinin-1 to be an important regulator in cell adhesions, epithelial cell plasticity and promoting cell migration thus implying that α-actinin-1 could contribute to cancer progression.

3.3 α-actinin-1 and α-actinin-4 have different roles in cancer

Due to high occurrence of breast cancer, there are a lot of prognostic survival data available in user-friendly databases for the research community to exploit (Gyorffy et al., 2010). Moreover, in respect to breast cancer, significant improvements in diagnosis and prognosis are coming from subtype classifications. Initially immunohistochemistry based hormone receptor status (e.g. estrogen receptor (ER), progesterone receptors (PR), human epidermal growth factor receptor 2 (HER2)), and

increasingly through so-called intrinsic subtype classifications based on mRNA expression signature (e.g. luminal A, basal-like subtypes) (Mihaly and Gyorffy, 2013). Estrogen receptor negative (ER-) breast cancers are a group of breast tumors associated with decreased survival in comparison to estrogen receptor positive (ER+) breast cancers as well as fewer prevention and treatment possibilities (Putti et al., 2005). Moreover, basal-like breast cancer subtype (ER-, PR-, HER2-) is of particular clinical interest due to lack of effective targeted therapies and poor baseline prognosis (Prat et al., 2013). In comparison, luminal A breast cancer subtype (ER+, HER2-) tend to have best prognosis, with fairly high survival rates and low recurrence (Voduc et al., 2010). Therefore it was of great interest to use these available prognostic survival data sources in our studies.

Kaplan-Meier analysis of α-actinin-1 and -4 revealed that α-actinin-4 expression levels do not correlate with ER or intrinsic subtypes whereas high α-actinin-1 shows an interesting correlation with ER negative status (III, Figure 3 and S3). It is noteworthy to mention that in a previous study the prognostic value of α-actinin-4 has been shown to be dependent on α-actinin-4 subcellular localization suggesting that there is a difference between α-actinin-1 and-4 (Honda et al., 1998; Honda et al., 2005). Unfortunately, our patient material was too limited to address this question experimentally. However, the evident difference between α-actinin-1 and -4 is that only high α-actinin-1 associates with poor prognosis with ER negative patient group. This is interesting finding since the commonly used hormone-based treatment cannot be used with these patients, thus new target for treatments are needed. α-actinin-1 is not likely to be the driver gene, but in addition to a possible prognostic value, understanding its cancer promoting cellular functions may help to increased knowledge of ER negative breast cancer.

Considering a possibility how ER levels are maintained in cells, it has been reported that transforming growth factor β (TGF-β) is a signaling molecule that can crosstalk to estrogen receptor α (ERα) by inhibiting ERα functions thus leading to increased levels of TGF-β in the cells. The established crosstalk between TGF-β and ERα works in a bidirectional manner (Knabbe et al., 1987; Stope et al., 2010). This crosstalk has been reported to be essential in various conditions such as pathophysiology of kidney functions and especially breast cancer (Band and Laiho, 2011; Matsuda et al., 2001). TGF-β belongs to the superfamily of cytokines and has numerous functions including proliferation, differentiation, migration, immune response and apoptosis. TGF-β signaling deregulation is frequent in tumors and promotes tumor growth and invasion, evasion of immune surveillance, and cancer cell dissemination and metastasis. TGF-β is most know for the ability to promote EMT (Massague, 2008; Papageorgis, 2015). Furthermore, TGF-β can induce a rapid reorganization of the actin cytoskeleton, leading to membrane ruffling at the cell edges and prolonged incubation with TGF-β results in the formation of actin stress fibers through the activation of Rho GTPase signaling (Edlund et al., 2002).

Difference between actinin-1 and -4 in ER negative breast cancer cell lines is noted in a mRNA profiling of previously published breast cancer panel (Neve et al., 2006), and our Western blotting analysis of selected breast cancer cell lines. Both of the results show that α-actinin-1 expression is higher in ER negative cells compared to ER positive (III, Figure 3). This correlation is not noted with α-actinin-4. This data is interesting and in support with the survival analysis in ER negative breast cancer patients. An evident difference between α-actinin-1 and -4 can also be detected in our cell culture studies, where we utilized two different breast cancer cell lines both ER negative and have high expression of α-actinin-1. The noted re-organization of the actin cytoskeleton and destabilization of E-cadherin based adhesions are specific for α-actinin-1, implying that α-actinin-1 and -4 regulate E-cadherin based adhesions differently (III, Figure 4 and S4).

Possible difference between α-actinin-1 and -4 in regulating E-cadherin based adhesions could be explained through their binding partners. α-actinin-1 binds to α-catenin (Knudsen et al., 1995), whereas α-actinin-4 binds to β-catenin (Hayashida et al., 2005). Association between α-actinin-1 and α-catenin anchors the cadherin-catenin complex to the cytoplasmic actin cytoskeleton. Binding of vinculin to α-catenin under applied tension allows transforming force into a sustainable biochemical signal (le Duc et al., 2010; Yao et al., 2014; Yonemura et al., 2010). Binding of actin filaments to the cadherin-catenin complex is essential as actin filaments can guide the cadherin-catenin cluster assembly, stability and movement (Hong et al., 2013). In comparison, association between α-actinin-4 and β-catenin occurs in the absence of E-cadherin and has been suggested to have a role in EMT (Hayashida et al., 2005). These findings are suggesting that α-actinin-1 and -4 contribute in regulating E-cadherin based adhesions in distinct ways that are isoform specific. This further implies that α-actinin-1 and -4 contribute to cancer progression in diverse ways. α-actinin-4 can contribute through gene amplification and increased copy number leading to increased cell migration and invasion as reported (Watabe et al., 2014; Yamamoto et al., 2012).

Database searches do not support that α-actinin-1 is amplified, instead an interesting observation is that it is induced by TGF-β (Bakin et al., 2004; Chambers et al., 2003; Levy and Hill, 2005; Ranganathan et al., 2007). Furthermore, α-actinin-1 has been reported to be essential for promoting TGF-β induced migration and invasion of breast cancer cells through the interaction with lipoma preferred partner (LPP), which belongs to the zyxin family of LIM domain proteins (Ngan et al., 2013). Concluding from these studies, TGF-β could be a possible signaling pathway that is involved in maintaining high α-actinin-1 levels in ER negative breast cancers. However, these interesting observation require further and more detail investigation.

In cancer context, our studies suggest that α-actinin-1 remodels the actin cytoskeleton and epithelial cell plasticity by destabilizing E-cadherin based adhesions in two- and three-dimensional environment. These changes are likely to promote migratory potential of collectively migrating cancer cells.

Figure 6 contains a summary of our findings in epithelial cells upon increased α-actinin-1 expression. Taken together, our studies suggest that α-actinin-1 could be a candidate prognostic biomarker in breast cancer, especially in ER negative breast cancer patients.

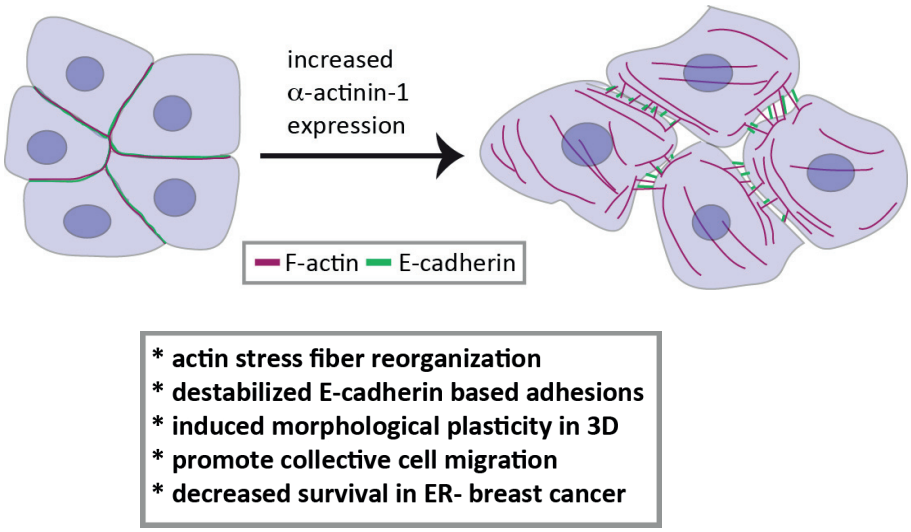


Figure 6. Summary of the key findings upon increased α-actinin-1 expression in epithelial cells. Schematic illustration of epithelial cells upon increased α-actinin-1 expression leading to various morphological changes. F-actin (red) and E-cadherin (green) alterations are highlighted in the schematic cell representation.

CONCLUDING REMARKS

Cell migration is a dynamic and adaptive process integrating actin stress fiber dynamics, cell-matrix and cell-cell adhesions. During this thesis project, we have identified critical players contributing to these events and thus providing further understanding how actin stress fibers, cell contractility and adhesions are regulated. Identifying the association between NUAK2 kinase and MRIP, has reveled a novel mechanism in regulating actin stress fiber assembly as well as cell contractility. These findings are interesting because cells need to maintain a tensional balance between the externally applied forces and actomyosin contractility generated inside the cell. If this balance is deregulated it can lead to cell detachment and increased cell migration. Identifying a kinase as an actin stress fiber regulator is of great interest as kinases can potentially be used as drug therapy targets and have valuable clinical applications.

Study of the two abundant actin crosslinking proteins, α-actinin-1 and α-actinin-4 has revealed distinct, isoform-specific functions in mesenchymal and epithelial cells. We identified α-actinin-1 to be essential for the assembly of actin stress fibers associated with cell-matrix and cell-cell adhesions. Our findings are expanding the knowledge of specific actin stress fiber subtypes varying in dynamics, molecular composition and signaling pathways regulating their functions. Understanding how distinct actin stress fibers are regulated is important as it provides the possibility of targeting specific molecules involved in particular functions. Moreover, our findings provide additional molecular-scale insight into how α-actinin isoforms contributes to biophysical interactions between tumor cells and the extracellular matrix thus regulating cell migration and cell plasticity.

Interestingly, we discovered during the course of this thesis that α-actinin-1 is upregulated in various cancers. In breast cancer especially contributes to cancer progression and associates with decreased survival in ER negative breast cancer patients. In the light of our findings it provides the possibility to also pursue α-actinin-1 as a possible drug target and as a candidate prognostic biomarker in ER negative breast cancer patients.

ACKNOWLEDGEMENTS

This thesis work was carried out in the Research Programs Unit and Genome Scale Biology Research Program at the Faculty of Medicine and in the Institute of Biotechnology, University of Helsinki. All present and former directors are acknowledged for providing excellent research facilities, infrastructure, and a great atmosphere to work in. This work has been financially supported by the Integrative Life Science Doctoral Programme, the Academy of Finland, Biocentrum Helsinki, The Finnish Medical Foundation, the Finnish Cancer Foundation, Sigrid Juselius Foundation, Instrumentarium Science Foundation, the Maud Kuistila Memorial Foundation, K. Albin Johansson Foundation, the University of Helsinki Funds, Emil Aaltonen Foundation, University of Helsinki Chancellor´s Funds, Biomedicum Helsinki Foundation, Ida Montin Foundation and Paulo Foundation. A warm thank you to all the funding organizations as conducting research wouldn't have been possible without this significant support.

My deepest gratitude goes to my thesis supervisors, academy research fellow Tea Vallenius and professor Tomi Mäkelä, for recruiting me initially as a master student, and for giving me this opportunity to pursue my dreams in becoming a scientist. It has been a huge privilege to have you both as my supervisors and to work in your labs. I have learned so much from both of you but most importantly the scientific thinking and writing as well as persistence and enthusiasm for science. I would like to express my most sincere gratitude to both of you and thank you for your support and guidance throughout the years and for being part of this journey.

I would like to express my gratitude to associate professor Sanna Lehtonen for her willingness to act as the opponent. Thank you for taking this time to discuss with me and being part of this exciting experience. Professor Juha Partanen is acknowledged for agreeing to serve as the custodian and for all his help with practical matters during the final steps of this thesis process. I would like to warmly thank the members of my thesis committee, professor Olli Carpén and docent Maria Vartiainen, for the fruitful discussions and valuable comments during the course of my PhD studies. I am very grateful to my pre-examiners, professor Jari Ylänne and docent Pirta Hotulainen, for their willingness to agree to my tight schedule and insightful suggestions on how to improve this thesis.

I would like to thank the scientific community at the Biomedicum Helsinki and at the Institute of Biotechnology, all the co-authors involved and collaborators that has shared reagents, support and scientific knowledge throughout the years. I want to warmly thank all the neighboring labs and especially the people from Juha Klefström, Päivi Ojala and Mikko Frilander labs that has contributed to my PhD journey and made it exciting, fun and memorable. This couldn't have been possible without you all and without the amazing people in our scientific community. Thank you also Sini, Essi,

Tiina and Johanna for all the laughter, parties and crazy moments during my PhD journey. To the people I have unwillingly forgotten from the list– it has been a pleasure working with you.

I would like to acknowledge the extremely helpful staff of Biomedicum Stem Cell Center, Genome Biology Unit (GBU), Biomedicum Imaging Unit (BIU) and Light Microscopy Unit (LMU) for their excellent services and imaging help. Ville Rantanen, Jussi Kenkkilä, Mikko Liljeström, Mika Molin and Kimmo Tanhuanpää are acknowledged for their essential help with imaging, image analysis and microscopy related support. A special thank you to Satu Sankkila and Anu Taulio for helping with administrative issues and the countless advises on all areas of life.

Saana Ruusulampi, Outi Heikkilä, Kirsi Mänttäri, Jenny Bärlund, Markus Keskitalo, Niina Roine, Susanna Räsänen and Kaisa Laajanen are acknowledged for their essential and valuable technical assistance and support throughout the years and for keeping the lab running. A special thank you to Saana for being my lab mentor from the beginning and teaching me everything I know in the lab. A huge thank you to all the Makelab members past and present, thank you for creating a great and enjoyable scientific working atmosphere and most of all for surviving my endless humor and jokes regardless if good or bad. To the Makelab girls - Kaisa, Susanna, Emilia, Saana, Elina, Niina, Saara, Heini, Eva, Katja, Ying, Lina, Jing, Iris, Alisa, Michelle, Anou, Ana-Maria and Rashmi thank you for the fun times and endless discussions about kids, pregnancies, family and of course science. To the amazing “juniors”, Heini and Eva. Heini, your laughter, energy and personality are great! Eva, thank you for introducing me to the world of R, or for me just EasyR! A special thank you to my core complex composed of Kaisa, Saana, Susanna, Elina, Niina and Emilia – you guys formed an essential support system throughout my PhD journey, you all made this journey memorable and full of joy and laughter. Thank you all for your friendship! To the Y chromosome representatives of the Makelab – Sushil, Yan, Kari, Pekka K, Tima, Markus, Dima, Johan, Pekka P. and Ryan – thank you for balancing the woman power of the lab, or at least trying to!

This journey wouldn’t have been possible without the support network and amazing friends outside the science world. A huge thank you to all my friends for being there for me all these years. A special thank you to Hanna, my dearest childhood friend, thank you for being a true friend and always being there for me. Zoe, thank you for your friendship, and for being my fellow scientist who understands me outside work and of course for all the “mega breakfasts” that kept us going during our early science days in Cardiff.

I would like to express my deepest gratitude to my loving family. To my parents, Doina and Iuri, thank you for always knowing how to advice me and for teaching me to never give up. Thank you for your endless support and unconditional love. I am and will always be grateful for all the sacrifices you both had to make to provide a better life and future for me, and my brother Mark. I am forever grateful for

your love, support and guidance in life. Mark, my dearest little brother, remember that whatever you decide to do in life you will always have the support of your loving family.

Finally, to the most energetic and amazing boys in the world, Lucas and Elias, thank you for keeping us busy and filling our lives with love, endless curiosity and passion for sports and speed. To my husband and love of my life, Peter, this journey would have not been possible without you by my side. Thank you for trying to understand the life of a scientist and for endlessly supporting me during the ups and downs. You showed me the true meaning of life and what really matters and that is a loving family! To the three musketeers of my life – thank you for completing my life and for your endless and unconditional love and support. I love you!

Helsinki, January 22nd 2018

REFERENCES

Abe, K., and M. Takeichi. 2008. EPLIN mediates linkage of the cadherin catenin complex to F-actin and stabilizes the circumferential actin belt. *Proc Natl Acad Sci U S A*. 105:13-19.

Abercrombie, M., and G.A. Dunn. 1975. Adhesions of fibroblasts to substratum during contact inhibition observed by interference reflection microscopy. *Exp Cell Res*. 92:57-62.

Abercrombie, M., J.E. Heaysman, and S.M. Pegrum. 1971. The locomotion of fibroblasts in culture. IV. Electron microscopy of the leading lamella. *Exp Cell Res*. 67:359-367.

Aguilar-Cuenca, R., C. Llorente-Gonzalez, C. Vicente, and M. Vicente-Manzanares. 2017. Microfilament-coordinated adhesion dynamics drives single cell migration and shapes whole tissues. *F1000Research*. 6:160.

Aksenova, V., L. Turoverova, M. Khotin, K.E. Magnusson, E. Tulchinsky, G. Melino, G.P. Pinaev, N. Barlev, and D. Tentler. 2013. Actin-binding protein alpha-actinin 4 (ACTN4) is a transcriptional co-activator of RelA/p65 sub-unit of NF-kB. *Oncotarget*. 4:362-372.

Alessi, D., L.K. MacDougall, M.M. Sola, M. Ikebe, and P. Cohen. 1992. The control of protein phosphatase-1 by targetting subunits. The major myosin phosphatase in avian smooth muscle is a novel form of protein phosphatase-1. *European journal of biochemistry*. 210:1023-1035.

Alexandrova, A.Y., K. Arnold, S. Schaub, J.M. Vasiliev, J.J. Meister, A.D. Bershadsky, and A.B. Verkhovsky. 2008. Comparative dynamics of retrograde actin flow and focal adhesions: formation of nascent adhesions triggers transition from fast to slow flow. *PLoS One*. 3:e3234.

Amano, M., M. Ito, K. Kimura, Y. Fukata, K. Chihara, T. Nakano, Y. Matsuura, and K. Kaibuchi. 1996. Phosphorylation and activation of myosin by Rho-associated kinase (Rho-kinase). *J Biol Chem*. 271:20246-20249.

Ananthakrishnan, R., and A. Ehrlicher. 2007. The forces behind cell movement. *International journal of biological sciences*. 3:303-317.

Ang, S.F., Z.S. Zhao, L. Lim, and E. Manser. 2010. DAAM1 is a formin required for centrosome re-orientation during cell migration. *PLoS One*. 5.

Angst, B.D., C. Marcozzi, and A.I. Magee. 2001. The cadherin superfamily: diversity in form and function. *J Cell Sci*. 114:629-641.

Aplin, A.E., A. Howe, S.K. Alahari, and R.L. Juliano. 1998. Signal transduction and signal modulation by cell adhesion receptors: the role of integrins, cadherins, immunoglobulin-cell adhesion molecules, and selectins. *Pharmacological reviews*. 50:197-263.

Araki, N., T. Hatae, T. Yamada, and S. Hirohashi. 2000. Actinin-4 is preferentially involved in circular ruffling and macropinocytosis in mouse macrophages: analysis by fluorescence ratio imaging. *J Cell Sci*. 113 (Pt 18):3329-3340.

Aspenström, P. 2010. Formin-binding proteins: modulators of formin-dependent actin polymerization. *Biochim Biophys Acta*. 1803:174-182.

Ayollo, D.V., I.Y. Zhitnyak, J.M. Vasiliev, and N.A. Gloushankova. 2009. Rearrangements of the actin cytoskeleton and E-cadherin-based adherens junctions caused by neoplasic transformation change cell-cell interactions. *PLoS One*. 4:e8027.

Azatov, M., S.M. Goicoechea, C.A. Otey, and A. Upadhyaya. 2016. The actin crosslinking protein palladin modulates force generation and mechanosensitivity of tumor associated fibroblasts. *Scientific reports*. 6:28805.

Babakov, V.N., O.A. Petukhova, L.V. Turoverova, I.V. Kropacheva, D.G. Tentler, A.V. Bolshakova, E.P. Podolskaya, K.E. Magnusson, and G.P. Pinaev. 2008. RelA/NF-kappaB transcription factor associates with alpha-actinin-4. *Exp Cell Res*. 314:1030-1038.

Bachir, A.I., A.R. Horwitz, W.J. Nelson, and J.M. Bianchini. 2017. Actin-Based Adhesion Modules Mediate Cell Interactions with the Extracellular Matrix and Neighboring Cells. *Cold Spring Harb Perspect Biol*. 9.

Bakin, A.V., A. Safina, C. Rinehart, C. Daroqui, H. Darbary, and D.M. Helfman. 2004. A critical role of tropomyosins in TGF-beta regulation of the actin cytoskeleton and cell motility in epithelial cells. *Mol Biol Cell*. 15:4682-4694.

Band, A.M., and M. Laiho. 2011. Crosstalk of TGF-beta and estrogen receptor signaling in breast cancer. *Journal of mammary gland biology and neoplasia*. 16:109-115.

Barbolina, M.V., B.P. Adley, D.L. Kelly, A.J. Fought, D.M. Scholtens, L.D. Shea, and M.S. Stack. 2008. Motility-related actinin alpha-4 is associated with advanced and metastatic ovarian carcinoma. *Lab Invest*. 88:602-614.

Bauer, K., M. Kratzer, M. Otte, K.L. de Quintana, J. Hagmann, G.J. Arnold, C. Eckerskorn, F. Lottspeich, and W. Siess. 2000. Human CLP36, a PDZ-domain and LIM-domain protein, binds to alpha-actinin-1 and associates with actin filaments and stress fibers in activated platelets and endothelial cells. *Blood*. 96:4236-4245.

Beach, J.R., L. Shao, K. Remmert, D. Li, E. Betzig, and J.A. Hammer, 3rd. 2014. Nonmuscle myosin II isoforms coassemble in living cells. *Curr Biol*. 24:1160-1166.

Beggs, A.H., T.J. Byers, J.H. Knoll, F.M. Boyce, G.A. Bruns, and L.M. Kunkel. 1992. Cloning and characterization of two human skeletal muscle alpha-actinin genes located on chromosomes 1 and 11. *J Biol Chem*. 267:9281-9288.

Bellanger, J.M., C. Astier, C. Sardet, Y. Ohta, T.P. Stossel, and A. Debant. 2000. The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. *Nat Cell Biol*. 2:888-892.

Bershadsky, A.D., C. Ballestrem, L. Carramusa, Y. Zilberman, B. Gilquin, S. Khochbin, A.Y. Alexandrova, A.B. Verkhovsky, T. Shemesh, and M.M. Kozlov. 2006. Assembly and mechanosensory function of focal adhesions: experiments and models. *Eur J Cell Biol*. 85:165-173.

Bertocchi, C., Y. Wang, A. Ravasio, Y. Hara, Y. Wu, T. Sailov, M.A. Baird, M.W. Davidson, R. Zaidel-Bar, Y. Toyama, B. Ladoux, R.M. Mege, and P. Kanchanawong. 2017. Nanoscale architecture of cadherin-based cell adhesions. *Nat Cell Biol*. 19:28-37.

Bertucci, F., P. Finetti, N. Cervera, E. Charafe-Jauffret, E. Mamessier, J. Adelaide, S. Debono, G. Houvenaeghel, D. Maraninchi, P. Viens, C. Charpin, J. Jacquemier, and D. Birnbaum. 2006. Gene expression profiling shows medullary breast cancer is a subgroup of basal breast cancers. *Cancer research*. 66:4636-4644.

Biswas, K.H., and R. Zaidel-Bar. 2017. Early events in the assembly of E-cadherin adhesions. *Exp Cell Res*. 358:14-19.

Blanchard, A., V. Ohanian, and D. Critchley. 1989. The structure and function of alpha-actinin. *J Muscle Res Cell Motil*. 10:280-289.

Blanchoin, L., R. Boujemaa-Paterski, C. Sykes, and J. Plastino. 2014. Actin dynamics, architecture, and mechanics in cell motility. *Physiol Rev*. 94:235-263.

Blume, C., P.M. Benz, U. Walter, J. Ha, B.E. Kemp, and T. Renne. 2007. AMP-activated protein kinase impairs endothelial actin cytoskeleton assembly by phosphorylating vasodilator-stimulated phosphoprotein. *J Biol Chem*. 282:4601-4612.

Bresnick, A.R. 1999. Molecular mechanisms of nonmuscle myosin-II regulation. *Curr Opin Cell Biol*. 11:26-33.

Brieher, W.M., and A.S. Yap. 2013. Cadherin junctions and their cytoskeleton(s). *Curr Opin Cell Biol*. 25:39-46.

Broderick, M.J., and S.J. Winder. 2005. Spectrin, alpha-actinin, and dystrophin. *Advances in protein chemistry*. 70:203-246.

Broschat, K.O. 1990. Tropomyosin prevents depolymerization of actin filaments from the pointed end. *J Biol Chem*. 265:21323-21329.

Buckley, C.D., J. Tan, K.L. Anderson, D. Hanein, N. Volkmann, W.I. Weis, W.J. Nelson, and A.R. Dunn. 2014. Cell adhesion. The minimal cadherin-catenin complex binds to actin filaments under force. *Science*. 346:1254211.

Buckley, I.K., and K.R. Porter. 1967. Cytoplasmic fibrils in living cultured cells. A light and electron microscope study. *Protoplasma*. 64:349-380.

Burnette, D.T., S. Manley, P. Sengupta, R. Sougrat, M.W. Davidson, B. Kachar, and J. Lippincott-Schwartz. 2011. A role for actin arcs in the leading-edge advance of migrating cells. *Nat Cell Biol*. 13:371-381.

Burnette, D.T., L. Shao, C. Ott, A.M. Pasapera, R.S. Fischer, M.A. Baird, C. Der Loughian, H. Delanoe-Ayari, M.J. Paszek, M.W. Davidson, E. Betzig, and J. Lippincott-Schwartz. 2014. A contractile and counterbalancing adhesion system controls the 3D shape of crawling cells. *J Cell Biol*. 205:83-96.

Burridge, K. 1981. Are stress fibres contractile? *Nature*. 294:691-692.

Burridge, K., and M. Chrzanowska-Wodnicka. 1996. Focal adhesions, contractility, and signaling. *Annu Rev Cell Dev Biol*. 12:463-518.

Burridge, K., and C. Guilluy. 2016. Focal adhesions, stress fibers and mechanical tension. *Exp Cell Res*. 343:14-20.

Burridge, K., and K. Wennerberg. 2004. Rho and Rac take center stage. *Cell*. 116:167-179.

Cai, Y., N. Biais, G. Giannone, M. Tanase, G. Jiang, J.M. Hofman, C.H. Wiggins, P. Silberzan, A. Buguin, B. Ladoux, and M.P. Sheetz. 2006. Nonmuscle myosin IIA-dependent force inhibits cell spreading and drives F-actin flow. *Biophys J*. 91:3907-3920.

Cai, Y., O. Rossier, N.C. Gauthier, N. Biais, M.A. Fardin, X. Zhang, L.W. Miller, B. Ladoux, V.W. Cornish, and M.P. Sheetz. 2010. Cytoskeletal coherence requires myosin-IIA contractility. *J Cell Sci*. 123:413-423.

Case, L.B., and C.M. Waterman. 2015. Integration of actin dynamics and cell adhesion by a three-dimensional, mechanosensitive molecular clutch. *Nat Cell Biol*. 17:955-963.

Cavey, M., and T. Lecuit. 2009. Molecular bases of cell-cell junctions stability and dynamics. *Cold Spring Harb Perspect Biol*. 1:a002998.

Cavey, M., M. Rauzi, P.F. Lenne, and T. Lecuit. 2008. A two-tiered mechanism for stabilization and immobilization of E-cadherin. *Nature*. 453:751-756.

Chakraborty, S., E.L. Reineke, M. Lam, X. Li, Y. Liu, C. Gao, S. Khurana, and H.Y. Kao. 2006. Alpha-actinin 4 potentiates myocyte enhancer factor-2 transcription activity by antagonizing histone deacetylase 7. *J Biol Chem*. 281:35070-35080.

Chambers, R.C., P. Leoni, N. Kaminski, G.J. Laurent, and R.A. Heller. 2003. Global expression profiling of fibroblast responses to transforming growth factor-beta1 reveals the induction of inhibitor of differentiation-1 and provides evidence of smooth muscle cell phenotypic switching. *Am J Pathol*. 162:533-546.

Chan, Y., H.Q. Tong, A.H. Beggs, and L.M. Kunkel. 1998. Human skeletal muscle-specific alpha-actinin-2 and -3 isoforms form homodimers and heterodimers in vitro and in vivo. *Biochem Biophys Res Commun*. 248:134-139.

Chao, Y., Q. Wu, M. Acquafondata, R. Dhir, and A. Wells. 2012. Partial mesenchymal to epithelial reverting transition in breast and prostate cancer metastases. *Cancer microenvironment : official journal of the International Cancer Microenvironment Society*. 5:19-28.

Chen, W.T. 1981. Mechanism of retraction of the trailing edge during fibroblast movement. *J Cell Biol*. 90:187-200.

Chesarone, M.A., and B.L. Goode. 2009. Actin nucleation and elongation factors: mechanisms and interplay. *Curr Opin Cell Biol*. 21:28-37.

Chhabra, E.S., and H.N. Higgs. 2007. The many faces of actin: matching assembly factors with cellular structures. *Nat Cell Biol*. 9:1110-1121.

Choi, C.K., M. Vicente-Manzanares, J. Zareno, L.A. Whitmore, A. Mogilner, and A.R. Horwitz. 2008. Actin and alpha-actinin orchestrate the assembly and maturation of nascent adhesions in a myosin II motor-independent manner. *Nat Cell Biol*. 10:1039-1050.

Chrzanowska-Wodnicka, M., and K. Burridge. 1996. Rho-stimulated contractility drives the formation of stress fibers and focal adhesions. *J Cell Biol*. 133:1403-1415.

Ciobanasu, C., B. Faivre, and C. Le Clainche. 2012. Actin dynamics associated with focal adhesions. *Int J Cell Biol*. 2012:941292.

Clark, K., M. Langeslag, C.G. Figdor, and F.N. van Leeuwen. 2007. Myosin II and mechanotransduction: a balancing act. *Trends in cell biology*. 17:178-186.

Clark, K.A., A.S. McElhinny, M.C. Beckerle, and C.C. Gregorio. 2002. Striated muscle cytoarchitecture: an intricate web of form and function. *Annu Rev Cell Dev Biol*. 18:637-706.

Craig, D.H., C. Downey, and M.D. Basson. 2008. SiRNA-mediated reduction of alpha-actinin-1 inhibits pressure-induced murine tumor cell wound implantation and enhances tumor-free survival. *Neoplasia*. 10:217-222.

Craig, D.H., B. Haimovich, and M.D. Basson. 2007a. Alpha-actinin-1 phosphorylation modulates pressure-induced colon cancer cell adhesion through regulation of focal adhesion kinase-Src interaction. *Am J Physiol Cell Physiol*. 293:C1862-1874.

Craig, D.H., J. Zhang, and M.D. Basson. 2007b. Cytoskeletal signaling by way of alpha-actinin-1 mediates ERK1/2 activation by repetitive deformation in human Caco2 intestinal epithelial cells. *Am J Surg*. 194:618-622.

Cramer, L.P., M. Siebert, and T.J. Mitchison. 1997. Identification of novel graded polarity actin filament bundles in locomoting heart fibroblasts: implications for the generation of motile force. *J Cell Biol*. 136:1287-1305.

Crowley, E., and A.F. Horwitz. 1995. Tyrosine phosphorylation and cytoskeletal tension regulate the release of fibroblast adhesions. *J Cell Biol*. 131:525-537.

Davis, M.A., R.C. Ireton, and A.B. Reynolds. 2003. A core function for p120-catenin in cadherin turnover. *J Cell Biol*. 163:525-534.

De Pascalis, C., and S. Etienne-Manneville. 2017. Single and collective cell migration: the mechanics of adhesions. *Mol Biol Cell*. 28:1833-1846.

Debnath, J., and J.S. Brugge. 2005. Modelling glandular epithelial cancers in three-dimensional cultures. *Nat Rev Cancer*. 5:675-688.

Djinovic-Carugo, K., M. Gautel, J. Ylanne, and P. Young. 2002. The spectrin repeat: a structural platform for cytoskeletal protein assemblies. *FEBS letters*. 513:119-123.

Djinovic-Carugo, K., P. Young, M. Gautel, and M. Saraste. 1999. Structure of the alpha-actinin rod: molecular basis for cross-linking of actin filaments. *Cell*. 98:537-546.

Dominguez, R., and K.C. Holmes. 2011. Actin structure and function. *Annual review of biophysics*. 40:169-186.

dos Remedios, C.G., D. Chhabra, M. Kekic, I.V. Dedova, M. Tsubakihara, D.A. Berry, and N.J. Nosworthy. 2003. Actin binding proteins: regulation of cytoskeletal microfilaments. *Physiol Rev*. 83:433-473.

Downey, C., D.H. Craig, and M.D. Basson. 2011. Isoform-specific modulation of pressure-stimulated cancer cell proliferation and adhesion by alpha-actinin. *Am J Surg*. 202:520-523.

Doyle, A.D., N. Carvajal, A. Jin, K. Matsumoto, and K.M. Yamada. 2015. Local 3D matrix microenvironment regulates cell migration through spatiotemporal dynamics of contractility-dependent adhesions. *Nature communications*. 6:8720.

Drmota Prebil, S., U. Slapsak, M. Pavsic, G. Ilc, V. Puz, E. de Almeida Ribeiro, D. Anrather, M. Hartl, L. Backman, J. Plavec, B. Lenarcic, and K. Djinovic-Carugo. 2016. Structure and calcium-binding studies of calmodulin-like domain of human non-muscle alpha-actinin-1. *Scientific reports*. 6:27383.

Dugina, V., I. Zwaenepoel, G. Gabbiani, S. Clement, and C. Chaponnier. 2009. {beta}- and {gamma}-cytoplasmic actins display distinct distribution and functional diversity. *J Cell Sci*. 122:2980-2988.

Ebashi, S., and F. Ebashi. 1964. A New Protein Factor Promoting Contraction of Actomyosin. *Nature*. 203:645-646.

Edelman, G.M. 1986. Cell adhesion molecules in the regulation of animal form and tissue pattern. *Annual review of cell biology*. 2:81-116.

Edlund, M., M.A. Lotano, and C.A. Otey. 2001. Dynamics of alpha-actinin in focal adhesions and stress fibers visualized with alpha-actinin-green fluorescent protein. *Cell Motil Cytoskeleton*. 48:190-200.

Edlund, S., M. Landstrom, C.H. Heldin, and P. Aspenstrom. 2002. Transforming growth factor-beta-induced mobilization of actin cytoskeleton requires signaling by small GTPases Cdc42 and RhoA. *Mol Biol Cell*. 13:902-914.

Ehrlicher, A.J., R. Krishnan, M. Guo, C.M. Bidan, D.A. Weitz, and M.R. Pollak. 2015. Alpha-actinin binding kinetics modulate cellular dynamics and force generation. *Proc Natl Acad Sci U S A*. 112:6619-6624.

El-Sibai, M., O. Pertz, H. Pang, S.C. Yip, M. Lorenz, M. Symons, J.S. Condeelis, K.M. Hahn, and J.M. Backer. 2008. RhoA/ROCK-mediated switching between Cdc42- and Rac1-dependent protrusion in MTLn3 carcinoma cells. *Exp Cell Res*. 314:1540-1552.

Endlich, N., C.A. Otey, W. Kriz, and K. Endlich. 2007. Movement of stress fibers away from focal adhesions identifies focal adhesions as sites of stress fiber assembly in stationary cells. *Cell Motil Cytoskeleton*. 64:966-976.

Endo, T., and T. Masaki. 1984. Differential expression and distribution of chicken skeletal- and smooth-muscle-type alpha-actinins during myogenesis in culture. *J Cell Biol*. 99:2322-2332.

Etienne-Manneville, S. 2004. Cdc42--the centre of polarity. *J Cell Sci*. 117:1291-1300.

Etienne-Manneville, S., and A. Hall. 2002. Rho GTPases in cell biology. *Nature*. 420:629-635.

Even-Ram, S., A.D. Doyle, M.A. Conti, K. Matsumoto, R.S. Adelstein, and K.M. Yamada. 2007. Myosin IIA regulates cell motility and actomyosin-microtubule crosstalk. *Nat Cell Biol*. 9:299-309.

Farquhar, M.G., and G.E. Palade. 1963. Junctional complexes in various epithelia. *J Cell Biol*. 17:375-412.

Fath, K.R., C.J. Edgell, and K. Burridge. 1989. The distribution of distinct integrins in focal contacts is determined by the substratum composition. *J Cell Sci*. 92 (Pt 1):67-75.

Faul, C., K. Asanuma, E. Yanagida-Asanuma, K. Kim, and P. Mundel. 2007. Actin up: regulation of podocyte structure and function by components of the actin cytoskeleton. *Trends in cell biology*. 17:428-437.

Fellenberg, J., L. Bernd, G. Delling, D. Witte, and A. Zahlten-Hinguranage. 2007. Prognostic significance of drug-regulated genes in high-grade osteosarcoma. *Mod Pathol*. 20:1085-1094.

Feng, Y., H. Ngu, S.K. Alford, M. Ward, F. Yin, and G.D. Longmore. 2013. alpha-actinin1 and 4 tyrosine phosphorylation is critical for stress fiber establishment, maintenance and focal adhesion maturation. *Exp Cell Res*. 319:1124-1135.

Fife, C.M., J.A. McCarroll, and M. Kavallaris. 2014. Movers and shakers: cell cytoskeleton in cancer metastasis. *British journal of pharmacology*. 171:5507-5523.

Firat-Karalar, E.N., and M.D. Welch. 2011. New mechanisms and functions of actin nucleation. *Curr Opin Cell Biol*. 23:4-13.

Fischer, R.S., M. Gardel, X. Ma, R.S. Adelstein, and C.M. Waterman. 2009. Local cortical tension by myosin II guides 3D endothelial cell branching. *Curr Biol*. 19:260-265.
Fletcher, D.A., and R.D. Mullins. 2010. Cell mechanics and the cytoskeleton. *Nature*. 463:485-492.
Foley, K.S., and P.W. Young. 2013. An analysis of splicing, actin-binding properties, heterodimerization and molecular interactions of the non-muscle alpha-actinins. *Biochem J*. 452:477-488.
Foley, K.S., and P.W. Young. 2014. The non-muscle functions of actinins: an update. *Biochem J*. 459:1-13.
Franzot, G., B. Sjoblom, M. Gautel, and K. Djinovic Carugo. 2005. The crystal structure of the actin binding domain from alpha-actinin in its closed conformation: structural insight into phospholipid regulation of alpha-actinin. *J Mol Biol*. 348:151-165.
Friedl, P., and S. Alexander. 2011. Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell*. 147:992-1009.
Friedl, P., J. Locker, E. Sahai, and J.E. Segall. 2012. Classifying collective cancer cell invasion. *Nat Cell Biol*. 14:777-783.
Friedl, P., and K. Wolf. 2003. Tumour-cell invasion and migration: diversity and escape mechanisms. *Nat Rev Cancer*. 3:362-374.
Friedl, P., and K. Wolf. 2010. Plasticity of cell migration: a multiscale tuning model. *J Cell Biol*. 188:11-19.
Fukumoto, M., S. Kurisu, T. Yamada, and T. Takenawa. 2015. alpha-Actinin-4 enhances colorectal cancer cell invasion by suppressing focal adhesion maturation. *PLoS One*. 10:e0120616.
Gallin, W.J., G.M. Edelman, and B.A. Cunningham. 1983. Characterization of L-CAM, a major cell adhesion molecule from embryonic liver cells. *Proc Natl Acad Sci U S A*. 80:1038-1042.
Gardel, M.L., I.C. Schneider, Y. Aratyn-Schaus, and C.M. Waterman. 2010. Mechanical integration of actin and adhesion dynamics in cell migration. *Annu Rev Cell Dev Biol*. 26:315-333.
Gardina, P.J., T.A. Clark, B. Shimada, M.K. Staples, Q. Yang, J. Veitch, A. Schweitzer, T. Awad, C. Sugnet, S. Dee, C. Davies, A. Williams, and Y. Turpaz. 2006. Alternative splicing and differential gene expression in colon cancer detected by a whole genome exon array. *BMC genomics*. 7:325.
Gateva, G., S. Tojkander, S. Koho, O. Carpen, and P. Lappalainen. 2014. Palladin promotes assembly of non-contractile dorsal stress fibers through VASP recruitment. *J Cell Sci*. 127:1887-1898.
Geiger, B., A. Bershadsky, R. Pankov, and K.M. Yamada. 2001. Transmembrane crosstalk between the extracellular matrix--cytoskeleton crosstalk. *Nat Rev Mol Cell Biol*. 2:793-805.
Geiger, B., J.P. Spatz, and A.D. Bershadsky. 2009. Environmental sensing through focal adhesions. *Nat Rev Mol Cell Biol*. 10:21-33.
Geiger, B., and K.M. Yamada. 2011. Molecular architecture and function of matrix adhesions. *Cold Spring Harb Perspect Biol*. 3.
Giannone, G., B.J. Dubin-Thaler, O. Rossier, Y. Cai, O. Chaga, G. Jiang, W. Beaver, H.G. Dobereiner, Y. Freund, G. Borisy, and M.P. Sheetz. 2007. Lamellipodial actin mechanically links myosin activity with adhesion-site formation. *Cell*. 128:561-575.
Gimona, M., R. Buccione, S.A. Courtneidge, and S. Linder. 2008. Assembly and biological role of podosomes and invadopodia. *Curr Opin Cell Biol*. 20:235-241.
Giuliano, K.A., and D.L. Taylor. 1990. Formation, transport, contraction, and disassembly of stress fibers in fibroblasts. *Cell Motil Cytoskeleton*. 16:14-21.
Gloushankova, N.A., S.N. Rubtsova, and I.Y. Zhitnyak. 2017. Cadherin-mediated cell-cell interactions in normal and cancer cells. *Tissue barriers*. 5:e1356900.
Gluck, U., and A. Ben-Ze'ev. 1994. Modulation of alpha-actinin levels affects cell motility and confers tumorigenicity on 3T3 cells. *J Cell Sci*. 107 (Pt 7):1773-1782.
Gluck, U., D.J. Kwiatkowski, and A. Ben-Ze'ev. 1993. Suppression of tumorigenicity in simian virus 40-transformed 3T3 cells transfected with alpha-actinin cDNA. *Proc Natl Acad Sci U S A*. 90:383-387.
Goley, E.D., and M.D. Welch. 2006. The ARP2/3 complex: an actin nucleator comes of age. *Nat Rev Mol Cell Biol*. 7:713-726.
Goode, B.L., and M.J. Eck. 2007. Mechanism and function of formins in the control of actin assembly. *Annual review of biochemistry*. 76:593-627.
Gordon, W.E., 3rd. 1978. Immunofluorescent and ultrastructural studies of "sarcomeric" units in stress fibers of cultured non-muscle cells. *Exp Cell Res*. 117:253-260.
Gottardi, C.J., E. Wong, and B.M. Gumbiner. 2001. E-cadherin suppresses cellular transformation by inhibiting beta-catenin signaling in an adhesion-independent manner. *J Cell Biol*. 153:1049-1060.
Green, K.J., and J.C. Jones. 1996. Desmosomes and hemidesmosomes: structure and function of molecular components. *FASEB J*. 10:871-881.
Guan, N., J. Ding, J. Zhang, and J. Yang. 2003. Expression of nephrin, podocin, alpha-actinin, and WT1 in children with nephrotic syndrome. *Pediatr Nephrol*. 18:1122-1127.
Gudjonsson, T., M.C. Adriance, M.D. Sternlicht, O.W. Petersen, and M.J. Bissell. 2005. Myoepithelial cells: their origin and function in breast morphogenesis and neoplasia. *Journal of mammary gland biology and neoplasia*. 10:261-272.
Gueguen, P., K. Rouault, J.M. Chen, O. Raguenes, Y. Fichou, E. Hardy, E. Gobin, B. Pan-Petesch, M. Kerbiriou, P. Trouve, P. Marcorelles, J.F. Abgrall, C. Le Marechal, and C. Ferec. 2013. A missense mutation in the alpha-actinin 1 gene (ACTN1) is the cause of autosomal dominant macrothrombocytopenia in a large French family. *PLoS One*. 8:e74728.
Gul, I.S., P. Hulpiau, Y. Saeys, and F. van Roy. 2017. Evolution and diversity of cadherins and catenins. *Exp Cell Res*. 358:3-9.
Gumbiner, B.M. 1996. Cell adhesion: the molecular basis of tissue architecture and morphogenesis. *Cell*. 84:345-357.
Gumbiner, B.M. 2005. Regulation of cadherin-mediated adhesion in morphogenesis. *Nat Rev Mol Cell Biol*. 6:622-634.
Guo, F., M. Debidda, L. Yang, D.A. Williams, and Y. Zheng. 2006. Genetic deletion of Rac1 GTPase reveals its critical role in actin stress fiber formation and focal adhesion complex assembly. *J Biol Chem*. 281:18652-18659.
Guo, Z., L.J. Neilson, H. Zhong, P.S. Murray, S. Zanivan, and R. Zaidel-Bar. 2014. E-cadherin interactome complexity and robustness resolved by quantitative proteomics. *Science signaling*. 7:rs7.

Gyorffy, B., A. Lanczky, A.C. Eklund, C. Denkert, J. Budczies, Q. Li, and Z. Szallasi. 2010. An online survival analysis tool to rapidly assess the effect of 22,277 genes on breast cancer prognosis using microarray data of 1,809 patients. *Breast cancer research and treatment*. 123:725-731.

Haeger, A., M. Krause, K. Wolf, and P. Friedl. 2014. Cell jamming: collective invasion of mesenchymal tumor cells imposed by tissue confinement. *Biochim Biophys Acta*. 1840:2386-2395.

Haga, R.B., and A.J. Ridley. 2016. Rho GTPases: Regulation and roles in cancer cell biology. *Small GTPases*. 7:207-221.

Halbleib, J.M., and W.J. Nelson. 2006. Cadherins in development: cell adhesion, sorting, and tissue morphogenesis. *Genes Dev*. 20:3199-3214.

Hall, A. 2009. The cytoskeleton and cancer. *Cancer metastasis reviews*. 28:5-14.

Hall, A., and C.D. Nobes. 2000. Rho GTPases: molecular switches that control the organization and dynamics of the actin cytoskeleton. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*. 355:965-970.

Hamill, K.J., S. Hiroyasu, Z.T. Colburn, R.V. Ventrella, S.B. Hopkinson, O. Skalli, and J.C.R. Jones. 2015. Alpha actinin-1 regulates cell-matrix adhesion organization in keratinocytes: consequences for skin cell motility. *J Invest Dermatol*. 135:1043-1052.

Hamill, K.J., S.B. Hopkinson, O. Skalli, and J.C. Jones. 2013. Actinin-4 in keratinocytes regulates motility via an effect on lamellipodia stability and matrix adhesions. *FASEB J*. 27:546-556.

Han, M.K., and J. de Rooij. 2016. Converging and Unique Mechanisms of Mechanotransduction at Adhesion Sites. *Trends in cell biology*. 26:612-623.

Hara, T., K. Honda, M. Shitashige, M. Ono, H. Matsuyama, K. Naito, S. Hirohashi, and T. Yamada. 2007. Mass spectrometry analysis of the native protein complex containing actinin-4 in prostate cancer cells. *Mol Cell Proteomics*. 6:479-491.

Harris, T.J., and U. Tepass. 2010. Adherens junctions: from molecules to morphogenesis. *Nat Rev Mol Cell Biol*. 11:502-514.

Harrison, O.J., X. Jin, S. Hong, F. Bahna, G. Ahlsen, J. Brasch, Y. Wu, J. Vendome, K. Felsovalyi, C.M. Hampton, R.B. Troyanovsky, A. Ben-Shaul, J. Frank, S.M. Troyanovsky, L. Shapiro, and B. Honig. 2011. The extracellular architecture of adherens junctions revealed by crystal structures of type I cadherins. *Structure*. 19:244-256.

Hartsock, A., and W.J. Nelson. 2008. Adherens and tight junctions: structure, function and connections to the actin cytoskeleton. *Biochim Biophys Acta*. 1778:660-669.

Hayashida, Y., K. Honda, M. Idogawa, Y. Ino, M. Ono, A. Tsuchida, T. Aoki, S. Hirohashi, and T. Yamada. 2005. E-cadherin regulates the association between beta-catenin and actinin-4. *Cancer research*. 65:8836-8845.

Hazan, R.B., L. Kang, S. Roe, P.I. Borgen, and D.L. Rimm. 1997. Vinculin is associated with the E-cadherin adhesion complex. *J Biol Chem*. 272:32448-32453.

Heasman, S.J., and A.J. Ridley. 2008. Mammalian Rho GTPases: new insights into their functions from in vivo studies. *Nat Rev Mol Cell Biol*. 9:690-701.

Heath, J.P. 1983. Behaviour and structure of the leading lamella in moving fibroblasts. I. Occurrence and centripetal movement of arc-shaped microfilament bundles beneath the dorsal cell surface. *J Cell Sci*. 60:331-354.

Heath, J.P., and G.A. Dunn. 1978. Cell to substratum contacts of chick fibroblasts and their relation to the microfilament system. A correlated interference-reflexion and high-voltage electron-microscope study. *J Cell Sci*. 29:197-212.

Heath, J.P., and B.F. Holifield. 1991. Cell locomotion: new research tests old ideas on membrane and cytoskeletal flow. *Cell Motil Cytoskeleton*. 18:245-257.

Hemminki, A., D. Markie, I. Tomlinson, E. Avizienyte, S. Roth, A. Loukola, G. Bignell, W. Warren, M. Aminoff, P. Hoglund, H. Jarvinen, P. Kristo, K. Pelin, M. Ridanpaa, R. Salovaara, T. Toro, W. Bodmer, S. Olschwang, A.S. Olsen, M.R. Stratton, A. de la Chapelle, and L.A. Aaltonen. 1998. A serine/threonine kinase gene defective in Peutz-Jeghers syndrome. *Nature*. 391:184-187.

Herman, I.M., N.J. Crisona, and T.D. Pollard. 1981. Relation between cell activity and the distribution of cytoplasmic actin and myosin. *J Cell Biol*. 90:84-91.

Hirokawa, N., and J.E. Heuser. 1981. Quick-freeze, deep-etch visualization of the cytoskeleton beneath surface differentiations of intestinal epithelial cells. *J Cell Biol*. 91:399-409.

Holmes, K.C., D. Popp, W. Gebhard, and W. Kabsch. 1990. Atomic model of the actin filament. *Nature*. 347:44-49.

Honda, K. 2015. The biological role of actinin-4 (ACTN4) in malignant phenotypes of cancer. *Cell & bioscience*. 5:41.

Honda, K., T. Yamada, R. Endo, Y. Ino, M. Gotoh, H. Tsuda, Y. Yamada, H. Chiba, and S. Hirohashi. 1998. Actinin-4, a novel actin-bundling protein associated with cell motility and cancer invasion. *J Cell Biol*. 140:1383-1393.

Honda, K., T. Yamada, Y. Hayashida, M. Idogawa, S. Sato, F. Hasegawa, Y. Ino, M. Ono, and S. Hirohashi. 2005. Actinin-4 increases cell motility and promotes lymph node metastasis of colorectal cancer. *Gastroenterology*. 128:51-62.

Honda, K., T. Yamada, M. Seike, Y. Hayashida, M. Idogawa, T. Kondo, Y. Ino, and S. Hirohashi. 2004. Alternative splice variant of actinin-4 in small cell lung cancer. *Oncogene*. 23:5257-5262.

Hong, S., R.B. Troyanovsky, and S.M. Troyanovsky. 2013. Binding to F-actin guides cadherin cluster assembly, stability, and movement. *J Cell Biol*. 201:131-143.

Horwitz, R., and D. Webb. 2003. Cell migration. *Curr Biol*. 13:R756-759.

Hotulainen, P., and C.C. Hoogenraad. 2010. Actin in dendritic spines: connecting dynamics to function. *J Cell Biol*. 189:619-629.

Hotulainen, P., and P. Lappalainen. 2006. Stress fibers are generated by two distinct actin assembly mechanisms in motile cells. *J Cell Biol*. 173:383-394.

Hu, K., L. Ji, K.T. Applegate, G. Danuser, and C.M. Waterman-Storer. 2007. Differential transmission of actin motion within focal adhesions. *Science*. 315:111-115.

Huber, A.H., D.B. Stewart, D.V. Laurents, W.J. Nelson, and W.I. Weis. 2001. The cadherin cytoplasmic domain is unstructured in the absence of beta-catenin. A possible mechanism for regulating cadherin turnover. *J Biol Chem*. 276:12301-12309.

Hulpiau, P., and F. van Roy. 2009. Molecular evolution of the cadherin superfamily. *Int J Biochem Cell Biol*. 41:349-369.

Huveneers, S., J. Oldenburg, E. Spanjaard, G. van der Krogt, I. Grigoriev, A. Akhmanova, H. Rehmann, and J. de Rooij. 2012. Vinculin associates with endothelial VE-cadherin junctions to control force-dependent remodeling. *J Cell Biol*. 196:641-652.

Hynes, R.O. 1987. Integrins: a family of cell surface receptors. *Cell*. 48:549-554.

Hynes, R.O. 1992. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell*. 69:11-25.

Hynes, R.O. 2002. Integrins: bidirectional, allosteric signaling machines. *Cell*. 110:673-687.

Ikenouchi, J., M. Furuse, K. Furuse, H. Sasaki, S. Tsukita, and S. Tsukita. 2005. Tricellulin constitutes a novel barrier at tricellular contacts of epithelial cells. *J Cell Biol*. 171:939-945.

Izaguirre, G., L. Aguirre, P. Ji, B. Aneskievich, and B. Haimovich. 1999. Tyrosine phosphorylation of alpha-actinin in activated platelets. *The Journal of biological chemistry*. 274:37012-37020.

Izzard, C.S., and L.R. Lochner. 1980. Formation of cell-to-substrate contacts during fibroblast motility: an interference-reflexion study. *J Cell Sci*. 42:81-116.

Jacinto, A., W. Wood, S. Woolner, C. Hiley, L. Turner, C. Wilson, A. Martinez-Arias, and P. Martin. 2002. Dynamic analysis of actin cable function during Drosophila dorsal closure. *Curr Biol*. 12:1245-1250.

Jaffe, A.B., and A. Hall. 2005. Rho GTPases: biochemistry and biology. *Annu Rev Cell Dev Biol*. 21:247-269.

Jana, S.S., S. Kawamoto, and R.S. Adelstein. 2006. A specific isoform of nonmuscle myosin II-C is required for cytokinesis in a tumor cell line. *J Biol Chem*. 281:24662-24670.

Jansen, K.A., P. Atherton, and C. Ballestrem. 2017. Mechanotransduction at the cell-matrix interface. *Seminars in cell & developmental biology*.

Jolly, M.K., S.C. Tripathi, D. Jia, S.M. Mooney, M. Celiktas, S.M. Hanash, S.A. Mani, K.J. Pienta, E. Ben-Jacob, and H. Levine. 2016. Stability of the hybrid epithelial/mesenchymal phenotype. *Oncotarget*. 7:27067-27084.

Kabsch, W., H.G. Mannherz, D. Suck, E.F. Pai, and K.C. Holmes. 1990. Atomic structure of the actin:DNase I complex. *Nature*. 347:37-44.

Kakuya, T., T. Mori, S. Yoshimoto, Y. Watabe, N. Miura, H. Shoji, K. Onidani, T. Shibahara, and K. Honda. 2017. Prognostic significance of gene amplification of ACTN4 in stage I and II oral tongue cancer. *International journal of oral and maxillofacial surgery*. 46:968-976.

Kanchanawong, P., G. Shtengel, A.M. Pasapera, E.B. Ramko, M.W. Davidson, H.F. Hess, and C.M. Waterman. 2010. Nanoscale architecture of integrin-based cell adhesions. *Nature*. 468:580-584.

Kaplan, J.M., S.H. Kim, K.N. North, H. Rennke, L.A. Correia, H.Q. Tong, B.J. Mathis, J.C. Rodriguez-Perez, P.G. Allen, A.H. Beggs, and M.R. Pollak. 2000. Mutations in ACTN4, encoding alpha-actinin-4, cause familial focal segmental glomerulosclerosis. *Nat Genet*. 24:251-256.

Kassianidou, E., and S. Kumar. 2015. A biomechanical perspective on stress fiber structure and function. *Biochim Biophys Acta*. 1853:3065-3074.

Katoh, K., Y. Kano, M. Amano, H. Onishi, K. Kaibuchi, and K. Fujiwara. 2001. Rho-kinase--mediated contraction of isolated stress fibers. *J Cell Biol*. 153:569-584.

Katz, A.M., D. Rosenthal, and D.N. Sauder. 1991. Cell adhesion molecules. Structure, function, and implication in a variety of cutaneous and other pathologic conditions. *International journal of dermatology*. 30:153-160.

Katz, B.Z., E. Zamir, A. Bershadsky, Z. Kam, K.M. Yamada, and B. Geiger. 2000. Physical state of the extracellular matrix regulates the structure and molecular composition of cell-matrix adhesions. *Mol Biol Cell*. 11:1047-1060.

Kawauchi, T. 2012. Cell adhesion and its endocytic regulation in cell migration during neural development and cancer metastasis. *International journal of molecular sciences*. 13:4564-4590.

Khatau, S.B., C.M. Hale, P.J. Stewart-Hutchinson, M.S. Patel, C.L. Stewart, P.C. Searson, D. Hodzic, and D. Wirtz. 2009. A perinuclear actin cap regulates nuclear shape. *Proc Natl Acad Sci U S A*. 106:19017-19022.

Khotin, M., L. Turoverova, V. Aksenova, N. Barlev, V.V. Borutinskaite, A. Vener, O. Bajenova, K.E. Magnusson, G.P. Pinaev, and D. Tentler. 2010. Proteomic analysis of ACTN4-interacting proteins reveals it's a putative involvement in mRNA metabolism. *Biochem Biophys Res Commun*. 397:192-196.

Khurana, S., S. Chakraborty, X. Cheng, Y.T. Su, and H.Y. Kao. 2011. The actin-binding protein, actinin alpha 4 (ACTN4), is a nuclear receptor coactivator that promotes proliferation of MCF-7 breast cancer cells. *J Biol Chem*. 286:1850-1859.

Khurana, S., S. Chakraborty, M. Lam, Y. Liu, Y.T. Su, X. Zhao, M.A. Saleem, P.W. Mathieson, L.A. Bruggeman, and H.Y. Kao. 2012a. Familial focal segmental glomerulosclerosis (FSGS)-linked alpha-actinin 4 (ACTN4) protein mutants lose ability to activate transcription by nuclear hormone receptors. *J Biol Chem*. 287:12027-12035.

Khurana, S., S. Chakraborty, X. Zhao, Y. Liu, D. Guan, M. Lam, W. Huang, S. Yang, and H.Y. Kao. 2012b. Identification of a novel LXXLL motif in alpha-actinin 4-spliced isoform that is critical for its interaction with estrogen receptor alpha and co-activators. *J Biol Chem*. 287:35418-35429.

Kikuchi, S., K. Honda, H. Tsuda, N. Hiraoka, I. Imoto, T. Kosuge, T. Umaki, K. Onozato, M. Shitashige, U. Yamaguchi, M. Ono, A. Tsuchida, T. Aoki, J. Inazawa, S. Hirohashi, and T. Yamada. 2008. Expression and gene amplification of actinin-4 in invasive ductal carcinoma of the pancreas. *Clinical cancer research : an official journal of the American Association for Cancer Research*. 14:5348-5356.

Kilpinen, S., R. Autio, K. Ojala, K. Iljin, E. Bucher, H. Sara, T. Pisto, M. Saarela, R.I. Skotheim, M. Bjorkman, J.P. Mpindi, S. Haapa-Paananen, P. Vainio, H. Edgren, M. Wolf, J. Astola, M. Nees, S. Hautaniemi, and O. Kallioniemi. 2008. Systematic

bioinformatic analysis of expression levels of 17,330 human genes across 9,783 samples from 175 types of healthy and pathological tissues. *Genome biology*. 9:R139.

Kim, D.H., S.B. Khatau, Y. Feng, S. Walcott, S.X. Sun, G.D. Longmore, and D. Wirtz. 2012. Actin cap associated focal adhesions and their distinct role in cellular mechanosensing. *Scientific reports*. 2:555.

Kimura, K., M. Ito, M. Amano, K. Chihara, Y. Fukata, M. Nakafuku, B. Yamamori, J. Feng, T. Nakano, K. Okawa, A. Iwamatsu, and K. Kaibuchi. 1996. Regulation of myosin phosphatase by Rho and Rho-associated kinase (Rho-kinase). *Science*. 273:245-248.

Kimura, M., M. Toyoda, M. Kato, K. Kobayashi, M. Abe, T. Kobayashi, M. Miyauchi, N. Yamamoto, T. Umezono, and D. Suzuki. 2008. Expression of alpha-actinin-4 in human diabetic nephropathy. *Intern Med*. 47:1099-1106.

Kirfel, G., A. Rigort, B. Borm, and V. Herzog. 2004. Cell migration: mechanisms of rear detachment and the formation of migration tracks. *Eur J Cell Biol*. 83:717-724.

Knabbe, C., M.E. Lippman, L.M. Wakefield, K.C. Flanders, A. Kasid, R. Derynck, and R.B. Dickson. 1987. Evidence that transforming growth factor-beta is a hormonally regulated negative growth factor in human breast cancer cells. *Cell*. 48:417-428.

Knudsen, K.A., A.P. Soler, K.R. Johnson, and M.J. Wheelock. 1995. Interaction of alpha-actinin with the cadherin/catenin cell-cell adhesion complex via alpha-catenin. *J Cell Biol*. 130:67-77.

Kobielak, A., H.A. Pasolli, and E. Fuchs. 2004. Mammalian formin-1 participates in adherens junctions and polymerization of linear actin cables. *Nat Cell Biol*. 6:21-30.

Koestler, S.A., S. Auinger, M. Vinzenz, K. Rottner, and J.V. Small. 2008. Differentially oriented populations of actin filaments generated in lamellipodia collaborate in pushing and pausing at the cell front. *Nat Cell Biol*. 10:306-313.

Koga, Y., and M. Ikebe. 2005. p116Rip decreases myosin II phosphorylation by activating myosin light chain phosphatase and by inactivating RhoA. *J Biol Chem*. 280:4983-4991.

Koga, Y., and M. Ikebe. 2008. A novel regulatory mechanism of myosin light chain phosphorylation via binding of 14-3-3 to myosin phosphatase. *Mol Biol Cell*. 19:1062-1071.

Kos, C.H., T.C. Le, S. Sinha, J.M. Henderson, S.H. Kim, H. Sugimoto, R. Kalluri, R.E. Gerszten, and M.R. Pollak. 2003. Mice deficient in alpha-actinin-4 have severe glomerular disease. *J Clin Invest*. 111:1683-1690.

Kovac, B., J.L. Teo, T.P. Makela, and T. Vallenius. 2013. Assembly of non-contractile dorsal stress fibers requires alpha-actinin-1 and Rac1 in migrating and spreading cells. *J Cell Sci*. 126:263-273.

Kozma, R., S. Sarner, S. Ahmed, and L. Lim. 1997. Rho family GTPases and neuronal growth cone remodelling: relationship between increased complexity induced by Cdc42Hs, Rac1, and acetylcholine and collapse induced by RhoA and lysophosphatidic acid. *Mol Cell Biol*. 17:1201-1211.

Krause, M., E.W. Dent, J.E. Bear, J.J. Loureiro, and F.B. Gertler. 2003. Ena/VASP proteins: regulators of the actin cytoskeleton and cell migration. *Annu Rev Cell Dev Biol*. 19:541-564.

Kreis, T.E., and W. Birchmeier. 1980. Stress fiber sarcomeres of fibroblasts are contractile. *Cell*. 22:555-561.

Kubow, K.E., and A.R. Horwitz. 2011. Reducing background fluorescence reveals adhesions in 3D matrices. *Nat Cell Biol*. 13:3-5; author reply 5-7.

Kuga, W., K. Tsuchihara, T. Ogura, S. Kanehara, M. Saito, A. Suzuki, and H. Esumi. 2008. Nuclear localization of SNARK; its impact on gene expression. *Biochem Biophys Res Commun*. 377:1062-1066.

Kumar, S., and V.M. Weaver. 2009. Mechanics, malignancy, and metastasis: the force journey of a tumor cell. *Cancer metastasis reviews*. 28:113-127.

Kumeta, M., S.H. Yoshimura, M. Harata, and K. Takeyasu. 2010. Molecular mechanisms underlying nucleocytoplasmic shuttling of actinin-4. *J Cell Sci*.

Kunishima, S., Y. Okuno, K. Yoshida, Y. Shiraishi, M. Sanada, H. Muramatsu, K. Chiba, H. Tanaka, K. Miyazaki, M. Sakai, M. Ohtake, R. Kobayashi, A. Iguchi, G. Niimi, M. Otsu, Y. Takahashi, S. Miyano, H. Saito, S. Kojima, and S. Ogawa. 2013. ACTN1 mutations cause congenital macrothrombocytopenia. *American journal of human genetics*. 92:431-438.

Kuo, J.C., X. Han, C.T. Hsiao, J.R. Yates, 3rd, and C.M. Waterman. 2011. Analysis of the myosin-II-responsive focal adhesion proteome reveals a role for beta-Pix in negative regulation of focal adhesion maturation. *Nat Cell Biol*. 13:383-393.

Kwon, O., C.L. Phillips, and B.A. Molitoris. 2002. Ischemia induces alterations in actin filaments in renal vascular smooth muscle cells. *American journal of physiology. Renal physiology*. 282:F1012-1019.

Ladwein, M., and K. Rottner. 2008. On the Rho'd: the regulation of membrane protrusions by Rho-GTPases. *FEBS letters*. 582:2066-2074.

Lambert, A.W., D.R. Pattabiraman, and R.A. Weinberg. 2017. Emerging Biological Principles of Metastasis. *Cell*. 168:670-691.

Lammermann, T., and M. Sixt. 2009. Mechanical modes of 'amoeboid' cell migration. *Curr Opin Cell Biol*. 21:636-644.

Lammers, M., S. Meyer, D. Kuhlmann, and A. Wittinghofer. 2008. Specificity of interactions between mDia isoforms and Rho proteins. *J Biol Chem*. 283:35236-35246.

Lauffenburger, D.A., and A.F. Horwitz. 1996. Cell migration: a physically integrated molecular process. *Cell*. 84:359-369.

Lazarides, E. 1975a. Tropomyosin antibody: the specific localization of tropomyosin in nonmuscle cells. *J Cell Biol*. 65:549-561.

Lazarides, E., and K. Weber. 1974. Actin antibody: the specific visualization of actin filaments in non-muscle cells. *Proc Natl Acad Sci U S A*. 71:2268-2272.

Lazarides, E.a.B., K. 1975b. Alpha-actinin: immunofluorescent localization of a muscle structural protein in nonmuscle cell. *Cell*. 6:289-298.

Le Clainche, C., and M.F. Carlier. 2008. Regulation of actin assembly associated with protrusion and adhesion in cell migration. *Physiol Rev*. 88:489-513.

le Duc, Q., Q. Shi, I. Blonk, A. Sonnenberg, N. Wang, D. Leckband, and J. de Rooij. 2010. Vinculin potentiates E-cadherin mechanosensing and is recruited to actin-anchored sites within adherens junctions in a myosin II-dependent manner. *J Cell Biol*. 189:1107-1115.

Lecharpentier, A., P. Vielh, P. Perez-Moreno, D. Planchard, J.C. Soria, and F. Farace. 2011. Detection of circulating tumour cells with a hybrid (epithelial/mesenchymal) phenotype in patients with metastatic non-small cell lung cancer. *British journal of cancer*. 105:1338-1341.

Leckband, D.E., and J. de Rooij. 2014. Cadherin adhesion and mechanotransduction. *Annu Rev Cell Dev Biol*. 30:291-315.

Lecuit, T. 2005. Adhesion remodeling underlying tissue morphogenesis. *Trends in cell biology*. 15:34-42.

Lecuit, T., P.F. Lenne, and E. Munro. 2011. Force generation, transmission, and integration during cell and tissue morphogenesis. *Annu Rev Cell Dev Biol*. 27:157-184.

Lecuit, T., and A.S. Yap. 2015. E-cadherin junctions as active mechanical integrators in tissue dynamics. *Nat Cell Biol*. 17:533-539.

Lee, J.H., H. Koh, M. Kim, Y. Kim, S.Y. Lee, R.E. Karess, S.H. Lee, M. Shong, J.M. Kim, J. Kim, and J. Chung. 2007. Energy-dependent regulation of cell structure by AMP-activated protein kinase. *Nature*. 447:1017-1020.

Lee, S., and S. Kumar. 2016. Actomyosin stress fiber mechanosensing in 2D and 3D. *F1000Research*. 5.

Lee, S.H., and R. Dominguez. 2010. Regulation of actin cytoskeleton dynamics in cells. *Molecules and cells*. 29:311-325.

Leerberg, J.M., G.A. Gomez, S. Verma, E.J. Moussa, S.K. Wu, R. Priya, B.D. Hoffman, C. Grashoff, M.A. Schwartz, and A.S. Yap. 2014. Tension-sensitive actin assembly supports contractility at the epithelial zonula adherens. *Curr Biol*. 24:1689-1699.

Lefebvre, D.L., Y. Bai, N. Shahmolky, M. Sharma, R. Poon, D.J. Drucker, and C.F. Rosen. 2001. Identification and characterization of a novel sucrose-non-fermenting protein kinase/AMP-activated protein kinase-related protein kinase, SNARK. *Biochem J*. 355:297-305.

Lefebvre, D.L., and C.F. Rosen. 2005. Regulation of SNARK activity in response to cellular stresses. *Biochim Biophys Acta*. 1724:71-85.

Legembre, P., R. Schickel, B.C. Barnhart, and M.E. Peter. 2004. Identification of SNF1/AMP kinase-related kinase as an NF-kappaB-regulated anti-apoptotic kinase involved in CD95-induced motility and invasiveness. *J Biol Chem*. 279:46742-46747.

Letort, G., H. Ennomani, L. Gressin, M. Thery, and L. Blanchoin. 2015. Dynamic reorganization of the actin cytoskeleton. *F1000Research*. 4.

Levy, L., and C.S. Hill. 2005. Smad4 dependency defines two classes of transforming growth factor {beta} (TGF-{beta}) target genes and distinguishes TGF-{beta}-induced epithelial-mesenchymal transition from its antiproliferative and migratory responses. *Mol Cell Biol*. 25:8108-8125.

Lewis, W.H.a.L.M.R. 1924. Behavior of cells in tissue cultures. *General Cytology (E.V. Cowdry, editor), Chicago, Illinois: The University of Chicago Press*:385-447.

Liu, Z., Y. Zhan, Y. Tu, K. Chen, Z. Liu, and C. Wu. 2015. PDZ and LIM domain protein 1(PDLIM1)/CLP36 promotes breast cancer cell migration, invasion and metastasis through interaction with alpha-actinin. *Oncogene*. 34:1300-1311.

Lizcano, J.M., O. Goransson, R. Toth, M. Deak, N.A. Morrice, J. Boudeau, S.A. Hawley, L. Udd, T.P. Makela, D.G. Hardie, and D.R. Alessi. 2004. LKB1 is a master kinase that activates 13 kinases of the AMPK subfamily, including MARK/PAR-1. *EMBO J*. 23:833-843.

Lo, C.M., D.B. Buxton, G.C. Chua, M. Dembo, R.S. Adelstein, and Y.L. Wang. 2004. Nonmuscle myosin IIb is involved in the guidance of fibroblast migration. *Mol Biol Cell*. 15:982-989.

Lu, L., Y. Feng, W.J. Hucker, S.J. Oswald, G.D. Longmore, and F.C. Yin. 2008a. Actin stress fiber pre-extension in human aortic endothelial cells. *Cell Motil Cytoskeleton*. 65:281-294.

Lu, L., S.J. Oswald, H. Ngu, and F.C. Yin. 2008b. Mechanical properties of actin stress fibers in living cells. *Biophys J*. 95:6060-6071.

Machacek, M., L. Hodgson, C. Welch, H. Elliott, O. Pertz, P. Nalbant, A. Abell, G.L. Johnson, K.M. Hahn, and G. Danuser. 2009. Coordination of Rho GTPase activities during cell protrusion. *Nature*. 461:99-103.

Makrilia, N., A. Kollias, L. Manolopoulos, and K. Syrigos. 2009. Cell adhesion molecules: role and clinical significance in cancer. *Cancer investigation*. 27:1023-1037.

Maninova, M., J. Caslavsky, and T. Vomastek. 2017. The assembly and function of perinuclear actin cap in migrating cells. *Protoplasma*. 254:1207-1218.

Maninova, M., and T. Vomastek. 2016. Dorsal stress fibers, transverse actin arcs, and perinuclear actin fibers form an interconnected network that induces nuclear movement in polarizing fibroblasts. *FEBS J*. 283:3676-3693.

Martin, P., and S.M. Parkhurst. 2004. Parallels between tissue repair and embryo morphogenesis. *Development*. 131:3021-3034.

Martinez-Rico, C., F. Pincet, J.P. Thiery, and S. Dufour. 2010. Integrins stimulate E-cadherin-mediated intercellular adhesion by regulating Src-kinase activation and actomyosin contractility. *J Cell Sci*. 123:712-722.

Maruthamuthu, V., Y. Aratyn-Schaus, and M.L. Gardel. 2010. Conserved F-actin dynamics and force transmission at cell adhesions. *Curr Opin Cell Biol*. 22:583-588.

Massague, J. 2008. TGFbeta in Cancer. *Cell*. 134:215-230.

Matsuda, T., T. Yamamoto, A. Muraguchi, and F. Saatcioglu. 2001. Cross-talk between transforming growth factor-beta and estrogen receptor signaling through Smad3. *J Biol Chem*. 276:42908-42914.

Mattila, P.K., and P. Lappalainen. 2008. Filopodia: molecular architecture and cellular functions. *Nat Rev Mol Cell Biol*. 9:446-454.

McGough, A., M. Way, and D. DeRosier. 1994. Determination of the alpha-actinin-binding site on actin filaments by cryoelectron microscopy and image analysis. *J Cell Biol*. 126:433-443.

McNutt, N.S., L.A. Culp, and P.H. Black. 1971. Contact-inhibited revertant cell lines isolated from SV40-transformed cells. II. Ultrastructural study. *J Cell Biol*. 50:691-708.

Mege, R.M., J. Gavard, and M. Lambert. 2006. Regulation of cell-cell junctions by the cytoskeleton. *Curr Opin Cell Biol*. 18:541-548.

Mege, R.M., and N. Ishiyama. 2017. Integration of Cadherin Adhesion and Cytoskeleton at Adherens Junctions. *Cold Spring Harb Perspect Biol*. 9.

Meng, W., and M. Takeichi. 2009. Adherens junction: molecular architecture and regulation. *Cold Spring Harb Perspect Biol*. 1:a002899.

Michaud, J.L., L.I. Lemieux, M. Dube, B.C. Vanderhyden, S.J. Robertson, and C.R. Kennedy. 2003. Focal and segmental glomerulosclerosis in mice with podocyte-specific expression of mutant alpha-actinin-4. *Journal of the American Society of Nephrology : JASN*. 14:1200-1211.

Mihaly, Z., and B. Gyorffy. 2013. Improving Pathological Assessment of Breast Cancer by Employing Array-Based Transcriptome Analysis. *Microarrays*. 2:228-242.

Millan, J., R.J. Cain, N. Reglero-Real, C. Bigarella, B. Marcos-Ramiro, L. Fernandez-Martin, I. Correas, and A.J. Ridley. 2010. Adherens junctions connect stress fibres between adjacent endothelial cells. *BMC biology*. 8:11.

Mimura, N., and A. Asano. 1978. Actin-related gelation of Ehrlich tumour cell extracts is reversibly inhibited by low concentrations of Ca2+. *Nature*. 272:273-276.

Miyaguchi, K. 2000. Ultrastructure of the zonula adherens revealed by rapid-freeze deep-etching. *J Struct Biol*. 132:169-178.

Miyake, Y., N. Inoue, K. Nishimura, N. Kinoshita, H. Hosoya, and S. Yonemura. 2006. Actomyosin tension is required for correct recruitment of adherens junction components and zonula occludens formation. *Exp Cell Res*. 312:1637-1650.

Miyanaga, A., K. Honda, K. Tsuta, M. Masuda, U. Yamaguchi, G. Fujii, A. Miyamoto, S. Shinagawa, N. Miura, H. Tsuda, T. Sakuma, H. Asamura, A. Gemma, and T. Yamada. 2013. Diagnostic and prognostic significance of the alternatively spliced ACTN4 variant in high-grade neuroendocrine pulmonary tumours. *Annals of oncology : official journal of the European Society for Medical Oncology*. 24:84-90.

Miyoshi, J., and Y. Takai. 2008. Structural and functional associations of apical junctions with cytoskeleton. *Biochim Biophys Acta*. 1778:670-691.

Mochitate, K., P. Pawelek, and F. Grinnell. 1991. Stress relaxation of contracted collagen gels: disruption of actin filament bundles, release of cell surface fibronectin, and down-regulation of DNA and protein synthesis. *Exp Cell Res*. 193:198-207.

Mounier, N., J.C. Perriard, G. Gabbiani, and C. Chaponnier. 1997. Transfected muscle and non-muscle actins are differentially sorted by cultured smooth muscle and non-muscle cells. *J Cell Sci*. 110 (Pt 7):839-846.

Mulder, J., A. Ariaens, D. van den Boomen, and W.H. Moolenaar. 2004. p116Rip targets myosin phosphatase to the actin cytoskeleton and is essential for RhoA/ROCK-regulated neuritogenesis. *Mol Biol Cell*. 15:5516-5527.

Mulder, J., M. Poland, M.F. Gebbink, J. Calafat, W.H. Moolenaar, and O. Kranenburg. 2003. p116Rip is a novel filamentous actin-binding protein. *J Biol Chem*. 278:27216-27223.

Murphy, A.C., A.J. Lindsay, M.W. McCaffrey, K. Djinovic-Carugo, and P.W. Young. 2016. Congenital macrothrombocytopenia-linked mutations in the actin-binding domain of alpha-actinin-1 enhance F-actin association. *FEBS letters*. 590:685-695.

Namiki, T., A. Tanemura, J.C. Valencia, S.G. Coelho, T. Passeron, M. Kawaguchi, W.D. Vieira, M. Ishikawa, W. Nishijima, T. Izumo, Y. Kaneko, I. Katayama, Y. Yamaguchi, L. Yin, E.C. Polley, H. Liu, Y. Kawakami, Y. Eishi, E. Takahashi, H. Yokozeki, and V.J. Hearing. 2011. AMP kinase-related kinase NUAK2 affects tumor growth, migration, and clinical outcome of human melanoma. *Proc Natl Acad Sci U S A*. 108:6597-6602.

Naumanen, P., P. Lappalainen, and P. Hotulainen. 2008. Mechanisms of actin stress fibre assembly. *Journal of microscopy*. 231:446-454.

Nelson, W.J. 2008. Regulation of cell-cell adhesion by the cadherin-catenin complex. *Biochemical Society transactions*. 36:149-155.

Neve, R.M., K. Chin, J. Fridlyand, J. Yeh, F.L. Baehner, T. Fevr, L. Clark, N. Bayani, J.P. Coppe, F. Tong, T. Speed, P.T. Spellman, S. DeVries, A. Lapuk, N.J. Wang, W.L. Kuo, J.L. Stilwell, D. Pinkel, D.G. Albertson, F.M. Waldman, F. McCormick, R.B. Dickson, M.D. Johnson, M. Lippman, S. Ethier, A. Gazdar, and J.W. Gray. 2006. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes. *Cancer Cell*. 10:515-527.

Ngan, E., J.J. Northey, C.M. Brown, J. Ursini-Siegel, and P.M. Siegel. 2013. A complex containing LPP and alpha-actinin mediates TGFbeta-induced migration and invasion of ErbB2-expressing breast cancer cells. *J Cell Sci*. 126:1981-1991.

Niessen, C.M., and C.J. Gottardi. 2008. Molecular components of the adherens junction. *Biochim Biophys Acta*. 1778:562-571.

Niessen, C.M., D. Leckband, and A.S. Yap. 2011. Tissue organization by cadherin adhesion molecules: dynamic molecular and cellular mechanisms of morphogenetic regulation. *Physiol Rev*. 91:691-731.

Nieto, M.A., R.Y. Huang, R.A. Jackson, and J.P. Thiery. 2016. Emt: 2016. *Cell*. 166:21-45.

Nikolopoulos, S.N., B.A. Spengler, K. Kisselbach, A.E. Evans, J.L. Biedler, and R.A. Ross. 2000. The human non-muscle alpha-actinin protein encoded by the ACTN4 gene suppresses tumorigenicity of human neuroblastoma cells. *Oncogene*. 19:380-386.

Nobes, C.D., and A. Hall. 1995. Rho, rac, and cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with actin stress fibers, lamellipodia, and filopodia. *Cell*. 81:53-62.

Noegel, A., W. Witke, and M. Schleicher. 1987. Calcium-sensitive non-muscle alpha-actinin contains EF-hand structures and highly conserved regions. *FEBS letters*. 221:391-396.

Oakes, P.W., Y. Beckham, J. Stricker, and M.L. Gardel. 2012. Tension is required but not sufficient for focal adhesion maturation without a stress fiber template. *J Cell Biol*. 196:363-374.

Oda, H., and M. Takeichi. 2011. Evolution: structural and functional diversity of cadherin at the adherens junction. *J Cell Biol*. 193:1137-1146.

Oda, Y., T. Otani, J. Ikenouchi, and M. Furuse. 2014. Tricellulin regulates junctional tension of epithelial cells at tricellular contacts through Cdc42. *J Cell Sci*. 127:4201-4212.

Olson, M.F., and E. Sahai. 2009. The actin cytoskeleton in cancer cell motility. *Clinical & experimental metastasis*. 26:273-287.

Ono, S., and K. Ono. 2002. Tropomyosin inhibits ADF/cofilin-dependent actin filament dynamics. *J Cell Biol*. 156:1065-1076.

Otey, C.A., and O. Carpen. 2004. Alpha-actinin revisited: a fresh look at an old player. *Cell Motil Cytoskeleton*. 58:104-111.

Otey, C.A., F.M. Pavalko, and K. Burridge. 1990. An interaction between alpha-actinin and the beta 1 integrin subunit in vitro. *J Cell Biol*. 111:721-729.

Pankov, R., E. Cukierman, B.Z. Katz, K. Matsumoto, D.C. Lin, S. Lin, C. Hahn, and K.M. Yamada. 2000. Integrin dynamics and matrix assembly: tensin-dependent translocation of alpha(5)beta(1) integrins promotes early fibronectin fibrillogenesis. *J Cell Biol*. 148:1075-1090.

Papageorgis, P. 2015. TGFbeta Signaling in Tumor Initiation, Epithelial-to-Mesenchymal Transition, and Metastasis. *Journal of oncology*. 2015:587193.

Parsons, J.T., A.R. Horwitz, and M.A. Schwartz. 2010. Cell adhesion: integrating cytoskeletal dynamics and cellular tension. *Nat Rev Mol Cell Biol*. 11:633-643.

Pasapera, A.M., I.C. Schneider, E. Rericha, D.D. Schlaepfer, and C.M. Waterman. 2010. Myosin II activity regulates vinculin recruitment to focal adhesions through FAK-mediated paxillin phosphorylation. *J Cell Biol*. 188:877-890.

Pellegrin, S.a.M., H. 2007. Actin stress fibers. *J Cell Sci*. 120:3491 - 3499.

Perdue, J.F. 1973. The distribution, ultrastructure, and chemistry of microfilaments in cultured chick embryo fibroblasts. *J Cell Biol*. 58:265-283.

Perez-Moreno, M., and E. Fuchs. 2006. Catenins: keeping cells from getting their signals crossed. *Dev Cell*. 11:601-612.

Perrin, B.J., and J.M. Ervasti. 2010. The actin gene family: function follows isoform. *Cytoskeleton*. 67:630-634.

Pertz, O., L. Hodgson, R.L. Klemke, and K.M. Hahn. 2006. Spatiotemporal dynamics of RhoA activity in migrating cells. *Nature*. 440:1069-1072.

Peterson, L.J., Z. Rajfur, A.S. Maddox, C.D. Freel, Y. Chen, M. Edlund, C. Otey, and K. Burridge. 2004. Simultaneous stretching and contraction of stress fibers in vivo. *Mol Biol Cell*. 15:3497-3508.

Peyrieras, N., F. Hyafil, D. Louvard, H.L. Ploegh, and F. Jacob. 1983. Uvomorulin: a nonintegral membrane protein of early mouse embryo. *Proc Natl Acad Sci U S A*. 80:6274-6277.

Podlubnaya, Z.A., L.A. Tskhovrebova, M.M. Zaalishtsbvili, and G.A. Stefanenko. 1975. Electron microscopic study of alpha-actinin. *J Mol Biol*. 92:357-359.

Pokutta, S., K. Herrenknecht, R. Kemler, and J. Engel. 1994. Conformational changes of the recombinant extracellular domain of E-cadherin upon calcium binding. *European journal of biochemistry*. 223:1019-1026.

Pollard, T.D., and G.G. Borisy. 2003. Cellular motility driven by assembly and disassembly of actin filaments. *Cell*. 112:453-465.

Pollard, T.D., and J.A. Cooper. 2009. Actin, a central player in cell shape and movement. *Science*. 326:1208-1212.

Ponti, A., M. Machacek, S.L. Gupton, C.M. Waterman-Storer, and G. Danuser. 2004. Two distinct actin networks drive the protrusion of migrating cells. *Science*. 305:1782-1786.

Prat, A., B. Adamo, M.C. Cheang, C.K. Anders, L.A. Carey, and C.M. Perou. 2013. Molecular characterization of basal-like and non-basal-like triple-negative breast cancer. *The oncologist*. 18:123-133.

Putti, T.C., D.M. El-Rehim, E.A. Rakha, C.E. Paish, A.H. Lee, S.E. Pinder, and I.O. Ellis. 2005. Estrogen receptor-negative breast carcinomas: a review of morphology and immunophenotypical analysis. *Mod Pathol*. 18:26-35.

Quick, Q., and O. Skalli. 2010. Alpha-actinin 1 and alpha-actinin 4: contrasting roles in the survival, motility, and RhoA signaling of astrocytoma cells. *Exp Cell Res*. 316:1137-1147.

Quinlan, R., P. Martin, and A. Graham. 2004. The role of actin cables in directing the morphogenesis of the pharyngeal pouches. *Development*. 131:593-599.

Ralphs, J.R., A.D. Waggett, and M. Benjamin. 2002. Actin stress fibres and cell-cell adhesion molecules in tendons: organisation in vivo and response to mechanical loading of tendon cells in vitro. *Matrix biology : journal of the International Society for Matrix Biology*. 21:67-74.

Rane, C.K., and A. Minden. 2014. P21 activated kinases: structure, regulation, and functions. *Small GTPases*. 5.

Ranganathan, P., A. Agrawal, R. Bhushan, A.K. Chavalmane, R.K. Kalathur, T. Takahashi, and P. Kondaiah. 2007. Expression profiling of genes regulated by TGF-beta: differential regulation in normal and tumour cells. *BMC genomics*. 8:98.

Ratheesh, A., and A.S. Yap. 2012. A bigger picture: classical cadherins and the dynamic actin cytoskeleton. *Nat Rev Mol Cell Biol*. 13:673-679.

Ribeiro Ede, A., Jr., N. Pinotsis, A. Ghisleni, A. Salmazo, P.V. Konarev, J. Kostan, B. Sjoblom, C. Schreiner, A.A. Polyansky, E.A. Gkougkoulia, M.R. Holt, F.L. Aachmann, B. Zagrovic, E. Bordignon, K.F. Pirker, D.I. Svergun, M. Gautel, and K. Djinovic-Carugo. 2014. The structure and regulation of human muscle alpha-actinin. *Cell*. 159:1447-1460.

Ridley, A.J. 2001. Rho GTPases and cell migration. *J Cell Sci*. 114:2713-2722.

Ridley, A.J. 2011. Life at the leading edge. *Cell*. 145:1012-1022.

Ridley, A.J., and A. Hall. 1992. The small GTP-binding protein rho regulates the assembly of focal adhesions and actin stress fibers in response to growth factors. *Cell*. 70:389-399.

Ridley, A.J., H.F. Paterson, C.L. Johnston, D. Diekmann, and A. Hall. 1992. The small GTP-binding protein rac regulates growth factor-induced membrane ruffling. *Cell*. 70:401-410.

Ridley, A.J., M.A. Schwartz, K. Burridge, R.A. Firtel, M.H. Ginsberg, G. Borisy, J.T. Parsons, and A.R. Horwitz. 2003. Cell migration: integrating signals from front to back. *Science*. 302:1704-1709.

Riento, K., and A.J. Ridley. 2003. Rocks: multifunctional kinases in cell behaviour. *Nat Rev Mol Cell Biol*. 4:446-456.

Riveline, D., E. Zamir, N.Q. Balaban, U.S. Schwarz, T. Ishizaki, S. Narumiya, Z. Kam, B. Geiger, and A.D. Bershadsky. 2001. Focal contacts as mechanosensors: externally applied local mechanical force induces growth of focal contacts by an mDia1-dependent and ROCK-independent mechanism. *J Cell Biol*. 153:1175-1186.

Rodriguez, F.J., L.J. Lewis-Tuffin, and P.Z. Anastasiadis. 2012. E-cadherin's dark side: possible role in tumor progression. *Biochim Biophys Acta*. 1826:23-31.

Rubenstein, P.A. 1990. The functional importance of multiple actin isoforms. *BioEssays : news and reviews in molecular, cellular and developmental biology*. 12:309-315.

Rubtsova, S.N., I.Y. Zhitnyak, and N.A. Gloushankova. 2015. A Novel Role of E-Cadherin-Based Adherens Junctions in Neoplastic Cell Dissemination. *PLoS One*. 10:e0133578.

Sahai, E. 2005. Mechanisms of cancer cell invasion. *Current opinion in genetics & development*. 15:87-96.

Saito, M., D.K. Tucker, D. Kohlhorst, C.M. Niessen, and A.P. Kowalczyk. 2012. Classical and desmosomal cadherins at a glance. *J Cell Sci*. 125:2547-2552.

Sanders, L.C., F. Matsumura, G.M. Bokoch, and P. de Lanerolle. 1999. Inhibition of myosin light chain kinase by p21-activated kinase. *Science*. 283:2083-2085.

Sandquist, J.C., K.I. Swenson, K.A. Demali, K. Burridge, and A.R. Means. 2006. Rho kinase differentially regulates phosphorylation of nonmuscle myosin II isoforms A and B during cell rounding and migration. *J Biol Chem*. 281:35873-35883.

Sanger, J.W., J.M. Sanger, and B.M. Jockusch. 1983. Differences in the stress fibers between fibroblasts and epithelial cells. *J Cell Biol*. 96:961-969.

Sanz-Moreno, V., G. Gadea, J. Ahn, H. Paterson, P. Marra, S. Pinner, E. Sahai, and C.J. Marshall. 2008. Rac activation and inactivation control plasticity of tumor cell movement. *Cell*. 135:510-523.

Schaefer, A.W., V.T. Schoonderwoert, L. Ji, N. Mederios, G. Danuser, and P. Forscher. 2008. Coordination of actin filament and microtubule dynamics during neurite outgrowth. *Dev Cell*. 15:146-162.

Schleicher, M., and B.M. Jockusch. 2008. Actin: its cumbersome pilgrimage through cellular compartments. *Histochemistry and cell biology*. 129:695-704.

Schmidt, A., and P.J. Koch. 2007. Desmosomes: just cell adhesion or is there more? *Cell Adh Migr*. 1:28-32.

Schulze, N., M. Graessl, A. Blancke Soares, M. Geyer, L. Dehmelt, and P. Nalbant. 2014. FHOD1 regulates stress fiber organization by controlling the dynamics of transverse arcs and dorsal fibers. *J Cell Sci*. 127:1379-1393.

Schwartz, M.A. 2010. Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harb Perspect Biol*. 2:a005066.

Schwartz, M.A., M.D. Schaller, and M.H. Ginsberg. 1995. Integrins: emerging paradigms of signal transduction. *Annu Rev Cell Dev Biol*. 11:549-599.

Sellers, J.R., M.D. Pato, and R.S. Adelstein. 1981. Reversible phosphorylation of smooth muscle myosin, heavy meromyosin, and platelet myosin. *J Biol Chem*. 256:13137-13142.

Sen, S., M. Dong, and S. Kumar. 2009. Isoform-specific contributions of alpha-actinin to glioma cell mechanobiology. *PLoS One*. 4:e8427.

Shackelford, D.B., and R.J. Shaw. 2009. The LKB1-AMPK pathway: metabolism and growth control in tumour suppression. *Nat Rev Cancer*. 9:563-575.

Shams, H., J. Golji, and M.R. Mofrad. 2012. A molecular trajectory of alpha-actinin activation. *Biophys J*. 103:2050-2059.

Shao, H., S. Li, S.C. Watkins, and A. Wells. 2014. alpha-Actinin-4 is required for amoeboid-type invasiveness of melanoma cells. *J Biol Chem*. 289:32717-32728.

Shao, H., J.H. Wang, M.R. Pollak, and A. Wells. 2010a. alpha-actinin-4 is essential for maintaining the spreading, motility and contractility of fibroblasts. *PLoS One*. 5:e13921.

Shao, H., C. Wu, and A. Wells. 2010b. Phosphorylation of alpha-actinin 4 upon epidermal growth factor exposure regulates its interaction with actin. *J Biol Chem*. 285:2591-2600.

Sheetz, M.P. 1994. Cell migration by graded attachment to substrates and contraction. *Seminars in cell biology*. 5:149-155.

Shipitsin, M., C. Small, S. Choudhury, E. Giladi, S. Friedlander, J. Nardone, S. Hussain, A.D. Hurley, C. Ernst, Y.E. Huang, H. Chang, T.P. Nifong, D.L. Rimm, J. Dunyak, M. Loda, D.M. Berman, and P. Blume-Jensen. 2014. Identification of proteomic biomarkers predicting prostate cancer aggressiveness and lethality despite biopsy-sampling error. *British journal of cancer*. 111:1201-1212.

Sjöblom, B., A. Salmazo, and K. Djinovic-Carugo. 2008. Alpha-actinin structure and regulation. *Cell Mol Life Sci*. 65:2688-2701.

Skau, C.T., R.S. Fischer, P. Gurel, H.R. Thiam, A. Tubbs, M.A. Baird, M.W. Davidson, M. Piel, G.M. Alushin, A. Nussenzweig, P.S. Steeg, and C.M. Waterman. 2016. FMN2 Makes Perinuclear Actin to Protect Nuclei during Confined Migration and Promote Metastasis. *Cell*. 167:1571-1585 e1518.

Skau, C.T., S.V. Plotnikov, A.D. Doyle, and C.M. Waterman. 2015. Inverted formin 2 in focal adhesions promotes dorsal stress fiber and fibrillar adhesion formation to drive extracellular matrix assembly. *Proc Natl Acad Sci U S A*. 112:E2447-2456.

Small, J.V., K. Rottner, I. Kaverina, and K.I. Anderson. 1998. Assembling an actin cytoskeleton for cell attachment and movement. *Biochim Biophys Acta*. 1404:271-281.

Smutny, M., H.L. Cox, J.M. Leerberg, E.M. Kovacs, M.A. Conti, C. Ferguson, N.A. Hamilton, R.G. Parton, R.S. Adelstein, and A.S. Yap. 2010. Myosin II isoforms identify distinct functional modules that support integrity of the epithelial zonula adherens. *Nat Cell Biol*. 12:696-702.

Spiering, D., and L. Hodgson. 2011. Dynamics of the Rho-family small GTPases in actin regulation and motility. *Cell Adh Migr*. 5:170-180.

Stope, M.B., S.L. Popp, C. Knabbe, and M.B. Buck. 2010. Estrogen receptor alpha attenuates transforming growth factor-beta signaling in breast cancer cells independent from agonistic and antagonistic ligands. *Breast cancer research and treatment*. 120:357-367.

Suleiman, H.Y., R. Roth, S. Jain, J.E. Heuser, A.S. Shaw, and J.H. Miner. 2017. Injury-induced actin cytoskeleton reorganization in podocytes revealed by super-resolution microscopy. *JCI insight*. 2.

Surks, H.K., C.T. Richards, and M.E. Mendelsohn. 2003. Myosin phosphatase-Rho interacting protein. A new member of the myosin phosphatase complex that directly binds RhoA. *J Biol Chem*. 278:51484-51493.

Surks, H.K., N. Riddick, and K. Ohtani. 2005. M-RIP targets myosin phosphatase to stress fibers to regulate myosin light chain phosphorylation in vascular smooth muscle cells. *J Biol Chem*. 280:42543-42551.

Suzuki, A., G. Kusakai, A. Kishimoto, Y. Minegichi, T. Ogura, and H. Esumi. 2003. Induction of cell-cell detachment during glucose starvation through F-actin conversion by SNARK, the fourth member of the AMP-activated protein kinase catalytic subunit family. *Biochem Biophys Res Commun*. 311:156-161.

Takeichi, M. 1977. Functional correlation between cell adhesive properties and some cell surface proteins. *J Cell Biol*. 75:464-474.

Takeichi, M. 2014. Dynamic contacts: rearranging adherens junctions to drive epithelial remodelling. *Nat Rev Mol Cell Biol*. 15:397-410.

Tamura, N., K. Ohno, T. Katayama, N. Kanayama, and K. Sato. 2007. The PDZ-LIM protein CLP36 is required for actin stress fiber formation and focal adhesion assembly in BeWo cells. *Biochem Biophys Res Commun*. 364:589-594.

Tan, I., J. Yong, J.M. Dong, L. Lim, and T. Leung. 2008. A tripartite complex containing MRCK modulates lamellar actomyosin retrograde flow. *Cell*. 135:123-136.

Tanaka, K., and K. Itoh. 1998. Reorganization of stress fiber-like structures in spreading platelets during surface activation. *J Struct Biol*. 124:13-41.

Tang, J., D.W. Taylor, and K.A. Taylor. 2001. The three-dimensional structure of alpha-actinin obtained by cryoelectron microscopy suggests a model for Ca(2+)-dependent actin binding. *J Mol Biol*. 310:845-858.

Tavares, S., A.F. Vieira, A.V. Taubenberger, M. Araujo, N.P. Martins, C. Bras-Pereira, A. Polonia, M. Herbig, C. Barreto, O. Otto, J. Cardoso, J.B. Pereira-Leal, J. Guck, J. Paredes, and F. Janody. 2017. Actin stress fiber organization promotes cell stiffening and proliferation of pre-invasive breast cancer cells. *Nature communications*. 8:15237.

Tee, Y.H., T. Shemesh, V. Thiagarajan, R.F. Hariadi, K.L. Anderson, C. Page, N. Volkmann, D. Hanein, S. Sivaramakrishnan, M.M. Kozlov, and A.D. Bershadsky. 2015. Cellular chirality arising from the self-organization of the actin cytoskeleton. *Nat Cell Biol*. 17:445-457.

Thiery, J.P., H. Acloque, R.Y. Huang, and M.A. Nieto. 2009. Epithelial-mesenchymal transitions in development and disease. *Cell*. 139:871-890.

Thomas, W.A., C. Boscher, Y.S. Chu, D. Cuvelier, C. Martinez-Rico, R. Seddiki, J. Heysch, B. Ladoux, J.P. Thiery, R.M. Mege, and S. Dufour. 2013. alpha-Catenin and vinculin cooperate to promote high E-cadherin-based adhesion strength. *J Biol Chem*. 288:4957-4969.

Thorsen, K., K.D. Sorensen, A.S. Brems-Eskildsen, C. Modin, M. Gaustadnes, A.M. Hein, M. Kruhoffer, S. Laurberg, M. Borre, K. Wang, S. Brunak, A.R. Krainer, N. Torring, L. Dyrskjot, C.L. Andersen, and T.F. Orntoft. 2008. Alternative splicing in colon, bladder, and prostate cancer identified by exon array analysis. *Mol Cell Proteomics*. 7:1214-1224.

Tojkander, S., G. Gateva, A. Husain, R. Krishnan, and P. Lappalainen. 2015. Generation of contractile actomyosin bundles depends on mechanosensitive actin filament assembly and disassembly. *eLife*. 4:e06126.

Tojkander, S., G. Gateva, and P. Lappalainen. 2012. Actin stress fibers - assembly, dynamics and biological roles. *J Cell Sci*. 125:1855-1864.

Tojkander, S., G. Gateva, G. Schevzov, P. Hotulainen, P. Naumanen, C. Martin, P.W. Gunning, and P. Lappalainen. 2011. A Molecular Pathway for Myosin II Recruitment to Stress Fibers. *Curr Biol*. 21:539-550.

Tomasek, J.J., G. Gabbiani, B. Hinz, C. Chaponnier, and R.A. Brown. 2002. Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol*. 3:349-363.

Totsukawa, G., Y. Yamakita, S. Yamashiro, D.J. Hartshorne, Y. Sasaki, and F. Matsumura. 2000. Distinct roles of ROCK (Rho-kinase) and MLCK in spatial regulation of MLC phosphorylation for assembly of stress fibers and focal adhesions in 3T3 fibroblasts. *J Cell Biol*. 150:797-806.

Tsuchihara, K., T. Ogura, R. Fujioka, S. Fujii, W. Kuga, M. Saito, T. Ochiya, A. Ochiai, and H. Esumi. 2008. Susceptibility of Snark-deficient mice to azoxymethane-induced colorectal tumorigenesis and the formation of aberrant crypt foci. *Cancer science*. 99:677-682.

Twiss, F., and J. de Rooij. 2013. Cadherin mechanotransduction in tissue remodeling. *Cell Mol Life Sci*. 70:4101-4116.

Twiss, F., Q. Le Duc, S. Van Der Horst, H. Tabdili, G. Van Der Krogt, N. Wang, H. Rehmann, S. Huveneers, D.E. Leckband, and J. De Rooij. 2012. Vinculin-dependent Cadherin mechanosensing regulates efficient epithelial barrier formation. *Biology open*. 1:1128-1140.

Vaahtomeri, K., E. Ventela, K. Laajanen, P. Katajisto, P.J. Wipff, B. Hinz, T. Vallenius, M. Tiainen, and T.P. Makela. 2008. Lkb1 is required for TGFbeta-mediated myofibroblast differentiation. *J Cell Sci*. 121:3531-3540.

Vaezi, A., C. Bauer, V. Vasioukhin, and E. Fuchs. 2002. Actin cable dynamics and Rho/Rock orchestrate a polarized cytoskeletal architecture in the early steps of assembling a stratified epithelium. *Dev Cell*. 3:367-381.

Valerius, N.H., O. Stendahl, J.H. Hartwig, and T.P. Stossel. 1981. Distribution of actin-binding protein and myosin in polymorphonuclear leukocytes during locomotion and phagocytosis. *Cell*. 24:195-202.

Vallenius, T. 2013. Actin stress fibre subtypes in mesenchymal-migrating cells. *Open biology*. 3:130001.

Vallenius, T., K. Luukko, and T.P. Mäkelä. 2000. CLP-36 PDZ-LIM protein associates with nonmuscle alpha-actinin-1 and alpha-actinin-4. *J Biol Chem*. 275:11100-11105.

Vallenius, T., K. Vaahtomeri, B. Kovac, A.M. Osiceanu, M. Viljanen, and T.P. Makela. 2011. An association between NUAK2 and MRIP reveals a novel mechanism for regulation of actin stress fibers. *J Cell Sci*. 124:384-393.

van Nieuw Amerongen, G.P., and V.W. van Hinsbergh. 2001. Cytoskeletal effects of rho-like small guanine nucleotide-binding proteins in the vascular system. *Arterioscler Thromb Vasc Biol*. 21:300-311.

van Roy, F., and G. Berx. 2008. The cell-cell adhesion molecule E-cadherin. *Cell Mol Life Sci*. 65:3756-3788.
Vandekerckhove, J., and K. Weber. 1978. At least six different actins are expressed in a higher mammal: an analysis based on the amino acid sequence of the amino-terminal tryptic peptide. *J Mol Biol*. 126:783-802.
Vasioukhin, V., C. Bauer, M. Yin, and E. Fuchs. 2000. Directed actin polymerization is the driving force for epithelial cell-cell adhesion. *Cell*. 100:209-219.
Vaughan, R.B., and J.P. Trinkaus. 1966. Movements of epithelial cell sheets in vitro. *J Cell Sci*. 1:407-413.
Vicente-Manzanares, M., and A.R. Horwitz. 2011. Adhesion dynamics at a glance. *J Cell Sci*. 124:3923-3927.
Vicente-Manzanares, M., M.A. Koach, L. Whitmore, M.L. Lamers, and A.F. Horwitz. 2008. Segregation and activation of myosin IIB creates a rear in migrating cells. *J Cell Biol*. 183:543-554.
Vicente-Manzanares, M., X. Ma, R.S. Adelstein, and A.R. Horwitz. 2009. Non-muscle myosin II takes centre stage in cell adhesion and migration. *Nat Rev Mol Cell Biol*. 10:778-790.
Vicente-Manzanares, M., J. Zareno, L. Whitmore, C.K. Choi, and A.F. Horwitz. 2007. Regulation of protrusion, adhesion dynamics, and polarity by myosins IIA and IIB in migrating cells. *J Cell Biol*. 176:573-580.
Virel, A., and L. Backman. 2007. A comparative and phylogenetic analysis of the alpha-actinin rod domain. *Mol Biol Evol*. 24:2254-2265.
Voduc, K.D., M.C. Cheang, S. Tyldesley, K. Gelmon, T.O. Nielsen, and H. Kennecke. 2010. Breast cancer subtypes and the risk of local and regional relapse. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology*. 28:1684-1691.
Wachsstock, D.H., J.A. Wilkins, and S. Lin. 1987. Specific interaction of vinculin with alpha-actinin. *Biochem Biophys Res Commun*. 146:554-560.
Watabe, Y., T. Mori, S. Yoshimoto, T. Nomura, T. Shibahara, T. Yamada, and K. Honda. 2014. Copy number increase of ACTN4 is a prognostic indicator in salivary gland carcinoma. *Cancer medicine*. 3:613-622.
Watanabe, N., T. Kato, A. Fujita, T. Ishizaki, and S. Narumiya. 1999. Cooperation between mDia1 and ROCK in Rho-induced actin reorganization. *Nat Cell Biol*. 1:136-143.
Webb, D.J., J.T. Parsons, and A.F. Horwitz. 2002. Adhesion assembly, disassembly and turnover in migrating cells -- over and over and over again. *Nat Cell Biol*. 4:E97-100.
Weber, K., and U. Groeschel-Stewart. 1974. Antibody to myosin: the specific visualization of myosin-containing filaments in nonmuscle cells. *Proc Natl Acad Sci U S A*. 71:4561-4564.
Wehland, J., M. Osborn, and K. Weber. 1979. Cell-to-substratum contacts in living cells: a direct correlation between interference-reflexion and indirect-immunofluorescence microscopy using antibodies against actin and alpha-actinin. *J Cell Sci*. 37:257-273.
Weins, A., P. Kenlan, S. Herbert, T.C. Le, I. Villegas, B.S. Kaplan, G.B. Appel, and M.R. Pollak. 2005. Mutational and Biological Analysis of alpha-actinin-4 in focal segmental glomerulosclerosis. *Journal of the American Society of Nephrology : JASN*. 16:3694-3701.
Weins, A., J.S. Schlondorff, F. Nakamura, B.M. Denker, J.H. Hartwig, T.P. Stossel, and M.R. Pollak. 2007. Disease-associated mutant alpha-actinin-4 reveals a mechanism for regulating its F-actin-binding affinity. *Proc Natl Acad Sci U S A*. 104:16080-16085.
Welch, M.D., and R.D. Mullins. 2002. Cellular control of actin nucleation. *Annu Rev Cell Dev Biol*. 18:247-288.
Welsch, T., S. Keleg, F. Bergmann, S. Bauer, U. Hinz, and J. Schmidt. 2009. Actinin-4 expression in primary and metastasized pancreatic ductal adenocarcinoma. *Pancreas*. 38:968-976.
Winkler, J., H. Lunsdorf, and B.M. Jockusch. 1997. Flexibility and fine structure of smooth-muscle alpha-actinin. *European journal of biochemistry*. 248:193-199.
Wong, A.J., T.D. Pollard, and I.M. Herman. 1983. Actin filament stress fibers in vascular endothelial cells in vivo. *Science*. 219:867-869.
Worth, D.C., and M. Parsons. 2008. Adhesion dynamics: mechanisms and measurements. *Int J Biochem Cell Biol*. 40:2397-2409.
Wyckoff, J.B., S.E. Pinner, S. Gschmeissner, J.S. Condeelis, and E. Sahai. 2006. ROCK- and myosin-dependent matrix deformation enables protease-independent tumor-cell invasion in vivo. *Curr Biol*. 16:1515-1523.
Xia, D., J.T. Stull, and K.E. Kamm. 2005. Myosin phosphatase targeting subunit 1 affects cell migration by regulating myosin phosphorylation and actin assembly. *Exp Cell Res*. 304:506-517.
Xia, S., and P. Kanchanawong. 2017. Nanoscale mechanobiology of cell adhesions. *Seminars in cell & developmental biology*.
Yamada, S., S. Yanamoto, H. Yoshida, I. Yoshitomi, G. Kawasaki, A. Mizuno, and T.K. Nemoto. 2010. RNAi-mediated down-regulation of alpha-actinin-4 decreases invasion potential in oral squamous cell carcinoma. *International journal of oral and maxillofacial surgery*. 39:61-67.
Yamaguchi, H., and J. Condeelis. 2007. Regulation of the actin cytoskeleton in cancer cell migration and invasion. *Biochim Biophys Acta*. 1773:642-652.
Yamaguchi, H., Y. Ito, N. Miura, Y. Nagamura, A. Nakabo, K. Fukami, K. Honda, and R. Sakai. 2017. Actinin-1 and actinin-4 play essential but distinct roles in invadopodia formation by carcinoma cells. *Eur J Cell Biol*. 96:685-694.
Yamamoto, H., S. Takashima, Y. Shintani, S. Yamazaki, O. Seguchi, A. Nakano, S. Higo, H. Kato, Y. Liao, Y. Asano, T. Minamino, Y. Matsumura, H. Takeda, and M. Kitakaze. 2008. Identification of a novel substrate for TNFalpha-induced kinase NUAK2. *Biochem Biophys Res Commun*. 365:541-547.
Yamamoto, S., H. Tsuda, K. Honda, T. Kita, M. Takano, S. Tamai, J. Inazawa, T. Yamada, and O. Matsubara. 2007. Actinin-4 expression in ovarian cancer: a novel prognostic indicator independent of clinical stage and histological type. *Mod Pathol*. 20:1278-1285.
Yamamoto, S., H. Tsuda, K. Honda, K. Onozato, M. Takano, S. Tamai, I. Imoto, J. Inazawa, T. Yamada, and O. Matsubara. 2009. Actinin-4 gene amplification in ovarian cancer: a candidate oncogene associated with poor patient prognosis and tumor chemoresistance. *Mod Pathol*. 22:499-507.

Yamamoto, S., H. Tsuda, K. Honda, M. Takano, S. Tamai, I. Imoto, J. Inazawa, T. Yamada, and O. Matsubara. 2012. ACTN4 gene amplification and actinin-4 protein overexpression drive tumour development and histological progression in a high-grade subset of ovarian clear-cell adenocarcinomas. *Histopathology*. 60:1073-1083.

Yang, C., and W.F. Glass, 2nd. 2008. Expression of alpha-actinin-1 in human glomerular mesangial cells in vivo and in vitro. *Exp Biol Med (Maywood)*. 233:689-693.

Yao, M., W. Qiu, R. Liu, A.K. Efremov, P. Cong, R. Seddiki, M. Payre, C.T. Lim, B. Ladoux, R.M. Mege, and J. Yan. 2014. Force-dependent conformational switch of alpha-catenin controls vinculin binding. *Nature communications*. 5:4525.

Yasutomi, M., S. Kunishima, S. Okazaki, A. Tanizawa, S. Tsuchida, and Y. Ohshima. 2016. ACTN1 rod domain mutation associated with congenital macrothrombocytopenia. *Annals of hematology*. 95:141-144.

Ye, X., and R.A. Weinberg. 2015. Epithelial-Mesenchymal Plasticity: A Central Regulator of Cancer Progression. *Trends in cell biology*. 25:675-686.

Yi, J., X.S. Wu, T. Crites, and J.A. Hammer, 3rd. 2012. Actin retrograde flow and actomyosin II arc contraction drive receptor cluster dynamics at the immunological synapse in Jurkat T cells. *Mol Biol Cell*. 23:834-852.

Ylänne, J., K. Scheffzek, P. Young, and M. Saraste. 2001. Crystal structure of the alpha-actinin rod reveals an extensive torsional twist. *Structure*. 9:597-604.

Yonemura, S. 2011. Cadherin-actin interactions at adherens junctions. *Curr Opin Cell Biol*. 23:515-522.

Yonemura, S. 2017. Actin filament association at adherens junctions. *The journal of medical investigation : JMI*. 64:14-19.

Yonemura, S., M. Itoh, A. Nagafuchi, and S. Tsukita. 1995. Cell-to-cell adherens junction formation and actin filament organization: similarities and differences between non-polarized fibroblasts and polarized epithelial cells. *J Cell Sci*. 108 (Pt 1):127-142.

Yonemura, S., Y. Wada, T. Watanabe, A. Nagafuchi, and M. Shibata. 2010. alpha-Catenin as a tension transducer that induces adherens junction development. *Nat Cell Biol*. 12:533-542.

Yoshida-Noro, C., N. Suzuki, and M. Takeichi. 1984. Molecular nature of the calcium-dependent cell-cell adhesion system in mouse teratocarcinoma and embryonic cells studied with a monoclonal antibody. *Developmental biology*. 101:19-27.

Yumura, S., H. Mori, and Y. Fukui. 1984. Localization of actin and myosin for the study of ameboid movement in Dictyostelium using improved immunofluorescence. *J Cell Biol*. 99:894-899.

Zagorska, A., M. Deak, D.G. Campbell, S. Banerjee, M. Hirano, S. Aizawa, A.R. Prescott, and D.R. Alessi. 2010. New roles for the LKB1-NUAK pathway in controlling myosin phosphatase complexes and cell adhesion. *Science signaling*. 3:ra25.

Zaidel-Bar, R. 2013. Cadherin adhesome at a glance. *J Cell Sci*. 126:373-378.

Zaidel-Bar, R., C. Ballestrem, Z. Kam, and B. Geiger. 2003. Early molecular events in the assembly of matrix adhesions at the leading edge of migrating cells. *J Cell Sci*. 116:4605-4613.

Zaidel-Bar, R., S. Itzkovitz, A. Ma'ayan, R. Iyengar, and B. Geiger. 2007. Functional atlas of the integrin adhesome. *Nat Cell Biol*. 9:858-867.

Zamir, E., and B. Geiger. 2001. Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci*. 114:3583-3590.

Zhang, J., M. Betson, J. Erasmus, K. Zeikos, M. Bailly, L.P. Cramer, and V.M. Braga. 2005. Actin at cell-cell junctions is composed of two dynamic and functional populations. *J Cell Sci*. 118:5549-5562.

Zimerman, B., T. Volberg, and B. Geiger. 2004. Early molecular events in the assembly of the focal adhesion-stress fiber complex during fibroblast spreading. *Cell Motil Cytoskeleton*. 58:143-159.

ISBN 978-951-51-4053-1 (paperback)
ISBN 978-951-51-4054-8 (PDF)
http://ethesis.helsinki.fi

Hansaprint
Helsinki 2018