

UC Davis

UC Davis Previously Published Works

Title

The dual role of filamin A in cancer: can't live with (too much of) it, can't live without it.

Permalink

<https://escholarship.org/uc/item/8wn4d86w>

Journal

Endocrine-related cancer, 20(6)

ISSN

1351-0088

Authors

Savoy, Rosalinda M

Ghosh, Paramita M

Publication Date

2013-12-01

DOI

10.1530/erc-13-0364

Peer reviewed

The dual role of filamin A in cancer: can't live with (too much of) it, can't live without it

Rosalinda M Savoy¹ and Paramita M Ghosh^{1,2}

¹Department of Urology, University of California Davis School of Medicine, University of California, 4860 Y Street, Suite 3500, Sacramento, California 95817, USA

²VA Northern California Health Care System, Mather, California, USA

Correspondence should be addressed to P M Ghosh

Email
Paramita.Ghosh@ucdmc.ucdavis.edu

Abstract

Filamin A (FlnA) has been associated with actin as cytoskeleton regulator. Recently its role in the cell has come under scrutiny for FlnA's involvement in cancer development. FlnA was originally revealed as a cancer-promoting protein, involved in invasion and metastasis. However, recent studies have also found that under certain conditions, it prevented tumor formation or progression, confusing the precise function of FlnA in cancer development. Here, we try to decipher the role of FlnA in cancer and the implications for its dual role. We propose that differences in subcellular localization of FlnA dictate its role in cancer development. In the cytoplasm, FlnA functions in various growth signaling pathways, such as vascular endothelial growth factor, in addition to being involved in cell migration and adhesion pathways, such as R-Ras and integrin signaling. Involvement in these pathways and various others has shown a correlation between high cytoplasmic FlnA levels and invasive cancers. However, an active cleaved form of FlnA can localize to the nucleus rather than the cytoplasm and its interaction with transcription factors has been linked to a decrease in invasiveness of cancers. Therefore, overexpression of FlnA has a tumor-promoting effect, only when it is localized to the cytoplasm, whereas if FlnA undergoes proteolysis and the resulting C-terminal fragment localizes to the nucleus, it acts to suppress tumor growth and inhibit metastasis. Development of drugs to target FlnA and cause cleavage and subsequent localization to the nucleus could be a new and potent field of research in treating cancer.

Key Words

- ▶ filamin A
- ▶ metastasis
- ▶ cancer
- ▶ transcription
- ▶ nucleus
- ▶ cytoplasm
- ▶ localization

Endocrine-Related Cancer
(2013) 20, R341–R356

Introduction

Filamin A (FlnA), also known as actin-binding protein 280 (ABP 280), was first identified as a non-muscle actin filament cross-linking protein (Stossel & Hartwig 1975). Subsequent reports revealed the importance of this protein in cytoplasmic gelation, cell contraction, and spreading (Hartwig & Stossel 1975). Since then, two other paralogous genes, filamin B (FlnB) and filamin C (FlnC), have been established as part of the filamin family. Filamins largely act as scaffolding molecules, facilitating

protein–protein interactions and influencing protein cellular localization. Studies revealed the protein structures of the filamins and identified over 90 filamin-binding proteins involved in cell signaling, cell migration and adhesion, phosphorylation, proteolysis, ion channel regulation, transcription regulation, receptor activation, muscle development, and other important cell functions. Mutations in these molecules have can result in a wide range of disease phenotypes, including terminal osseous

dysplasia, cardiovascular malformations, and neural defects (Nakamura *et al.* 2011).

The filamins, in particular, FlnA, are highly susceptible to proteolysis. Multiple studies showed that, while full-length FlnA is mainly localized to the cytoplasm, it can be cleaved to a 90-kDa fragment that can then localize to the nucleus (Ozanne *et al.* 2000, Loy *et al.* 2003, Wang *et al.* 2007, Bedolla *et al.* 2009). In the cytoplasm, full-length FlnA promotes the development of metastasis (recently reviewed in Yue *et al.* (2013)), whereas nuclear FlnA was shown to be necessary for inhibition of transcription and susceptibility to therapeutic interventions (Loy *et al.* 2003, Wang *et al.* 2007). In this review, we therefore focus on the opposing roles of FlnA in cancer progression depending on its localization. Reports in the literature have proposed blocking FlnA expression as a means of cancer therapy; however, we and others showed that completely eliminating FlnA will have serious deleterious consequences. Here, we propose, instead, to promote FlnA proteolysis – which was shown to have the required cancer-suppression effect.

FlnA is important for organogenesis during development

Early studies indicated that loss of FlnA was embryonically lethal (Feng *et al.* 2006). Absence of FlnA resulted in male lethality because of incomplete septation of the outflow tract of the heart, which produces common arterial trunk and midline fusion defects manifesting as sternum and palate abnormalities (Hart *et al.* 2006). In addition, carrier females exhibit misshapen pupils while a proportion of both male and female mutant mice have other cardiac defects including ventricular septal defect (Hart *et al.* 2006). However, mice that lack FlnA only in the megakaryocyte (MK) lineage were generated by pairing FlnA^{loxP} mice with PF4-Cre mice and were shown to have severe macrothrombocytopenia because of the rapid clearance of FlnA-null platelets from circulation (Jurak Begonja *et al.* 2011). Further, FlnA was important for platelet formation because FlnA^{loxP} PF4-Cre bone marrows and spleens had increased megakaryopoiesis and FlnA-null proplatelets released platelets more readily than controls *in vitro* (Jurak Begonja *et al.* 2011).

Mutations in the *FlnA* gene were linked to a large number of developmental diseases. Periventricular heterotopia is an X-linked dominant disorder, in which neurons fail to migrate into the cerebral cortex, but remain as nodules lining the ventricular surface. This disease has been linked to various mutations in *FlnA*, including a

frameshift mutation (3045del5) in exon 21 (de Wit *et al.* 2009) and a cytosine-to-thymidine missense mutation (c. C1286T) in exon 9, resulting in a threonine-to-methionine amino acid substitution (Masruha *et al.* 2006). Various other mutations in other parts of the gene, resulting in protein misfolding, has been reported (Sheen *et al.* 2001, 2005, Parrini *et al.* 2006). Most hemizygous affected males die early during embryogenesis, whereas heterozygous females have normal intelligence but suffer from various neurological and vascular conditions. FlnA mutations have been linked to not only neurological disorders but cardiovascular problems as well (described in details in Zhou *et al.* (2007)), including familial cardiac valvular dystrophy (Kyndt *et al.* 2007).

The high rate of neurological and cardiovascular developmental disorders due to mutations in various parts of the *FlnA* gene has highlighted its importance in cell migration during development (Price *et al.* 1994, de Wit *et al.* 2009). Axonal pathfinding during embryogenesis is based on the migration of nerve growth cones, which relies strongly on f-actin, filamin, α -actinin, myosin, tropomyosin, talin, and vinculin (Letourneau & Shattuck 1989). Shortly thereafter, it was discovered that mutations in *FlnA* prevented migration of cerebral cortical neurons that led to incomplete neurological development (Fox *et al.* 1998). Because of this, *FlnA* mutations have been linked to a broad range of congenital malformations, affecting craniofacial structures, skeleton, brain, viscera, and urogenital tract, in four X-linked human disorders: otopalatodigital syndrome types 1 (OPD1) and 2 (OPD2), frontometaphyseal dysplasia, and Melnick–Needles syndrome (Robertson *et al.* 2003). Interestingly, the majority of the mutations are clustered into four regions of the gene: the actin-binding domain (ABD) and rod domain repeats 3, 10, and 14/15 (described later) (Robertson *et al.* 2003). Mutations in *FlnA* are also linked to an X-linked recessive form of chronic idiopathic intestinal pseudo-obstruction (CIIPX; Gargiulo *et al.* 2007) and to an X-inactivation mosaicism in the corneal epithelia (Douvaras *et al.* 2012).

The varieties of disorders that are associated with mutations in FlnA indicate its importance in cell and organ development. High filamin expression was reported in diverse organs including esophagus, stomach, intestine, aorta, lung, bladder, uterus, and ovary (Brown & Binder 1993). Filamin expression is somewhat tissue specific: FlnA and FlnB are ubiquitously expressed, while FlnC is limited to cardiac and skeletal cells (Maestrini *et al.* 1993, van der Flier & Sonnenberg 2001). Proper functioning of FlnA is ubiquitously required for maintenance and organ development (Zhou *et al.* 2010).

FlnA regulates organogenesis by its ability to induce cell migration through its actin-binding properties

FlnA's role in organogenesis likely arises from its ability to regulate cell migration. Within the human genome, FlnA is located on chromosome Xq28 and consists of 48 exons spanning a little over 26 kb (Fig. 1A). The two other paralogous Fln genes in humans, FlnB and FlnC, are located on chromosome 3p14.3 and chromosome 7q32–35 respectively (Fig. 1A). The wide distribution of filamins throughout the human genome points to its high importance in development; it is likely that this arrangement prevents complete inactivation of all filamins due to malfunction of any one part. The FlnA gene has two known full-length mRNA variants. The two FlnA alternative splicing variants are very similar and only differ in the deletion of 24 nucleotides coding for amino acids 1649–1656, skipping an entire exon (Maestrini *et al.* 1993). The similarity between the two full-length FlnA transcripts has prevented studies to distinguish the expression

patterns between the two variants (Maestrini *et al.* 1993, Uhlen *et al.* 2010). In addition, FlnA-spliced variants were identified, which could result in smaller C-terminal fragments of FlnA (predicted protein size 120, 106, or 85 kDa fragments) (Fig. 1B; Wiemann *et al.* 2001).

The more common full-length transcript encodes a 280-kDa protein consisting of 2647 amino acids with several different domains. The full-length filamin contains an ABD at the N-terminal end followed by 24 immunoglobulin (Ig)-like repeat domains, which are folded into β -sheets and together form a rod structure (Ruskamo *et al.* 2012; Fig. 2A). The ABD of FlnA is similar to other ABDs and contains two calponin homology (CH) domains, with two actin-binding sites (ABS) in CH1 and one ABS in CH2 (Ruskamo & Ylanne 2009). These CH domains are conserved in FlnB and FlnC (Fig. 2A). In both FlnA and FlnC, there are also transmembrane domains in the ABD, not present in FlnB. Therefore, FlnA and FlnC, but not FlnB, are predicted to localize to the plasma membrane. On the other hand, FlnA and FlnB both contain nuclear localization signals (NLS) in the ABD, not present in FlnC,

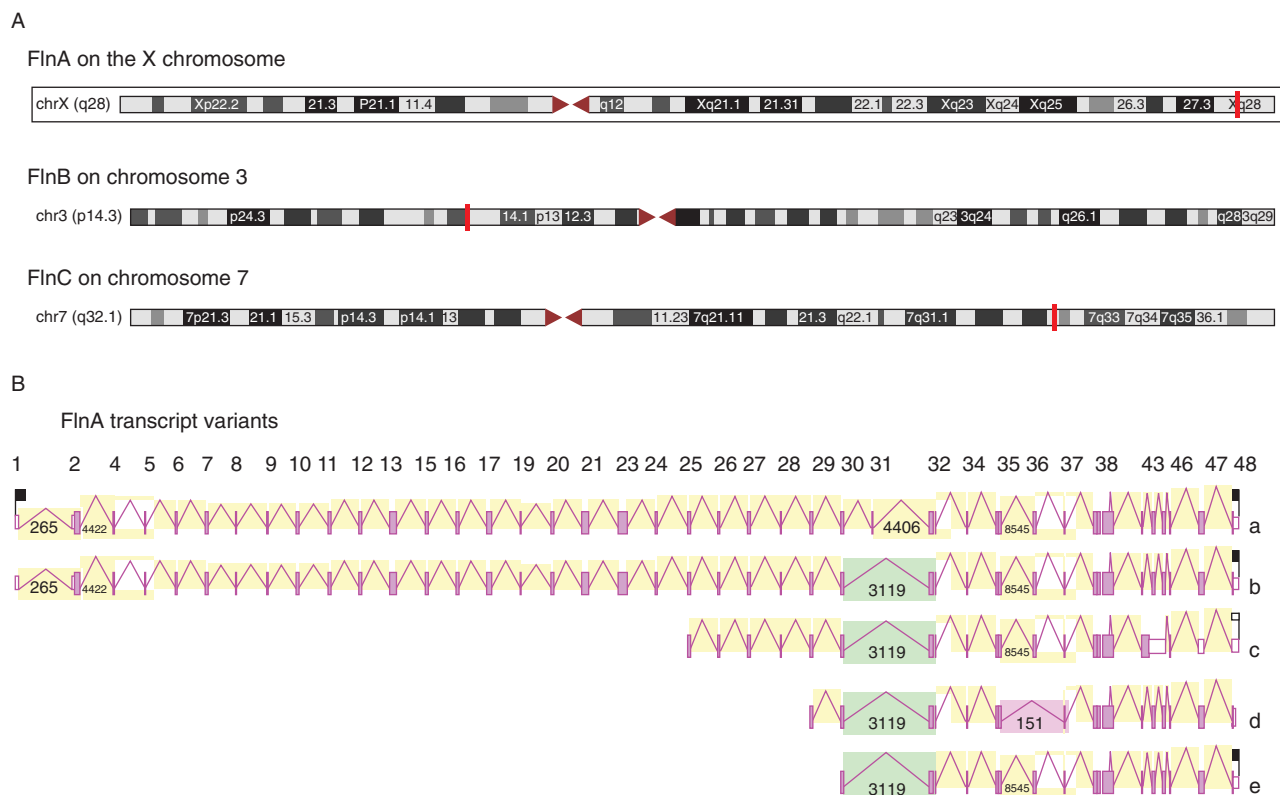
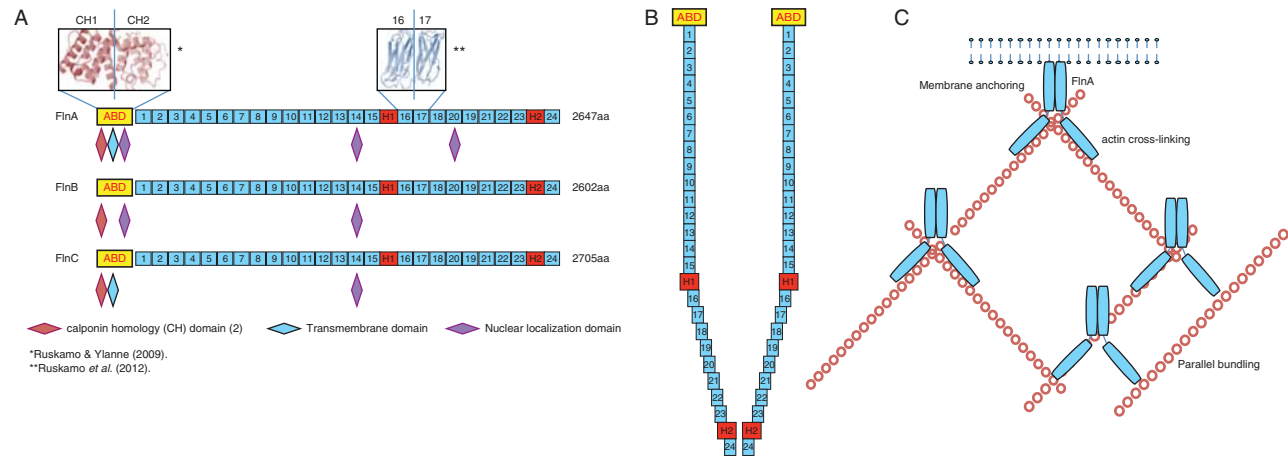


Figure 1

(A) Within the human genome, FlnA is located on chromosome Xq28, while FlnB is located on chromosome 3p14.3, and FlnC is located on chromosome 7q32–35. (B) The FlnA gene has two known full-length mRNA variants, which only differ in the deletion of 24 nucleotides coding for amino acids

1649–1656 – skipping an entire exon. In addition, cDNA clones were identified that could result in smaller C-terminal fragments of FlnA (predicted 120, 106, or 85 kDa fragments).

**Figure 2**

(A) FlnA is a 280 kDa protein consisting of 2647 amino acids with several different domains. There is an actin-binding domain (ABD) at the N-terminal end followed by 24 immunoglobulin (Ig)-like repeat domains, which are folded into β -sheets and together form a rod structure. The ABD of FlnA contains two calponin homology (CH) domains, with two actin-binding sites (ABS) in CH1 and one ABS in CH2. FlnA also contains a transmembrane domain in the ABD and three nuclear localization domains, located in repeats 1, 14, and 21. While the CH domains are conserved in FlnB and FlnC, FlnB does not contain the transmembrane domain and has only two nuclear localizations, located in repeats 1 and 14. FlnC does contain the transmembrane domain, but only has one nuclear localization in repeat 14. In all three, the 24 repeat domains following the

though all three isoforms have an NLS in repeat 14, and FlnA has a third NLS in repeat 20 (Fig. 2A).

The 24 repeat domains at the C-terminal end of the filamins are interrupted by two hinge regions, the first (H1) between repeats 15 and 16 and the second (H2) between repeats 23 and 24 (Fig. 2A). The repeat domains within FlnA have high sequence similarity and there is also high homology between the three filamin isoforms, with $\sim 70\%$ of sequence similarity overall, and the lowest homology of only 45% at the hinge regions (van der Flier & Sonnenberg 2001). The molecules homodimerize through repeat 24 and the hinge domains allow the protein to bend, so that when it dimerizes, the structure is similar to a Y, with dimerization occurring at the 24th repeat (Robertson 2005; Fig. 2B).

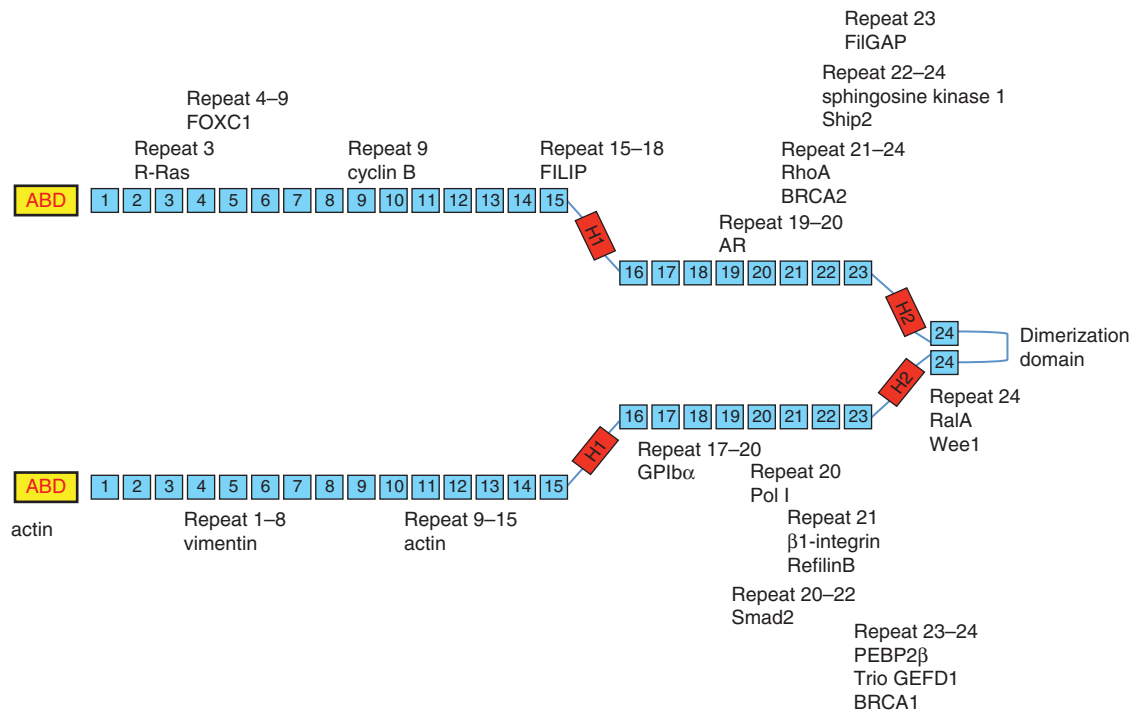
Dimerization allows FlnA to regulate the structure of the cell through actin binding in multiple ways (Fig. 2C). The best known function of FlnA is to crosslink actin, providing organization and stability to actin that forms the cytoskeleton. This function of FlnA involves formation of homodimers at the C-terminal and binding to actin filaments with the N-terminal ABD (van der Flier & Sonnenberg 2001). As an integral part of the cytoskeleton, FlnA maintains cell rigidity and protein localization, and is of particular importance in cell adhesion and migration.

ABD are interrupted by two hinge regions, the first (H1) is between repeats 15 and 16 and the second (H2) is between repeats 23 and 24. The repeat domains within FlnA have high sequence similarity and there is also high sequence similarity between the three filamin isoforms, with $\sim 70\%$ sequence similarity overall, and the lowest homology of only 45% at the hinge regions. (B) The molecules homodimerize by binding at repeat 24 and the hinge domains allow the protein to bend, so that when it dimerizes the structure is similar to a Y. (C) Dimerization allows FlnA to regulate the structure of the cell through actin binding in multiple ways. FlnA anchors the actin filaments at the membrane and can form them into perpendicular or parallel strands.

In addition, FlnA also increases branching of actin proteins, which is required to organize the cytoskeleton and maintain the flexibility and rigidity of the cell (Stossel *et al.* 2001). FlnA is also crucial in cell migration – it is recruited to the sites of migration, such as filopodia and lamellipodia, and brings other proteins involved with this function to the cell surface to allow cell movement (Nakamura *et al.* 2011). These reports indicate that the major functions of FlnA in the cytoplasm or at the membrane appear to be the promotion of cell adhesion, migration, or rigidity. On the other hand, WT FlnA overexpression also inhibited neuronal migration, thereby indicating that an optimum levels of FlnA is required for proper development (Sarkisian *et al.* 2006).

FlnA acts as a scaffolding molecule to regulate various cell functions

FlnA has over 90 known protein-binding partners, many of which regulate the actin cytoskeleton (Fig. 3). Many of these proteins interact with FlnA as part of the signaling pathways to reorganize the actin cytoskeleton as a reaction to stress or other stimuli (Zhou *et al.* 2010). As FlnA is a large molecule, it is used as scaffolding for other proteins

**Figure 3**

FlnA has over 90 known protein-binding partners, many of which regulate the actin cytoskeleton. Actin binding occurs at the ABD and also between repeats 9 and 15. Most other proteins bind closer to the C-terminal domain, between repeats 16 and 24.

to bind and regulate the response to cell signaling. Most proteins interact with one of the Ig repeat domains of FlnA and because of the similarity between repeats, proteins can bind at multiple sites (Nakamura *et al.* 2011). In addition, most protein binding occurs at the C-terminal end, while the ABD is present at the N-terminal (van der Flier & Sonnenberg 2001, Stossel *et al.* 2001).

β 1-integrin, which binds to repeat 21 of FlnA, is of critical importance in cell adhesion. FlnA binds to the cytoplasmic domain of β 1-integrin and promotes cell adhesion and migration by facilitating ligand binding via inside-out signaling. FlnA and β 1-integrin maintain a positively correlated relationship during cell spreading and migration, when one is decreased, so is the other (Kim *et al.* 2008). Disruption of this interaction by knockdown of FlnA decreases the amount of β 1-integrin present at the cell membrane at the cell's initial contact with the extracellular matrix, showing the importance of FlnA in cell adhesion (Kim & McCulloch 2011). Deregulation of this pathway is often found in various types of cancer and has been implicated for therapeutic targets (Barkan & Chambers 2011).

FlnA is often found co-localized with vimentin and both are found at sites of cell protrusions (Kim & McCulloch 2011). Overexpression of proteins such as

RalA normally stimulates migration, but FlnA deficient cells were unable to migrate, indicating the crucial role of FlnA (Ohta *et al.* 1999). In addition, FlnA acts at these sites to bring multiple proteins including PtdIns(3,4,5)P(3), sphingosine kinase 1, and small GTPases to the sites of lamellipodia formation to promote cell migration (Maceyka *et al.* 2008, Takabayashi *et al.* 2010, Zhou *et al.* 2010). FlnA anchors the GTPases to the cell membrane, acting as a scaffolding protein for downstream targets to remodel the actin cytoskeleton and allow cell motility (Zhou *et al.* 2010).

Tissue factor (TF), the cellular receptor and cofactor for clotting factor VII/VIIa (FVII/VIIa), which plays a role in fibrin formation in embryogenesis, was found to co-localize with FlnA at the leading edge of spreading cells (Muller *et al.* 1999). FlnA is considered to be a ligand for the TF cytoplasmic domain and recruitment to TF adhesion contacts is associated with the reorganization of actin filaments, but these interactions are not associated with FlnA interactions with integrins (Ott *et al.* 1998). Lack of TF–FlnA interactions may result in incomplete implantation of the embryo into the endometrium and covering of the implantation location by a fibrin plug, which may also contribute to embryonic lethality in FlnA-null mice mentioned earlier (Feng *et al.* 2006). FlnA's role

in platelet adhesion also involves interaction with glycoprotein (GP) Ib α , which plays an important role in the regulation of the membrane skeleton and stability (Cranmer *et al.* 2011). The slit–diaphragm junctional adhesion protein, nephrin, is necessary for the development of podocyte morphology and transduces phosphorylation-dependent signals that regulate cytoskeletal dynamics (Venkatareddy *et al.* 2011). Upon activation, nephrin recruits and regulates a protein complex that includes SH2 domain containing 5' inositol phosphatase (Ship2), filamin, and lamellipodin (Venkatareddy *et al.* 2011), resulting in lamellipodia formation. Finally, FlnA is also shown to regulate the cell cycle. Embryonic FlnA-null mice had reduced brain size and decreased neural progenitors, which were attributed to prolonged cell cycle (Lian *et al.* 2012). Suppression of FlnA led to prolongation of the cell cycle, principally in the M phase, whereas the cdk1 kinase Wee1 bound FlnA, demonstrated to show increased expression levels after loss of FlnA function, was associated with increased phosphorylation of cdk1 (Lian *et al.* 2012).

Regulation of FlnA expression and localization

A number of regulators of FlnA expression have been identified that determine which organs would express this molecule and at what phases of development. One protein that appears to regulate the functionality of FlnA is the FlnA-interacting protein (FILIP) that regulates cortical cell migration out of the ventricular zone (Nagano *et al.* 2002). FILIP interacts with FlnA and induces its degradation – therefore, most ventricular zone cells that overexpress FILIP fail to migrate in explants. FILIP siRNA results in FlnA overexpression and promotes the development and maintenance of a bipolar shape also in the subventricular and intermediate zones (Nagano *et al.* 2004). Thus FILIP regulates FlnA levels and determine the mode of migration of neurons entering the cortical plate (Sato & Nagano 2005).

Another regulator of FlnA expression is the vesicle transport ADP-ribosylation factor guanine exchange factor 2 gene (*ARFGEF2*). Transfection of a dominant-negative construct of *ARFGEF2* in neuroblastoma cells partially blocked FlnA transport from the Golgi apparatus to the cell membrane, underscoring the importance of this protein in targeted transport of FlnA to the cell surface within neural progenitors (Lu *et al.* 2006). Refilins, a unique family of actin regulators, act as molecular switches to convert FlnA from an actin branching protein into one that bundles (Gay *et al.* 2011). The RefilinB/FlnA complex organizes the perinuclear actin filament bundles forming

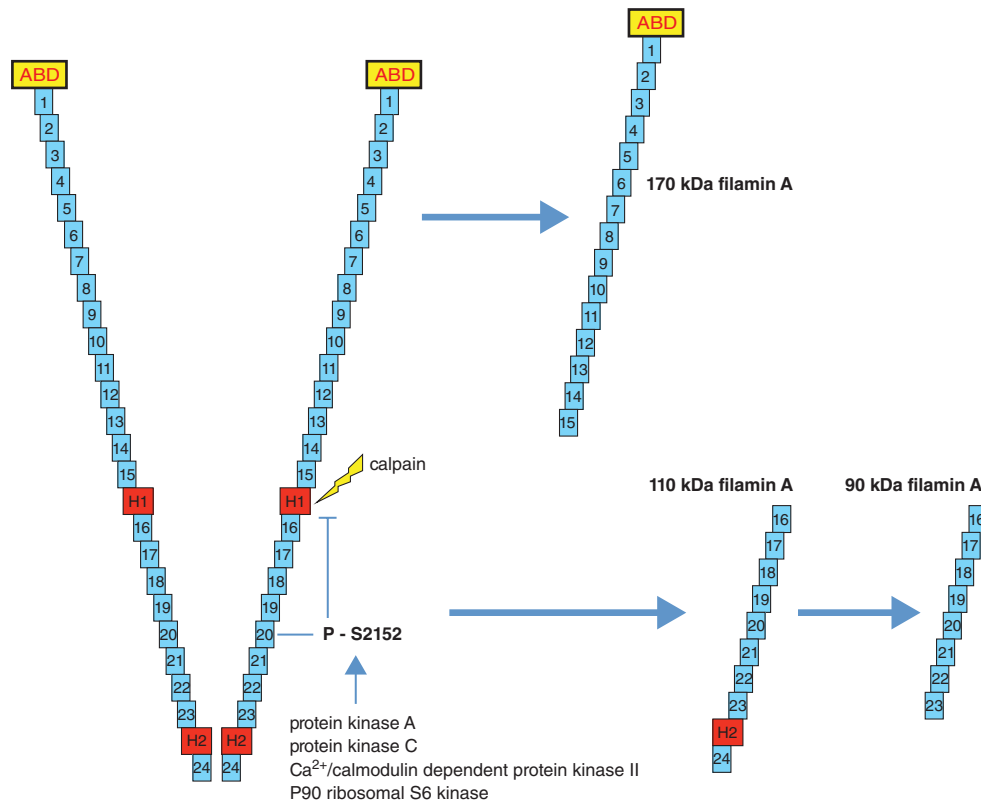
an actin cap. and the nuclear shape changes during epithelial–mesenchymal transition (EMT; Gay *et al.* 2011).

FlnA expression is also regulated by signal transduction pathways involving the MAP kinase pathway. MEKK4 (MAP3K4)(–/–) mice developed periventricular heterotopia associated with breaches in the neuroependymal lining, which largely comprised neurons that failed to reach the cortical plate. The expression of FlnA was elevated in MEKK4(–/–) forebrain, most notably near sites of failed neuronal migration while the recombinant MKK4 protein promoted the interaction between MEKK4 and Fln-A (Sarkisian *et al.* 2006). A novel group of evolutionarily conserved HECT ubiquitin ligases with an N-terminal filamin domain (HFNs) were deemed to regulate the filamin complex activity and cell type-specific motility through the breakdown of filamin complexes (Blagg *et al.* 2011). FlnA can also be regulated by proteasomal degradation, which is mediated by the E3 ubiquitin ligases ASB2 α and ASB2 β (Heuze *et al.* 2008). This regulation is of particular importance in the maturation of dendritic cells and inhibits their mobility by causing the degradation of FlnA (Lamsoul *et al.* 2013).

FlnA localization within the cell was found to be regulated by inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein (IKBKAP), which encodes IKAP (ELP1), a protein associated with familial dysautonomia (FD), with defective neuronal development and maintenance (Johansen *et al.* 2008). Cytosolic IKAP that co-localized with FlnA along membrane ruffles, whereas downregulation of IKAP expression resulted in decreased FlnA membrane ruffles localization and defective actin cytoskeleton organization. Another mitochondria-anchored protein, protein kinase C, and casein kinase substrate in neurons protein 2 (PACSIN2) prevent filamin localization to membrane ruffles but not to stress fiber (Cousin *et al.* 2008). Thus, a family of proteins is involved in the regulation of FlnA expression in various parts of the cell.

Proteolysis of FlnA

One way by which FlnA can be quickly removed once it is no longer required is by proteolysis. The hinge regions are sites at which proteolysis occurs (van der Flier & Sonnenberg 2001). FlnA is susceptible to cleavage by calpain and caspase at the two hinge regions that can produce two proteins, a 170 kDa protein that includes the ABD and repeats 1–15 and a 110-kDa protein that is further cleaved to a 90-kDa protein that contains repeats 16–23 (Gorlin *et al.* 1990, Browne *et al.* 2000; Fig. 4).

**Figure 4**

FlnA is susceptible to proteolysis at the hinge regions. This cleavage can occur by calpain and caspase producing two proteins, a 170 kDa protein that includes the ABD and repeats 1–15 and a 110 kDa protein that is further cleaved to a 90 kDa protein that contains repeats 16–23. Susceptibility of FlnA to cleavage is regulated by its phosphorylation. Specific phosphorylation at S2152 in the 20th repeat is of particular importance because it is known to protect FlnA against cleavage.

Susceptibility of FlnA to cleavage is regulated by its phosphorylation. Specific phosphorylation at S2152 in the 20th repeat is of particular importance because it is known to protect FlnA against cleavage (Garcia *et al.* 2006). FlnA can be phosphorylated at S2152 by several different kinases, including protein kinase A, protein kinase C, Ca²⁺/calmodulin-dependent protein kinase II, and p90 ribosomal S6 kinase (Wallach *et al.* 1978, Kawamoto & Hidaka 1984, Chen & Stracher 1989, Ohta & Hartwig 1996, Jay *et al.* 2000, Raynaud *et al.* 2006).

Ca²⁺/calmodulin-dependent protein kinase II can phosphorylate S2523, which can result in a change of location from the cytosol to the membrane, regulating endothelial cell barrier function (Borbiev *et al.* 2001). p56^{lck} can also cause phosphorylation of FlnA, but does not protect it against calpain cleavage, indicating that it phosphorylates a site outside of S2152. p56^{lck} regulates the interaction between FlnA and surface receptors, including

FlnA can be phosphorylated at S2152 by several different kinases including protein kinase A (cAMP-dependent protein kinase), protein kinase C, Ca²⁺/calmodulin-dependent protein kinase II, and p90 ribosomal S6 kinase. Other phosphorylation sites, including S2523, can influence location from the cytosol to the membrane and the interaction between FlnA surface receptors, but does not protect against cleavage.

β2-integrins, and can also influence actin crosslinking (Goldmann 2002, Pal Sharma & Goldmann 2004). It is projected that following the completion of its function in the organ, FlnA undergoes proteolysis to prevent further activation of its downstream targets in order to prevent unwanted cell migration, cell adhesion, or cell cycle progression.

FlnA's involvement in cancer

Cancer has been described as the product of developmental error leading to the acquisition of a unique cell character (de-differentiation) (da Costa 2001). Lineage tracing has shown that stem cells are mobilized to repair skin wounds and that this process may contribute to skin tumor development (Arwert *et al.* 2012). Tumorigenesis and wound repair, both depend on communication between epithelial cells, mesenchymal cells, and bone

marrow-derived cells (Arwert *et al.* 2012). Similarly, exposure to certain chemicals and environmental factors, food, hormones, infectious agents, radiation, sunlight, and tobacco may trigger certain kinds of cancers. Genetics and propensity to obesity, diet, and physical activity determine the risks of developing cancer. Many of these factors may induce some type of injury to the affected organ resulting in de-differentiation (da Costa 2001).

Regeneration, or replacement, of lost or damaged cells involves the processes of de-differentiation, transdifferentiation, and reprogramming (Jopling *et al.* 2011), and in many cases, cancer can be thought of as continued and unwanted regeneration that does not know how to stop, resulting in overexpression of certain key proteins. Whatever the cause, FlnA was seen to be overexpressed in multiple types of cancer, including prostate (Bedolla *et al.* 2009), breast (Alper *et al.* 2009, Tian *et al.* 2013), lung cancer (Uramoto *et al.* 2010), hemangiomas (Hosaka *et al.* 1985), colon cancer (Porter *et al.* 1993, Larriba *et al.* 2009), melanoma (Flanagan *et al.* 2001), neuroblastoma (Bachmann *et al.* 2006), squamous cell carcinoma (Kamochi *et al.* 2008), hepatic cholangiocarcinoma (Guedj *et al.* 2009), etc.

However, the puzzling factor regarding filamin overexpression in cancer is the outcome. On one hand, cyclin D1 interacted with FlnA to promote migration and invasive potential of breast cancer cells (Zhong *et al.* 2010). On the other hand, FlnA regulated focal adhesion disassembly and suppressed breast cancer cell migration and invasion (Xu *et al.* 2010). Similarly, in prostate cancer, FlnA binding promoted androgen receptor (AR) localization to the nucleus (Ozanne *et al.* 2000), which is thought to promote prostate cancer progression; however, once in the nucleus, it inhibited the transcriptional activity of the same transcription factor (Loy *et al.* 2003). Therefore, we argue that overexpression of FlnA has a tumor-promoting effect only when it is localized to the cytoplasm or plasma membrane, where it has the ability to promote tumor metastasis. On the other hand, despite overexpression, if FlnA undergoes proteolysis and the proteolysed fragments localize to the nucleus, where they regulate transcription, then they may act to suppress tumor growth and inhibit metastasis (Fig. 5). Ultimately, the function of FlnA in the cell, whether in cancer or in other diseases, depend on the binding partners available for its interaction – FlnA remains as a scaffolding protein that only act, whether in the cytoplasm or the nucleus, to promote the interaction of these proteins to either promote or prevent cancer.

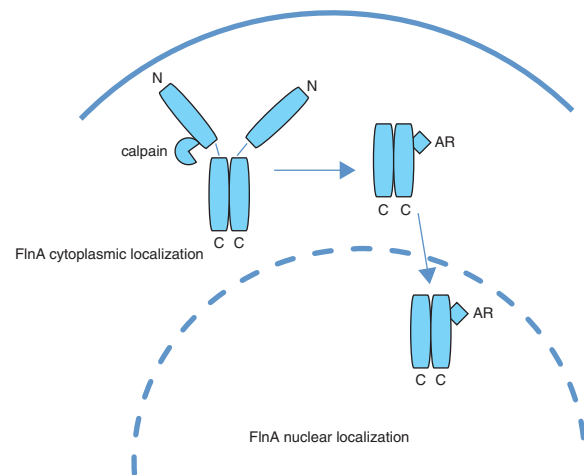


Figure 5

When FlnA is localized to the cytoplasm or plasma membrane, it has the ability to promote tumor metastasis through interaction with signaling molecules. If FlnA undergoes proteolysis and the proteolysed fragments localize to the nucleus, where they regulate transcription, then they may act to suppress tumor growth and inhibit metastasis.

FlnA promotes cancer growth and metastasis in the cytoplasm by interacting with signaling molecules

The development of cancer is a multistep process that transforms a normal cell to the one that evades apoptosis, grows uncontrollably, promotes angiogenesis to support the tumor, and finally invades surrounding tissue and metastasizes (Hanahan & Weinberg 2000). Through FlnA's involvement in multiple cellular pathways, it can be linked as an important factor in all of these cancer-promoting steps. FlnA's function in the cytoplasm as a scaffolding protein and its vital importance in cell adhesion and migration can transform it into an extremely potent cancer-promoting protein.

Targeting FlnA through knockdown experiments has given investigators an insight into FlnA's role in cancer development and has shown its importance in cancer cell growth and metastasis. Crossing a conditionally overexpressed K-RAS mouse model (to induce lung tumor formations) with a conditional knockout FlnA mouse showed that in the absence of FlnA (albeit conditional, as an FlnA null mouse is embryonically lethal), K-RAS overexpression did not lead to lung tumor formation (Nallapalli *et al.* 2012). Isolated mouse fibroblasts from the K-RAS(+/+) FlnA(-/-) mouse lines showed reduced ability to proliferate and reduced motility as well as a 25% reduction of p-ERK and p-Akt in comparison with those expressing FlnA (Nallapalli *et al.* 2012). As K-RAS is a powerful oncogenic protein, it is

very interesting to discover that without FlnA, K-RAS-driven lung cancer formation is halted.

Regulation of DNA damage

FlnA can be linked to an anti-apoptotic mechanism in cancer development through its involvement in double strand break repair (DSBR). Investigation of the role of FlnA in DSBR was initiated after an interaction between FlnA and BRCA2 was discovered by yeast two-hybrid screen and subsequent co-immunoprecipitation (Yuan & Shen 2001). A previous report showed that lack of FlnA led to susceptibility of DNA damage and G2 arrest (Meng *et al.* 2004). FlnA seems to act as a scaffolding protein for BRCA2 in the assembly of the repair complex. When cells were treated with radiation, lack of FlnA led to an increase of cell death, an increase in γ H2AX nuclear foci (indicating double strand break), and a decrease in RAD51 formation on chromatin (Yue *et al.* 2009). Through this mechanism FlnA promotes cell survival and protects cancer cells against drug treatment.

Angiogenesis

Angiogenesis is another crucial part of cancer development. Tumors give rise to new blood vessels to continue their growth (Ricci-Vitiani *et al.* 2010, Wang *et al.* 2010). FlnA has been implicated in angiogenesis through links with vascular endothelial growth factor (VEGF; Uramoto *et al.* 2010). The expression of FlnA and VEGF

was analyzed by immunohistochemistry in lung tumor samples. Positive expression of FlnA and VEGF was detected in the cytoplasm of tumor cells in 66/137 (48.2%) and 69/137 (50.4%) patients with lung cancer, respectively, while statistical analysis suggested that FlnA is mediating the angiogenesis pathway and is responsible for controlling the growth of lung tumors (Uramoto *et al.* 2010). In addition, studies on bone marrow angiogenesis found that four proteins: FlnA, vimentin, α -crystallin B, and 14-3-3 ζ/δ protein, were overexpressed in endothelial cells present in multiple myeloma compared with normal endothelial cells (Berardi *et al.* 2011). The expression of FlnA in multiple myeloma was increased by VEGF, fibroblast growth factor 2, and hepatocyte growth factor, while using siRNA to knockdown their expression inhibited angiogenesis-related functions, such as spreading, migration, and tubular morphogenesis (Berardi *et al.* 2011).

Metastasis

Table 1 outlines a number of molecules that are shown to regulate FlnA-mediated cell migration and metastasis and only a few of these are outlined below. One mechanism by which FlnA is involved in promoting cancer metastasis is through activation of c-Met. c-Met is the only receptor for the hepatocyte growth factor and is extremely important in cell growth and motility and is a very potent oncogene. An association between FlnA and c-Met was first identified when irradiated fibroblasts expressed increased levels of

Table 1 FlnA-interacting proteins that promote or inhibit metastasis

Inhibits metastasis	Promotes metastasis
AR (Wang)	B1 integrin (Kim <i>et al.</i> 2007)
IGFBP5 (Abrass & Hansen 2010)	PtdIns(3,4,5)P(3) (Takabayashi <i>et al.</i> 2010)
RNA polymerase, POL III (Deng <i>et al.</i> 2012)	Sphingosine kinase 1 (Maceyka <i>et al.</i> 2008)
ASB2 (Heuze <i>et al.</i> 2008, Razinia <i>et al.</i> 2011)	BRCA2 (Yuan & Shen 2001)
PEBP2 β (Yoshida <i>et al.</i> 2005)	VEGF (Uramoto <i>et al.</i> 2010)
FoxC1/PBX1 (Berry <i>et al.</i> 2005)	c-Met (Zhou)
Klotho (Camilli <i>et al.</i> 2011)	R-Ras (Gawecka <i>et al.</i> 2010)
Granzyme B (Browne <i>et al.</i> 2000)	RhoA
	Cyclin B1 (Cukier <i>et al.</i> 2007)
	K-Ras (Nallapalli <i>et al.</i> 2012)
	cdc25C (Telles <i>et al.</i> 2011)
	BRCA1 (Velkova <i>et al.</i> 2010)
	Cyclin D1/cyclin-dependent kinase 4 (Zhong <i>et al.</i> 2010)
	Vimentin (MacPherson & Fagerholm 2010)
	MLL (De Braekeleer <i>et al.</i> 2009)
	Caveolin-1 (Ravid <i>et al.</i> 2008)
	TGF- β (Mishra & Marshall 2006)
	Arp2/3 (Flanagan <i>et al.</i> 2001)
	Pro-PrP (Li <i>et al.</i> 2010)
	EGFR (Sy)
	P73 α (Ravid <i>et al.</i> 2008)

both proteins (Kamochi *et al.* 2008). Even more importantly these cells promoted invasion and growth of human squamous cell carcinoma cells (Kamochi *et al.* 2008). Investigation of the mechanism by which these proteins interacted and promoted metastatic cancer cells showed that when tumors cells are deficient for FlnA, expression of c-Met was also significantly reduced and the cells exhibited poor migration and invasion ability when exposed to hepatocyte growth factor (HGF). In addition, AKT, a downstream signaling molecule, is also reduced in FlnA-deficient cells. FlnA was found to regulate c-Met expression through its interaction with Smad2 (Zhou *et al.* 2011).

A second mechanism by which FlnA is involved in promoting cell migration and metastasis is through its interaction with the small GTPases, R-Ras and RhoA. Similar to FlnA, R-Ras also regulates cell adhesion and migration. Using a yeast two-hybrid assay, investigators showed that R-Ras and FlnA interact, specifically at the third repeat of FlnA. Deletion of the third repeat of FlnA prevented R-Ras from binding to FlnA and decreased cell migration. Furthermore, knockdown of R-Ras inhibited FlnA from interacting with the fibronectin matrix assembly (Gawecka *et al.* 2010). In addition to R-Ras, FlnA also interacts with RhoA and promotes migration in head and neck squamous cell carcinoma cells. RhoA causes modification to the cytoskeleton by phosphorylation of FlnA (Bourguignon *et al.* 2006). R-Ras and RhoA are important cell signaling molecules that are often mutated or overexpressed in cancer (Mora *et al.* 2007, He *et al.* 2010). Through their interaction with FlnA they can easily promote cell migration and metastasis in cancer (Table 1).

FlnA can regulate RhoA through its interaction with Trio, a small guanine nucleotide-exchange factor (GEF) that activates a number of Rho GTPases by assisting the switch from GDP for GTP (Bellanger *et al.* 2000). Trio has two GEF domains (GEFD1 and GEFD2) that can activate RhoG, Rac, and RhoA and is also important for axon guidance and cell migration in the nervous system (Bellanger *et al.* 1998, Blangy *et al.* 2000). A yeast two-hybrid screen showed that the GEFD1 of Trio interacted with the C-terminal end of FlnA, specifically on repeat 23 and 24 (Bellanger *et al.* 2000). GEFD1 but not GEFD2 interacted with FlnA. A previous study on 3T3 cells showed that GEFD1 caused ruffle formation of actin fibers that contained high levels of FlnA (Bellanger *et al.* 1998). Using two human malignant melanoma cell lines, one lacking FlnA (M2) and a subline that stably expressed FlnA (M2A7), investigators showed that expression of GEFD1 in M2 caused no ruffle formation, while expression of GEFD1

in M2A7 caused dorsal ruffles. This FlnA-dependent formation was specific for Trio GEFD1, while overexpression of its target proteins, RhoG and Rac, caused ruffle formation regardless of the presence of FlnA. Furthermore, the ruffle formation was only seen when Trio GEFD1 and FlnA were physically linked; constructs of GEFD1 and FlnA lacking the necessary regions for binding prevented ruffle formation. These changes in the cytoskeletal formation by Trio indicate its importance in cancer development and metastasis through its link to FlnA (Bellanger *et al.* 2000).

An important step in cancer metastasis is the contraction of the cell and regulation of cell polarity. Cells undergoing locomotion must first expand by lamellae protrusions and then contract to induce the rest of the cell to move. This involved complex reorganization of the cytoskeleton is regulated by ROCK inactivation of Rac. Investigation has shown that this regulation of Rac and lamellae formation is mediated by the FlnA GTPase-activating protein (FilGAP). The yeast two-hybrid assay revealed this FlnA-binding protein, which had homologous domains to a family of RhoGAP proteins. To determine the function of FilGAP, the GAP domain was microinjected into Swiss 3T3 cells that were stimulated with either epidermal growth factor (EGF) or lysophosphatidic acid (LPA). FilGAP abolished filopodia formation in EGF-stimulated cells, which involved the activation of Cdc42 and Rac1, but not in LPA-stimulated cells which involved the activation of RhoA. The expression of an FlnA plasmid lacking repeat 23, where FilGAP binds, prevented the GAP activity of FilGAP, indicating the importance of FlnA in this pathway. siRNA against FilGAP showed an increase in lamellae formation, demonstrating its importance in suppressing lamellae. ROCK was found to phosphorylate FilGAP, which inactivates Rac. This pathway shows both the importance of FlnA as a scaffolding molecule and as a remodeler of the cytoskeleton. Both of these have a large role in tumor development and spreading. The FilGAP pathway has been implicated in tumor cell migration and is largely dependent on FlnA to complete its activation (Saito *et al.* 2012).

Cancer is very rarely caused by the deregulation of one growth pathway alone. FlnA likely plays a role in cancer metastasis because of its involvement in multiple regulatory pathways (Gawecka *et al.* 2010, Zhou *et al.* 2011). As a result, overexpression of FlnA has been associated with highly metastatic cancers including prostate cancer, melanoma, and neuroblastoma (Bachmann *et al.* 2006, Coughlin *et al.* 2006, Zhu *et al.* 2007).

FlnA inhibits cancer growth and metastasis in the nucleus by interacting with transcription factors

FlnA's role in cell biology is largely as a scaffolding molecule that acts to bring proteins together so that they can interact. In the cytoplasm often this results in cell migration and proliferation. Upon interaction with transcription factors, both in the cytoplasm and the nucleus, FlnA can regulate their transcriptional activity. FlnA was found to interact with PEBP2 β in the cytoplasm and prevent its nuclear localization and interaction with Runx1 (Watanabe *et al.* 2005, Yoshida *et al.* 2005). Runx1 is an important transcription factor involved with hematopoietic cell differentiation (Okada *et al.* 1998). Furthermore Runx1 activity as a transcription factor is impaired without dimerization with PEBP2 β (Niki *et al.* 1997). It was previously established that PEBP2 β was present in the cytoplasm and brought into the nucleus by Runx1. FlnA was found to retain PEBP2 β in the cytoplasm and prevent its interaction with Runx1 (Yoshida *et al.* 2005).

FlnA can also localize to the nucleus and this corresponds with a repression of some cell activities. FlnA has several nuclear localization sequences in repeats 1, 14, and 20. Ozanne *et al.* (2000) first showed that FlnA was crucial in the translocation of the AR into the nucleus. During investigation of the mechanism by which the AR was transported to and from the nucleus, a yeast two-hybrid screen identified the C-terminal end of FlnA as an AR-interacting protein. Furthermore, in the absence of FlnA, AR was unable to enter the nucleus (Ozanne *et al.* 2000). FlnA's role in the nucleus and specifically AR was further examined by a different group (Loy *et al.* 2003). They reported that the FlnA repeats 16–24 interacted with the hinge domain of AR. Assessment of FlnA's role on AR transcriptional activity showed that increasing amounts of FlnA repressed AR induction of its transcriptional target prostate specific antigen (PSA) (Loy *et al.* 2003).

FlnA's new role in the nucleus has led researchers to evaluate the differences that arise from the localization of the protein. Cytoplasmic FlnA is often highly over-expressed in metastatic cancers including breast and prostate. Specifically in metastatic prostate cancer, cytoplasmic FlnA is phosphorylated at S2152, which prevents its cleavage at the hinge region to the 90 kDa fragment, whereas in less aggressive or benign prostate tumors the 90 kDa FlnA was found in the nucleus (Bedolla *et al.* 2009). Inhibition of the AR by anti-androgen hormonal therapy is the prevalent treatment for metastatic prostate cancer; however, patients frequently become resistant to such

treatment in advanced disease (hormone refractory prostate cancer, HRPC). Cytoplasmic full-length FlnA expression was increased in HRPC, indicating that lack of the nuclear 90 kDa FlnA could be a mechanism in which prostate cancer progresses (Bedolla *et al.* 2009). Transfection of a plasmid encoding the FlnA fragment that localizes to the nucleus caused a HRPC cell line, C4-2, to become sensitive to anti-androgen treatment (Wang *et al.* 2007). In addition to regulating AR in prostate cancer, FlnA has also been linked to other transcription factors and transcription machinery. Work with IGFBP5 showed that accumulation of the nuclear 90 kDa FlnA induced migration in glomerular mesangial cell by inducing transcription of laminin subunits (Berfield *et al.* 2006). Further research showed that IGFBP5 caused dephosphorylation of FlnA, leading to its cleavage by calpains. The C-terminal fragment of FlnA was attached to Smad3/4 and caused its nuclear translocation and binding to the promoter of the IGFBP5 target genes, which induced gene transcription (Abrass & Hansen 2010).

The 90 kDa FlnA fragment is not the only fragment that enters the nucleus. Full-length FlnA is also known to play a role in gene transcription in the nucleolus. It directly binds to RNA polymerase in the nucleolus to inhibit rRNA gene transcription (Deng *et al.* 2012). Knockdown of FlnA by siRNA caused increased rRNA expression, rDNA promoter activity, and cell proliferation. *In vitro* studies to immunodeplete FlnA from nuclear extracts caused a decrease in rDNA promoter-driven transcription. FlnA was also coimmunoprecipitated with several of the Pol I components; actin, TIF-IA, and RPA40, and in the absence of FlnA these proteins were found at a higher occupancy at the rDNA promoter (Deng *et al.* 2012). Full-length FlnA is also known to interact in the nucleus with the FOXC1 transcription factor (Berry *et al.* 2005). High levels of nuclear full-length FlnA in A7 melanoma cells inhibited FOXC1 from activating transcription. FOXC1 was further removed from transcription-rich areas in the nucleus and moved to a heterochromatin-rich region. FlnA is able to recruit PBX1 to the nucleus, which then interacted with FOXC1 and inhibited its transcriptional activity (Berry *et al.* 2005).

Conclusion

The examination of FlnA in cancer development is still underway, but already it is considered to have an important function in cancer development and the progression to metastasis. In the cytoplasm, FlnA binds to actin to give structure to and remodel the cytoskeleton

and, as importantly, acts as a scaffolding molecule, facilitating protein interaction (Zhou *et al.* 2010). FlnA has been reported to interact with over 90 proteins, which indicates the numerous pathways that FlnA can affect and its importance in the cell (Stossel *et al.* 2001). FlnA is of particular importance in facilitating cell adhesion and migration by directing $\beta 1$ integrin to the site of cell attachment and through its interaction with vimentin (Kim *et al.* 2008, Kim & McCulloch 2011). In addition, FlnA has been widely implicated in having an important role in cancer development and being a possible target for future therapies.

In regard to FlnA's involvement in cancer development, FlnA can cause two opposite outcomes of cancer depending on its subcellular localization. By being present in the cytoplasm and interacting with cell signaling molecules FlnA can promote cell growth and metastasis; on the other hand, when acting in the nucleus and with transcription factors, FlnA causes inhibition of cell growth and prevents metastasis. In the cytoplasm FlnA acts in various signaling pathways and interacts with several significant oncogenic proteins including K-RAS, R-Ras, VEGF, and c-Met (Gawecka *et al.* 2010, Uramoto *et al.* 2010, Zhou *et al.* 2011, Nallapalli *et al.* 2012). Through these interactions FlnA aids their functions and enhances cell growth. In the nucleus, FlnA interacts with several transcription factors including AR, Pol III, and FOXC1 (Ozanne *et al.* 2000, Berry *et al.* 2005, Deng *et al.* 2012). FlnA causes a general inhibition of their activities at the promoter regions on the DNA, causing growth inhibition or cell death. Targeting FlnA in the cytoplasm through the inhibition of the phosphorylation of S2152 would result in cleavage of the full-length protein into the 90 kDa form. Subsequent localization to the nucleus would then inhibit various transcription factors and result in the inhibition of cancer growth (Wang *et al.* 2007). Work toward this end is already in progress in prostate cancer. Genistein-combined polysaccharide is a soy derivative that can reduce phosphorylation of FlnA S2152 in prostate cancer cells (Mooso *et al.* 2012). This results in translocation of the 90 kDa FlnA and modification of AR transcription. Using this therapy or others to induce cleavage of FlnA, in other cancers, would cause subsequent localization to the nucleus and repress various transcription factors and induce cell quiescence or death.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

Funding

During the composition of this manuscript, salary support for one or more authors was provided partly by a Biomedical Laboratory Research and Development (BLRD) service Merit Award (I01BX000400) from the Department of Veterans Affairs and by Award # CA133209 from the National Cancer Institute. The work reported here does not represent the views or opinions of the Department of Veteran Affairs or the United States Government.

References

- Abrass CK & Hansen KM 2010 Insulin-like growth factor-binding protein-5-induced laminin gamma1 transcription requires filamin A. *Journal of Biological Chemistry* **285** 12925–12934. (doi:10.1074/jbc.M109.061754)
- Alper O, Stetler-Stevenson WG, Harris LN, Leitner WW, Ozdemirli M, Hartmann D, Raffeld M, Abu-Asab M, Byers S, Zhuang Z *et al.* 2009 Novel anti-filamin-A antibody detects a secreted variant of filamin-A in plasma from patients with breast carcinoma and high-grade astrocytoma. *Cancer Science* **100** 1748–1756. (doi:10.1111/j.1349-7006.2009.01244.x)
- Arwert EN, Hoste E & Watt FM 2012 Epithelial stem cells, wound healing and cancer. *Nature Reviews. Cancer* **12** 170–180. (doi:10.1038/nrc3217)
- Bachmann AS, Howard JP & Vogel CW 2006 Actin-binding protein filamin A is displayed on the surface of human neuroblastoma cells. *Cancer Science* **97** 1359–1365. (doi:10.1111/j.1349-7006.2006.00327.x)
- Barkan D & Chambers AF 2011 $\beta 1$ -integrin: a potential therapeutic target in the battle against cancer recurrence. *Clinical Cancer Research* **17** 7219–7223. (doi:10.1158/1078-0432.CCR-11-0642)
- Bedolla RG, Wang Y, Asuncion A, Chamie K, Siddiqui S, Mudryj MM, Prihoda TJ, Siddiqui J, Chinnaiyan AM, Mehra R *et al.* 2009 Nuclear versus cytoplasmic localization of filamin A in prostate cancer: immunohistochemical correlation with metastases. *Clinical Cancer Research* **15** 788–796. (doi:10.1158/1078-0432.CCR-08-1402)
- Bellanger JM, Lazaro JB, Diriong S, Fernandez A, Lamb N & Debant A 1998 The two guanine nucleotide exchange factor domains of Trio link the Rac1 and the RhoA pathways *in vivo*. *Oncogene* **16** 147–152. (doi:10.1038/sj.onc.1201532)
- Bellanger JM, Astier C, Sardet C, Ohta Y, Stossel TP & Debant A 2000 The Rac1- and RhoG-specific GEF domain of Trio targets filamin to remodel cytoskeletal actin. *Nature Cell Biology* **2** 888–892. (doi:10.1038/35046533)
- Berardi S, Caivano A, Ria R, Nico B, Savino R, Terracciano R, De Tullio G, Ferrucci A, De Luisi A, Moschetta M *et al.* 2011 Four proteins governing overangiogenic endothelial cell phenotype in patients with multiple myeloma are plausible therapeutic targets. *Oncogene* **31** 2258–2269. (doi:10.1038/onc.2011.412)
- Berfield AK, Hansen KM & Abrass CK 2006 Rat glomerular mesangial cells require laminin-9 to migrate in response to insulin-like growth factor binding protein-5. *American Journal of Physiology. Cell Physiology* **291** C589–C599. (doi:10.1152/ajpcell.00623.2005)
- Berry FB, O'Neill MA, Coca-Prados M & Walter MA 2005 FOXC1 transcriptional regulatory activity is impaired by PBX1 in a filamin A-mediated manner. *Molecular and Cellular Biology* **25** 1415–1424. (doi:10.1128/MCB.25.4.1415-1424.2005)
- Blagg SL, Battom SE, Annesley SJ, Keller T, Parkinson K, Wu JM, Fisher PR & Thompson CR 2011 Cell type-specific filamin complex regulation by a novel class of HECT ubiquitin ligase is required for normal cell motility and patterning. *Development* **138** 1583–1593. (doi:10.1242/dev.063800)
- Blangy A, Vignal E, Schmidt S, Debant A, Gauthier-Rouviere C & Fort P 2000 TrioGEF1 controls Rac- and Cdc42-dependent cell structures through the direct activation of rhoG. *Journal of Cell Science* **113** 729–739.
- Borbiev T, Verin AD, Shi S, Liu F & Garcia JG 2001 Regulation of endothelial cell barrier function by calcium/calmodulin-dependent protein kinase II.

- American Journal of Physiology. Lung Cellular and Molecular Physiology* **280** L983–L990.
- Bourguignon LY, Gilad E, Brightman A, Diedrich F & Singleton P 2006 Hyaluronan–CD44 interaction with leukemia-associated RhoGEF and epidermal growth factor receptor promotes Rho/Ras co-activation, phospholipase C epsilon-Ca²⁺ signaling, and cytoskeleton modification in head and neck squamous cell carcinoma cells. *Journal of Biological Chemistry* **281** 14026–14040. (doi:10.1074/jbc.M507734200)
- Brown KD & Binder LI 1993 Expression of the cytoskeletal-associated protein filamin in adult rat organs. *Experimental Cell Research* **209** 325–332. (doi:10.1006/excr.1993.1317)
- Browne KA, Johnstone RW, Jans DA & Trapani JA 2000 Filamin (280-kDa actin-binding protein) is a caspase substrate and is also cleaved directly by the cytotoxic T lymphocyte protease granzyme B during apoptosis. *Journal of Biological Chemistry* **275** 39262–39266. (doi:10.1074/jbc.C000622200)
- Camilli TC, Xu M, O'Connell MP, Chien B, Frank BP, Subaran S, Indig FE, Morin PJ, Hewitt SM & Weeraratna AT 2011 Loss of Klotho during melanoma progression leads to increased filamin cleavage, increased Wnt5A expression, and enhanced melanoma cell motility. *Pigment Cell & Melanoma Research* **24** 175–186. (doi:10.1111/j.1755-148X.2010.00792.x)
- Chen M & Stracher A 1989 *In situ* phosphorylation of platelet actin-binding protein by cAMP-dependent protein kinase stabilizes it against proteolysis by calpain. *Journal of Biological Chemistry* **264** 14282–14289.
- da Costa LF 2001 Return of de-differentiation: why cancer is a developmental disease. *Current Opinion in Oncology* **13** 58–62. (doi:10.1097/00001622-200101000-00012)
- Coughlin MF, Puig-de-Morales M, Bursac P, Mellema M, Millet E & Fredberg JJ 2006 Filamin-A and rheological properties of cultured melanoma cells. *Biophysical Journal* **90** 2199–2205. (doi:10.1529/biophysj.105.061267)
- Cousin H, Desimone DW & Alfandari D 2008 PACSIN2 regulates cell adhesion during gastrulation in *Xenopus laevis*. *Developmental Biology* **319** 86–99. (doi:10.1016/j.ydbio.REF4=10.1111/j.1349-7006.2006.00327.x)
- Cranmer SL, Ashworth KJ, Yao Y, Berndt MC, Ruggeri ZM, Andrews RK & Jackson SP 2011 High shear-dependent loss of membrane integrity and defective platelet adhesion following disruption of the GPIIb/3–filamin interaction. *Blood* **117** 2718–2727. (doi:10.1182/blood-2010-07-296194)
- Cukier IH, Li Y & Lee JM 2007 Cyclin B1/Cdk1 binds and phosphorylates Filamin A and regulates its ability to cross-link actin. *FEBS Letters* **581** 1661–1672. (doi:10.1016/j.febslet.2007.REF8=10.1038/35046533)
- De Braekeleer E, Douet-Guilbert N, Morel F, Le Bris MJ, Meyer C, Marschalek R, Ferec C & De Braekeleer M 2009 FLNA, a new partner gene fused to MLL in a patient with acute myelomonoblastic leukaemia. *British Journal of Haematology* **146** 693–695. (doi:10.1111/j.1365-2141.2009.07824.x)
- Deng W, Lopez-Camacho C, Tang JY, Mendoza-Villanueva D, Maya-Mendoza A, Jackson DA & Shore P 2012 Cytoskeletal protein filamin A is a nucleolar protein that suppresses ribosomal RNA gene transcription. *PNAS* **109** 1524–1529. (doi:10.1073/pnas.1107879109)
- Douvaras P, Liu W, Mort RL, McKie L, West KM, Cross SH, Morley SD & West JD 2012 Normal X-inactivation mosaicism in corneas of heterozygous FlnaDilp2/+ female mice – a model of human filamin A (FLNA) diseases. *BMC Research Notes* **5** 122. (doi:10.1186/1756-0500-5-122)
- Feng Y, Chen MH, Moskowitz IP, Mendonza AM, Vidali L, Nakamura F, Kwiatkowski DJ & Walsh CA 2006 Filamin A (FLNA) is required for cell–cell contact in vascular development and cardiac morphogenesis. *PNAS* **103** 19836–19841. (doi:10.1073/pnas.0609628104)
- Fiori JL, Zhu TN, O'Connell MP, Hoek KS, Indig FE, Frank BP, Morris C, Kole S, Hasskamp J, Elias G *et al.* 2009 Filamin A modulates kinase activation and intracellular trafficking of epidermal growth factor receptors in human melanoma cells. *Endocrinology* **150** 2551–2560. (doi:10.1210/en.2008-1344)
- Flanagan LA, Chou J, Falet H, Neujahr R, Hartwig JH & Stossel TP 2001 Filamin A, the Arp2/3 complex, and the morphology and function of cortical actin filaments in human melanoma cells. *Journal of Cell Biology* **155** 511–517. (doi:10.1083/jcb.200105148)
- van der Flier A & Sonnenberg A 2001 Structural and functional aspects of filamins. *Biochimica et Biophysica Acta* **1538** 99–117. (doi:10.1016/S0167-4889(01)00072-6)
- Fox JW, Lamperti ED, Eksioglu YZ, Hong SE, Feng Y, Graham DA, Scheffer IE, Dobyns WB, Hirsch BA, Radtke RA *et al.* 1998 Mutations in filamin 1 prevent migration of cerebral cortical neurons in human periventricular heterotopia. *Neuron* **21** 1315–1325. (doi:10.1016/S0896-6273(00)80651-0)
- Garcia E, Stracher A & Jay D 2006 Calcineurin dephosphorylates the C-terminal region of filamin in an important regulatory site: a possible mechanism for filamin mobilization and cell signaling. *Archives of Biochemistry and Biophysics* **446** 140–150. (doi:10.1016/j.abb.2005.12.006)
- Gargiulo A, Auricchio R, Barone MV, Cotugno G, Reardon W, Milla PJ, Ballabio A, Ciccodicola A & Auricchio A 2007 Filamin A is mutated in X-linked chronic idiopathic intestinal pseudo-obstruction with central nervous system involvement. *American Journal of Human Genetics* **80** 751–758. (doi:10.1086/513321)
- Gawecka JE, Griffiths GS, Ek-Rylander B, Ramos JW & Matter ML 2010 R-Ras regulates migration through an interaction with filamin A in melanoma cells. *PLoS ONE* **5** e11269. (doi:10.1371/journal.pone.0011269)
- Gay O, Gilquin B, Nakamura F, Jenkins ZA, McCartney R, Krakow D, Deshiere A, Assard N, Hartwig JH, Robertson SP *et al.* 2011 ReflinB (FAM101B) targets filamin A to organize perinuclear actin networks and regulates nuclear shape. *PNAS* **108** 11464–11469. (doi:10.1073/pnas.1104211108)
- Goldmann WH 2002 p56(lck) Controls phosphorylation of filamin (ABP-280) and regulates focal adhesion kinase (pp125(FAK)). *Cell Biology International* **26** 567–571. (doi:10.1006/cbir.2002.0900)
- Gorlin JB, Yamin R, Egan S, Stewart M, Stossel TP, Kwiatkowski DJ & Hartwig JH 1990 Human endothelial actin-binding protein (ABP-280, nonmuscle filamin): a molecular leaf spring. *Journal of Cell Biology* **111** 1089–1105. (doi:10.1083/jcb.111.3.1089)
- Guedj N, Zhan Q, Perigny M, Rautou PE, Degos F, Belghiti J, Farges O, Bedossa P & Paradis V 2009 Comparative protein expression profiles of hilar and peripheral hepatic cholangiocarcinomas. *Journal of Hepatology* **51** 93–101. (doi:10.1016/j.jhep.2009.03.017)
- Hanahan D & Weinberg RA 2000 The hallmarks of cancer. *Cell* **100** 57–70. (doi:10.1016/S0092-8674(00)81683-9)
- Hart AW, Morgan JE, Schneider J, West K, McKie L, Bhattacharya S, Jackson JJ & Cross SH 2006 Cardiac malformations and midline skeletal defects in mice lacking filamin A. *Human Molecular Genetics* **15** 2457–2467. (doi:10.1093/hmg/ddl168)
- Hartwig JH & Stossel TP 1975 Isolation and properties of actin, myosin, and a new actin-binding protein in rabbit alveolar macrophages. *Journal of Biological Chemistry* **250** 5696–5705.
- He M, Cheng Y, Li W, Liu Q, Liu J, Huang J & Fu X 2010 Vascular endothelial growth factor C promotes cervical cancer metastasis via up-regulation and activation of RhoA/ROCK-2/moesin cascade. *BMC Cancer* **10** 170. (doi:10.1186/1471-2407-10-170)
- Heuze ML, Lamsoul I, Baldassarre M, Lad Y, Leveque S, Razinia Z, Moog-Lutz C, Calderwood DA & Lutz PG 2008 ASB2 targets filamins A and B to proteasomal degradation. *Blood* **112** 5130–5140. (doi:10.1182/blood-2007-12-128744)
- Hosaka N, Murase N, Orito T & Mori M 1985 Immunohistochemical evaluation of factor VIII related antigen, filament proteins and lectin binding in haemangiomas. *Virchows Archiv. A, Pathological Anatomy and Histopathology* **407** 237–247. (doi:10.1007/BF00710649)
- Jay D, Garcia EJ, Lara JE, Medina MA & de la Luz Ibarra M 2000 Determination of a cAMP-dependent protein kinase phosphorylation site in the C-terminal region of human endothelial actin-binding

- protein. *Archives of Biochemistry and Biophysics* **377** 80–84. (doi:10.1006/abbi.2000.1762)
- Johansen LD, Naumanen T, Knudsen A, Westerlund N, Gromova I, Junttila M, Nielsen C, Bottzauw T, Tolkovsky A, Westermarck J *et al.* 2008 IKAP localizes to membrane ruffles with filamin A and regulates actin cytoskeleton organization and cell migration. *Journal of Cell Science* **121** 854–864. (doi:10.1242/jcs.013722)
- Jopling C, Boue S & Izpisua Belmonte JC 2011 Dedifferentiation, transdifferentiation and reprogramming: three routes to regeneration. *Nature Reviews. Molecular Cell Biology* **12** 79–89. (doi:10.1038/nrm3043)
- Jurak Begonja A, Hoffmeister KM, Hartwig JH & Falet H 2011 FlnA-null megakaryocytes prematurely release large and fragile platelets that circulate poorly. *Blood* **118** 2285–2295. (doi:10.1182/blood-2011-04-348482)
- Kamochi N, Nakashima M, Aoki S, Uchihashi K, Sugihara H, Toda S & Kudo S 2008 Irradiated fibroblast-induced bystander effects on invasive growth of squamous cell carcinoma under cancer–stromal cell interaction. *Cancer Science* **99** 2417–2427. (doi:10.1111/j.1349-7006.2008.00978.x)
- Kawamoto S & Hidaka H 1984 Ca²⁺-activated, phospholipid-dependent protein kinase catalyzes the phosphorylation of actin-binding proteins. *Biochemical and Biophysical Research Communications* **118** 736–742. (doi:10.1016/0006-291X(84)91456-6)
- Kim H & McCulloch CA 2011 Filamin A mediates interactions between cytoskeletal proteins that control cell adhesion. *FEBS Letters* **585** 18–22. (doi:10.1016/j.febslet.2010.11.033)
- Kim EJ, Park JS & Um SJ 2007 Filamin A negatively regulates the transcriptional activity of p73 α in the cytoplasm. *Biochemical and Biophysical Research Communications* **362** 1101–1106. (doi:10.1016/j.bbrc.2007.08.148)
- Kim H, Sengupta A, Glogauer M & McCulloch CA 2008 Filamin A regulates cell spreading and survival via β 1 integrins. *Experimental Cell Research* **314** 834–846. (doi:10.1016/j.yexcr.2007.11.022)
- Kyndt F, Gueffet JP, Probst V, Jaafar P, Legendre A, Le Bouffant F, Toquet C, Roy E, McGregor L, Lynch SA *et al.* 2007 Mutations in the gene encoding filamin A as a cause for familial cardiac valvular dystrophy. *Circulation* **115** 40–49. (doi:10.1161/CIRCULATIONAHA.106.622621)
- Lamsoul I, Metais A, Gouot E, Heuze ML, Lennon-Dumenil AM, Moog-Lutz C & Lutz PG 2013 ASB2 α regulates migration of immature dendritic cells. *Blood* **122** 533–541. (doi:10.1182/blood-2012-11-466649)
- Larriba MJ, Martin-Villar E, Garcia JM, Pereira F, Pena C, de Herreros AG, Bonilla F & Munoz A 2009 Snail2 cooperates with Snail1 in the repression of vitamin D receptor in colon cancer. *Carcinogenesis* **30** 1459–1468. (doi:10.1093/carcin/bgp140)
- Letourneau PC & Shattuck TA 1989 Distribution and possible interactions of actin-associated proteins and cell adhesion molecules of nerve growth cones. *Development* **105** 505–519.
- Li C, Yu S, Nakamura F, Pentikainen OT, Singh N, Yin S, Xin W & Sy MS 2010 Pro-prion binds filamin A, facilitating its interaction with integrin β 1, and contributes to melanomagenesis. *Journal of Biological Chemistry* **285** 30328–30339. (doi:10.1074/jbc.M110.147413)
- Lian G, Lu J, Hu J, Zhang J, Cross SH, Ferland RJ & Sheen VL 2012 Filamin A regulates neural progenitor proliferation and cortical size through Wee1-dependent Cdk1 phosphorylation. *Journal of Neuroscience* **32** 7672–7684. (doi:10.1523/JNEUROSCI.0894-12.2012)
- Loy CJ, Sim KS & Yong EL 2003 Filamin-A fragment localizes to the nucleus to regulate androgen receptor and coactivator functions. *PNAS* **100** 4562–4567. (doi:10.1073/pnas.0736237100)
- Lu J, Tiao G, Folkert H, Hecht J, Walsh C & Sheen V 2006 Overlapping expression of ARFGEF2 and Filamin A in the neuroependymal lining of the lateral ventricles: insights into the cause of periventricular heterotopia. *Journal of Comparative Neurology* **494** 476–484. (doi:10.1002/cne.20806)
- Maceyka M, Alvarez SE, Milstien S & Spiegel S 2008 Filamin A links sphingosine kinase 1 and sphingosine-1-phosphate receptor 1 at lamellipodia to orchestrate cell migration. *Molecular and Cellular Biology* **28** 5687–5697. (doi:10.1128/MCB.00465-08)
- MacPherson M & Fagerholm SC 2010 Filamin and filamin-binding proteins in integrin-regulation and adhesion. Focus on: "FilaminA is required for vimentin-mediated cell adhesion and spreading". *American Journal of Physiology. Cell Physiology* **298** C206–C208. (doi:10.1152/ajpcell.00505.2009)
- Maestrini E, Patrosso C, Mancini M, Rivella S, Rocchi M, Repetto M, Villa A, Frattini A, Zoppe M, Vezzoni P *et al.* 1993 Mapping of two genes encoding isoforms of the actin binding protein ABP-280, a dystrophin like protein, to Xq28 and to chromosome 7. *Human Molecular Genetics* **2** 761–766. (doi:10.1093/hmg/2.6.761)
- Masruha MR, Caboclo LO, Carrete H Jr, Cendes IL, Rodrigues MG, Garzon E, Yacubian EM, Sakamoto AC, Sheen V, Hamey M *et al.* 2006 Mutation in filamin A causes periventricular heterotopia, developmental regression, and West syndrome in males. *Epilepsia* **47** 211–214. (doi:10.1111/j.1528-1167.2006.00390.x)
- Meng X, Yuan Y, Maestas A & Shen Z 2004 Recovery from DNA damage-induced G2 arrest requires actin-binding protein filamin-A/actin-binding protein 280. *Journal of Biological Chemistry* **279** 6098–6105. (doi:10.1074/jbc.M306794200)
- Mishra L & Marshall B 2006 Adaptor proteins and ubiquinators in TGF- β signaling. *Cytokine & Growth Factor Reviews* **17** 75–87. (doi:10.1016/j.cytogfr.2005.09.001)
- Mooso BA, Vinnall RL, Tepper CG, Savoy RM, Cheung JP, Singh S, Siddiqui S, Wang Y, Bedolla RG, Martinez A *et al.* 2012 Enhancing the effectiveness of androgen deprivation in prostate cancer by inducing Filamin A nuclear localization. *Endocrine-Related Cancer* **19** 759–777. (doi:10.1530/ERC-12-0171)
- Mora N, Rosales R & Rosales C 2007 R-Ras promotes metastasis of cervical cancer epithelial cells. *Cancer Immunology and Immunotherapy* **56** 535–544. (doi:10.1007/s00262-006-0205-z)
- Muller M, Albrecht S, Golfert F, Hofer A, Funk RH, Magdolen V, Flossel C & Luther T 1999 Localization of tissue factor in actin-filament-rich membrane areas of epithelial cells. *Experimental Cell Research* **248** 136–147. (doi:10.1006/excr.1999.4395)
- Nagano T, Yoneda T, Hatanaka Y, Kubota C, Murakami F & Sato M 2002 Filamin A-interacting protein (FILIP) regulates cortical cell migration out of the ventricular zone. *Nature Cell Biology* **4** 495–501. (doi:10.1038/ncb808)
- Nagano T, Morikubo S & Sato M 2004 Filamin A and FILIP (Filamin A-Interacting Protein) regulate cell polarity and motility in neocortical subventricular and intermediate zones during radial migration. *Journal of Neuroscience* **24** 9648–9657. (doi:10.1523/JNEUROSCI.2363-04.2004)
- Nakamura F, Stossel TP & Hartwig JH 2011 The filamins: organizers of cell structure and function. *Cell Adhesion & Migration* **5** 160–169. (doi:10.4161/cam.5.2.14401)
- Nallapalli RK, Ibrahim MX, Zhou AX, Bandaru S, Naresh S, Redfors B, Pazooki D, Zhang Y, Boren J, Cao Y *et al.* 2012 Targeting filamin A reduces K-RAS-induced lung adenocarcinomas and endothelial response to tumor growth in mice. *Molecular Cancer* **11** 50. (doi:10.1186/1476-4598-11-50)
- Niki M, Okada H, Takano H, Kuno J, Tani K, Hibino H, Asano S, Ito Y, Satake M & Noda T 1997 Hematopoiesis in the fetal liver is impaired by targeted mutagenesis of a gene encoding a non-DNA binding subunit of the transcription factor, polyomavirus enhancer binding protein 2/core binding factor. *PNAS* **94** 5697–5702. (doi:10.1073/pnas.94.11.5697)
- Ohta Y & Hartwig JH 1996 Phosphorylation of actin-binding protein 280 by growth factors is mediated by p90 ribosomal protein S6 kinase. *Journal of Biological Chemistry* **271** 11858–11864. (doi:10.1074/jbc.271.27.16294)
- Ohta Y, Suzuki N, Nakamura S, Hartwig JH & Stossel TP 1999 The small GTPase Ra1A targets filamin to induce filopodia. *PNAS* **96** 2122–2128. (doi:10.1073/pnas.96.5.2122) (doi:10.1073/pnas.96.5.2122)
- Okada H, Watanabe T, Niki M, Takano H, Chiba N, Yanai N, Tani K, Hibino H, Asano S, Mucenski ML *et al.* 1998 AML1(–/–) embryos do not express

- certain hematopoiesis-related gene transcripts including those of the PU.1 gene. *Oncogene* **17** 2287–2293. (doi:10.1038/sj.onc.1202151)
- Ott I, Fischer EG, Miyagi Y, Mueller BM & Ruf W 1998 A role for tissue factor in cell adhesion and migration mediated by interaction with actin-binding protein 280. *Journal of Cell Biology* **140** 1241–1253. (doi:10.1083/jcb.140.5.1241)
- Ozanne DM, Brady ME, Cook S, Gaughan L, Neal DE & Robson CN 2000 Androgen receptor nuclear translocation is facilitated by the f-actin cross-linking protein filamin. *Molecular Endocrinology* **14** 1618–1626. (doi:10.1210/me.14.10.1618)
- Pal Sharma C & Goldmann WH 2004 Phosphorylation of actin-binding protein (ABP-280; filamin) by tyrosine kinase p56lck modulates actin filament cross-linking. *Cell Biology International* **28** 935–941. (doi:10.1016/j.cellbi.2004.09.006)
- Parrini E, Ramazzotti A, Dobyns WB, Mei D, Moro F, Veggiotti P, Marini C, Brillstra EH, Dalla Bernardina B, Goodwin L *et al.* 2006 Periventricular heterotopia: phenotypic heterogeneity and correlation with Filamin A mutations. *Brain* **129** 1892–1906. (doi:10.1093/brain/awl125)
- Porter RM, Holme TC, Newman EL, Hopwood D, Wilkinson JM & Cuschieri A 1993 Monoclonal antibodies to cytoskeletal proteins: an immunohistochemical investigation of human colon cancer. *Journal of Pathology* **170** 435–440. (doi:10.1002/path.1711700406)
- Price MG, Caprette DR & Gomer RH 1994 Different temporal patterns of expression result in the same type, amount, and distribution of filamin (ABP) in cardiac and skeletal myofibrils. *Cell Motility and the Cytoskeleton* **27** 248–261. (doi:10.1002/cm.970270306)
- Ravid D, Chuderland D, Landsman L, Lavie Y, Reich R & Liscovitch M 2008 Filamin A is a novel caveolin-1-dependent target in IGF-I-stimulated cancer cell migration. *Experimental Cell Research* **314** 2762–2773. (doi:10.1016/j.yexcr.2008.06.004)
- Raynaud F, Jond-Necand C, Marcilhac A, Furst D & Benyamin Y 2006 Calpain 1-gamma filamin interaction in muscle cells: a possible *in situ* regulation by PKC- α . *International Journal of Biochemistry & Cell Biology* **38** 404–413. (doi:10.1016/j.biocel.2006.01.001)
- Razinia Z, Baldassarre M, Bouaouina M, Lamsoul I, Lutz PG & Calderwood DA 2011 The E3 ubiquitin ligase specificity subunit ASB2 α targets filamins for proteasomal degradation by interacting with the filamin actin-binding domain. *Journal of Cell Science* **124** 2631–2641. (doi:10.1242/jcs.084343)
- Ricci-Vitiani L, Pallini R, Biffoni M, Todaro M, Invernici G, Cenci T, Maira G, Parati EA, Stassi G, Larocca LM *et al.* 2010 Tumour vascularization via endothelial differentiation of glioblastoma stem-like cells. *Nature* **468** 824–828. (doi:10.1038/nature09557)
- Robertson SP 2005 Filamin A: phenotypic diversity. *Current Opinion in Genetics & Development* **15** 301–307. (doi:10.1016/j.gde.2005.04.001)
- Robertson SP, Twigg SR, Sutherland-Smith AJ, Biancalana V, Gorlin RJ, Horn D, Kenwick SJ, Kim CA, Morava E, Newbury-Ecob R *et al.* 2003 Localized mutations in the gene encoding the cytoskeletal protein filamin A cause diverse malformations in humans. *Nature Genetics* **33** 487–491. (doi:10.1038/ng1119)
- Ruskamo S & Ylanne J 2009 Structure of the human filamin A actin-binding domain. *Acta Crystallographica. Section D. Biological Crystallography* **65** 1217–1221. (doi:10.1107/S0907444909037330)
- Ruskamo S, Gilbert R, Hofmann G, Jiang P, Campbell ID, Ylanne J & Pentikainen U 2012 The C-terminal rod 2 fragment of filamin A forms a compact structure that can be extended. *Biochemical Journal* **446** 261–269. (doi:10.1042/BJ20120361)
- Sarkisian MR, Bartley CM, Chi H, Nakamura F, Hashimoto-Torii K, Torii M, Flavell RA & Rakic P 2006 MEKK4 signaling regulates filamin expression and neuronal migration. *Neuron* **52** 789–801. (doi:10.1016/j.neuron.2006.10.024)
- Sato M & Nagano T 2005 Involvement of filamin A and filamin A-interacting protein (FILIP) in controlling the start and cell shape of radially migrating cortical neurons. *Anatomical Science International* **80** 19–29. (doi:10.1111/j.1447-073x.2005.00101.x)
- Saito K, Ozawa Y, Hibino K & Ohta Y 2012 FilGAP, a Rho/Rho-associated protein kinase-regulated GTPase-activating protein for Rac, controls tumor cell migration. *Molecular Biology of the Cell* **23** 4739–4750. (doi:10.1091/mbc.E12-04-0310)
- Sheen VL, Dixon PH, Fox JW, Hong SE, Kinton L, Sisodiya SM, Duncan JS, Dubeau F, Scheffer IE, Schachter SC *et al.* 2001 Mutations in the X-linked filamin 1 gene cause periventricular nodular heterotopia in males as well as in females. *Human Molecular Genetics* **10** 1775–1783.
- Sheen VL, Jansen A, Chen MH, Parrini E, Morgan T, Ravenscroft R, Ganesh V, Underwood T, Wiley J, Leventer R *et al.* 2005 Filamin A mutations cause periventricular heterotopia with Ehlers–Danlos syndrome. *Neurology* **64** 254–262.
- Stossel TP & Hartwig JH 1975 Interactions between actin, myosin, and an actin-binding protein from rabbit alveolar macrophages. Alveolar macrophage myosin Mg-2+ -adenosine triphosphatase requires a cofactor for activation by actin. *Journal of Biological Chemistry* **250** 5706–5712.
- Stossel TP, Condeelis J, Cooley L, Hartwig JH, Noegel A, Schleicher M & Shapiro SS 2001 Filamins as integrators of cell mechanics and signalling. *Nature Reviews. Molecular Cell Biology* **2** 138–145. (doi:10.1038/35052082)
- Takabayashi T, Xie MJ, Takeuchi S, Kawasaki M, Yagi H, Okamoto M, Tariqur RM, Malik F, Kuroda K, Kubota C *et al.* 2010 LL5 β directs the translocation of filamin A and SHIP2 to sites of phosphatidylinositol 3,4,5-triphosphate (PtdIns(3,4,5)P3) accumulation, and PtdIns(3,4,5)P3 localization is mutually modified by co-recruited SHIP2. *Journal of Biological Chemistry* **285** 16155–16165. (doi:10.1074/jbc.M109.081901)
- Telles E, Gurjar M, Ganti K, Gupta D & Dalal SN 2011 Filamin A stimulates cdc25C function and promotes entry into mitosis. *Cell Cycle* **10** 776–782. (doi:10.4161/cc.10.5.14954)
- Tian H-M, Liu X-H, Han W, Zhao L-L, Yuan B & Yuan C-J 2013 Differential expression of filamin A and its clinical significance in breast cancer. *Oncology Letters* **6** 681–686. (doi:10.3892/ol.2013.1454)
- Uhlen M, Oksvold P, Fagerberg L, Lundberg E, Jonasson K, Forsberg M, Zwahlen M, Kampf C, Wester K, Hober S *et al.* 2010 Towards a knowledge-based Human Protein Atlas. *Nature Biotechnology* **28** 1248–1250. (doi:10.1038/nbt1210-1248)
- Uramoto H, Akyurek LM & Hanagiri T 2010 A positive relationship between filamin and VEGF in patients with lung cancer. *Anticancer Research* **30** 3939–3944.
- Velkova A, Carvalho MA, Johnson JO, Tavtigan SV & Monteiro AN 2010 Identification of Filamin A as a BRCA1-interacting protein required for efficient DNA repair. *Cell Cycle* **9** 1421–1433. (doi:10.4161/cc.9.7.11256)
- Venkatareddy M, Cook L, Abuarquob K, Verma R & Garg P 2011 Nephren regulates lamellipodia formation by assembling a protein complex that includes Ship2, filamin and lamellipodin. *PLoS ONE* **6** e28710. (doi:10.1371/journal.pone.0028710)
- Wallach D, Davies PJ & Pastan I 1978 Cyclic AMP-dependent phosphorylation of filamin in mammalian smooth muscle. *Journal of Biological Chemistry* **253** 4739–4745.
- Wang Y, Kreisberg JI, Bedolla RG, Mikhailova M, deVere White RW & Ghosh PM 2007 A 90 kDa fragment of filamin A promotes Casodex-induced growth inhibition in Casodex-resistant androgen receptor positive C4-2 prostate cancer cells. *Oncogene* **26** 6061–6070. (doi:10.1038/sj.onc.1210435)
- Wang R, Chadalavada K, Wilshire J, Kowalik U, Hovinga KE, Geber A, Fligelman B, Leversha M, Brennan C & Tabar V 2010 Glioblastoma stem-like cells give rise to tumour endothelium. *Nature* **468** 829–833. (doi:10.1038/nature09624)
- Watanabe T, Yoshida N & Satake M 2005 Biological implications of filamin A-bound PEBP2 β /CBF β retention in the cytoplasm. *Critical Reviews in Eukaryotic Gene Expression* **15** 197–206. (doi:10.1615/CritRevEukarGeneExpr.v15.i3.20)
- Wiemann S, Weil B, Wellenreuther R, Gassenhuber J, Glassl S, Ansoerge W, Bocher M, Blocker H, Bauersachs S, Blum H *et al.* 2001 Toward a catalog

- of human genes and proteins: sequencing and analysis of 500 novel complete protein coding human cDNAs. *Genome Research* **11** 422–435. (doi:10.1101/gr.GR1547R)
- de Wit MC, Kros JM, Halley DJ, de Coo IF, Verdijk R, Jacobs BC & Mancini GM 2009 Filamin A mutation, a common cause for periventricular heterotopia, aneurysms and cardiac defects. *Journal of Neurology, Neurosurgery, and Psychiatry* **80** 426–428. (doi:10.1136/jnnp.2008.149419)
- Xu Y, Bismar TA, Su J, Xu B, Kristiansen G, Varga Z, Teng L, Ingber DE, Mammoto A, Kumar R *et al.* 2010 Filamin A regulates focal adhesion disassembly and suppresses breast cancer cell migration and invasion. *Journal of Experimental Medicine* **207** 2421–2437. (doi:10.1084/jem.20100433)
- Yoshida N, Ogata T, Tanabe K, Li S, Nakazato M, Kohu K, Takafuta T, Shapiro S, Ohta Y, Satake M *et al.* 2005 Filamin A-bound PEBP2 β /CBF β is retained in the cytoplasm and prevented from functioning as a partner of the Runx1 transcription factor. *Molecular and Cellular Biology* **25** 1003–1012. (doi:10.1128/MCB.25.3.1003-1012.2005)
- Yuan Y & Shen Z 2001 Interaction with BRCA2 suggests a role for filamin-1 (hsFLNa) in DNA damage response. *Journal of Biological Chemistry* **276** 48318–48324. (doi:10.1074/jbc.M102557200)
- Yue J, Wang Q, Lu H, Brenneman M, Fan F & Shen Z 2009 The cytoskeleton protein filamin-A is required for an efficient recombinational DNA double strand break repair. *Cancer Research* **69** 7978–7985. (doi:10.1158/0008-5472.CAN-09-2177)
- Yue J, Huhn S & Shen Z 2013 Complex roles of filamin-A mediated cytoskeleton network in cancer progression. *Cell & Bioscience* **3** 7. (doi:10.1186/2045-3701-3-7)
- Zhong Z, Yeow WS, Zou C, Wassell R, Wang C, Pestell RG, Quong JN & Quong AA 2010 Cyclin D1/cyclin-dependent kinase 4 interacts with filamin A and affects the migration and invasion potential of breast cancer cells. *Cancer Research* **70** 2105–2114. (doi:10.1158/0008-5472.CAN-08-1108)
- Zhou X, Boren J & Akyurek LM 2007 Filamins in cardiovascular development. *Trends in Cardiovascular Medicine* **17** 222–229. (doi:10.1016/j.tcm.2007.08.001)
- Zhou AX, Hartwig JH & Akyurek LM 2010 Filamins in cell signaling, transcription and organ development. *Trends in Cell Biology* **20** 113–123. (doi:10.1016/j.tcb.2009.12.001)
- Zhou AX, Toylu A, Nallapalli RK, Nilsson G, Atabey N, Heldin CH, Boren J, Bergo MO & Akyurek LM 2011 Filamin A mediates HGF/c-MET signaling in tumor cell migration. *International Journal of Cancer* **128** 839–846. (doi:10.1002/ijc.25417)
- Zhu TN, He HJ, Kole S, D'Souza T, Agarwal R, Morin PJ & Bernier M 2007 Filamin A-mediated down-regulation of the exchange factor Ras-GRF1 correlates with decreased matrix metalloproteinase-9 expression in human melanoma cells. *Journal of Biological Chemistry* **282** 14816–14826. (doi:10.1074/jbc.M61143020REF110=10.1615/CritRev-EukarGeneExpr.v15.i3.20)

Received in final form 6 October 2013

Accepted 9 October 2013

Made available online as an Accepted Preprint

9 October 2013